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GENOMIC EVALUATION OF BULLS FOR DAUGHTERS' MILK TRAITS IN RUSSIAN BLACK-AND-WHITE AND HOLSTEIN CATTLE POPULATION THROUGH THE VALIDATION PROCEDURE

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Abstract

The rapid development of molecular genetic methods in animal breeding over the past ten years has given rise to an increase of the selection intensity at the population level. Expansion of the economically useful traits spectrum of dairy cattle allowed increasing the opportunities for breeding to improve the cows' health and for studying the essence of the metabolic synthesis of milk components. The purpose of this study was to verify the effectiveness of genomic forecasting in the development of the concept of dairy cattle genetic assessment in the regional and national aspects. The study for bulls' estimations in Russian Black-and-White improved by Holstein and Holstein breeds by simulation of breeding process using 124 herds of the Moscow and Leningrad regions was carried out. The effectiveness of genomic prediction as compared to the parent averages (PA) and the estimated breeding values (EBV) of sires have been shown. The selection of testing bulls based on the genomic information corrects PA and is to refine EBV that is further obtained by using progeny. Repeatability of genomic EBV was obtained through validation of parentage and genomic information for 100 sires with data for at least 300 daughters. This dataset lay down the core of the newly created Russian regional reference group of dairy cattle. For calculating the additive relationship matrix 1050 ancestors was used. For genomic relationship matrix, 39818 nucleotide polymorphisms were taken into analysis. Based on the REML, BLUP SM, GBLUP methods the procedures to assess of animals were carried out. The average annual genetic trend for milk production traits in the studied populations from 1987 to 2006 was +60 kg, +2.5 kg, +1.5 kg by milk yield, milk fat and milk protein, respectively. It was found that the repeatability of genomic estimates was ranging from 0.371 to 0.606 for milk production traits, which on average exceeded the PA value by 0.147. The accuracy of the evaluation obtained by progeny tested bulls ranged from 0.879 to 0.900 that was higher than the genomic prediction by 0.405 units. The principles of creating the reference population based on the analysis of multi dimension scaling and genetic distances were studied. The distinction between two regional populations (Moscow and Leningrad regions) was $F_{st} = 0.0025$. The decay of the linkage disequilibrium between the markers at distances up to 1000 kb is shown. In distance from 5 up to 70 kb the linkage level was get the maximum values from 0.20 to 0.54. In the framework of the metabolic pathways study for the milk components synthesis genetic parameters and mean least square estimates

were obtained for the extended milk composition: lactose ($h^2 = 0.18$), dry matter ($h^2 = 0.10$), solids-not-fat ($h^2 = 0.19$), milk freezing point ($h^2 = 0.06$), somatic cells score ($h^2 = 0.10$) and milk urea ($h^2 = 0.04$). The values of additive genetic variances have been get indicating the objective possibilities of using them in Russian dairy cattle breeding sector. To obtain reliable whole-genome associations a further replenishment of the database of the cows' milk component will be carried out using additional spectra. The complex studies have grounded approaches to the use of genomic estimations, the principles of reference population extension and widening list of features for the quantitative and qualitative milk composition assessment.

Keywords: genomic breeding value, milk production, reference population, linkage disequilibrium, heritability, milk components

Development of modern techniques and approaches requires rethinking of the existing pedigree practice in livestock farming. Within the scope of breeding programs, animal breeding system becomes a priority since it largely depends on the selection pressure and on breeding value assessment tools by such categories as parents of sires, as well as parents of high-producing cows. Complex testing of animals in terms of their productivity, breeding record, total impact of the genetic markers across the entire genome, and progeny characterization are the integral part of the work with domestic stock.

The project for genome assessment in dairy cattle, which had started at the beginning of the XXI century, had opened new genome era in selection [1, 2]. First genome assessment had been obtained for bulls of Holstein and Jersey breeds [3, 4]. Nowadays, at least 32 countries participate in genomic multi-trait across country evaluation (GMACE). However, only in 11 of these countries a forecast of the genome pedigree value by milk performance has the official status for use in dairy cattle selection programs [5-7].

Practical efficiency of genomic selection depends on many factors, the main these are size of the reference population, contribution of information about each genotyped animal and its progeny in a series of generations, genetic variability of the analyzed signs (heritability), genome scanning density, distribution of the quantitative trait loci (QTL) and linkage disequilibrium (LD) between markers [8]. However, rather different objectives of development of the genome value estimation algorithms arise upon obtainment of such parameters, in particular by number of species in the reference population (for instance, over 1 million heads in USA). Thus, APY algorithm is used for formation of the population nucleus out of the animals tested by offspring quality and young (for breeding) genotyped animals and presupposes inversion of the smaller genome matrix. For Holstein breed the optimal number comprises at least 12 thousand heads depending on the efficient population size [9]. Issues of prompt access to information and time spend by computer for the procedure also appears to be the priority: from 2 hours upon use of the APY algorithm to 1 month upon using of the standard approach to obtainment of the genome relationship matrix (GRM) type G^{-1} [10].

Besides, studies are being conducted for identification of separate causal nucleotides (point mutations) for qualitative traits (QTN), which along with numerous known non-coding polymorphic replacements (single nucleotide polymorphism, SNP) may improve the accuracy of the genome forecast. The research is aimed at optimization of SNP number with high variability share of causal variants for construction of the GRM, enabling to obtain information on the large number of genotypes and 100 % approximation of the assessment accuracy [11]. Thus, causal SNPs validly associated with economically useful traits in dairy cattle have been detected on chromosomes 5, 6, 9, 14, 15, and 20. Polymorphisms located close to or inside the genes *DGATI* (BTA14), *GHR* (BTA20), *ABCG2* (BTA6) display the most genetic dispersion by milk production indicators [12-16]. At the same time, regions with genes *FAM181A*, *SLC24A4* and

NKX21 have also been found on chromosome 21 (47-59 million bps) for poorly inherited traits (age of first calving service period) [17]. It should be noted that milk and meat characteristics (protein fraction, fat acids) indicating on close relationship with several QTL regions in the genome have been obtained during study [18, 19]. This research is of high relevance for understanding how biosynthesis of primary structural elements occurs and what metabolic ways regulate expression of quantitative traits in animals.

Economic indicators of fat and protein content and the number of somatic cells are of the most interest to control the milk composition during selection. Recently, special emphasis is placed on the herded animal health indicators, which may be indirectly determined by composition of the feedstock [20]. Introduction of additional assessment parameters of the quantitative and qualitative composition of milk (traces of acetone and β -hydroxybutyric acid, urea content) shall have positive effect on realization of the genetic value in animals by normalization of the metabolic process (acidosis, ketosis), improvement of fertility, extension of productive use, and improvement of the quality of products obtained [21].

Genetic analysis of inheritance of lactose level, dry matter (DM) content and milk solids-not-fat (SNF) in cow milk had shown perspectives of these traits in selection: for lactose $h^2 = 0.281-0.340$, for DM $h^2 = 0.298$, for SNF $h^2 = 0.20-0.30$ [22, 23]. Genotype determinacy (h^2 from 0.072 to 0.130-0.220) had also been found for urea concentration in milk characterizing the balance between the energy value of a diet and protein consumption by an animal [24]. Other individual parameters such as milk freezing point (FP) allow assessing milking procedure in terms of excessive contents of water in milk. Due to significant influence of paratypic factors, e.g. age of animal, month of year, lactation stage, herd size, milking system) on the FP, heritability value did not exceed $h^2 = 0.120$ [25, 26; A. Costa et al., 2017, personal communication]. In-deep analysis of milk fractions would contribute to a better understanding of animal metabolism in order to find mechanisms of its genetic regulation.

Creation of the genome assessment system is one of the integration priorities of the Russian livestock breeding into the global structure for improving the competitiveness of the national breeding material. This problem may not be solved by simple reproduction of foreign models due to significant differences in environmental factors (feeding, keeping, used technologies, and climate conditions), genetic structure of population (genetic variability, LD), methods and ways of measurement of the economically valuable traits. Recently, there is accumulation of breeding records, and extension of the Russian reference population of Russian Black-and-White improved by Holsteins and Holstein breeds, as well as approbation of modern genotype assessment methodologies in animals. Best world practices in this domain may be effectively incorporated upon attainment of comparable information.

Genome forecasting methodology had been practiced in Moscow and Leningrad regions with high results on productive parameters and pedigree work in dairy stock farming. The article displays the results of selection modeling in sires by validation of the genomic breeding value forecasts for the first time implemented on the Russian Black-and-White improved by Holsteins and Holsteins. It was shown that within the scope of the regional component, genetic assessment values shall be corrected by the ancestor values, which in its turn are adjusted by the large number of SNP markers. Our findings describe additive variability on additional milk composition indicators which reflect qualitative and quantitative parameters of milk production.

Purpose of this study was estimation of the genomic breeding values for

milk production traits in core population of Russian Black-and-Whites improved by Holsteins and Holsteins based on full-genome data.

Techniques. Study was conducted in populations of Russian Black-and-Whites cattle improved by Holsteins and Holsteins in Moscow Province (MP) and Leningrad Province (LP). Fifty sires from each region (1987–2006 years of birth) were genotyped using biochip Illumina Bovine SNP50K v2 BeadChip with a total of 54609 bovine SNPs (Illumina, USA). The sample included bulls with at least 300 first-calf daughter cows, with known milk yielding for 305-day lactation, milk fat fraction (weigh%, MWF) and protein fraction (weigh%, PWF), milk fat (MF) and milk protein (MP). This sample was the core reference population used to simulate validation of bull sires by ancestors (based on breeding records), offspring quality, and full-genome data.

Estimated breeding values (EBVs) were calculated via BLUP Sire Model (BLUP SM) method according to earlier approved mixed type model [15]. The database summarized the information on 77375 first-calf cows from 124 pedigree herds, including 35190 heads from 77 herds in MP, and 42185 heads from 47 herds in LP. Parents' average estimates were recalculated by removing EBV values for 10 randomly selected sires from each regional population, 20 heads in total; direct genome value (DGV) was obtained by GBLUP (genomic BLUP) method [27]. The validated sample consisted of 68175 heads (31485 heads from Moscow region, and 36690 heads from Leningrad region). Variation-covariation components were assessed by the restricted maximum likelihood (REML) method using the population constants of heritability, i.e. 0.180 for milking, 0.221 for milk fat fraction, 0.177 for fat weight, 0.173 for milk protein fraction, and 0.142 for protein weight. EBV in sires was calculated with the use of software of BLUPF90 family, including REMLF90 [28].

Total number of ancestors in breeding records of sires comprised 1050 heads. Calculation of the genetic distances between populations (F_{st}) and multi-dimensional scaling (MDS) with assessment of the linkage disequilibrium between markers were performed with the use of Plink 1.07 and Plink 1.9 package software [29]. After quality control of genotyping, 39818 SNPs were analyzed. For determination of LD values, calculations were made for each chromosome with further averaging in sliding window of 1000 kilobase pairs (--chr ... --r2 --ld-window 1000 --ld-window-kb 1000 --ld-window-r2 0) [30].

Milk composition database obtained by infrared spectrometry of milk samples (a CombiFoss F+ device, FOSS, Denmark) from 7784 cows of 7 pedigree herds (Moscow Province, 2016) were analyzed to study the metabolic ways and regulation of milk component synthesis. Selection and genetic parameters for quantitative traits (lactose content in milk, %; dry matter content, %; fat free milk solids — FFS, %) and qualitative traits (freezing point of milk, °C; somatic cell score, CSC; urea concentration, mg/100 ml) were evaluated. Average values were assessed by method of least square (MLS) using Statistica 7.0 software. The interactions considered were herd × month of a control event, herd × age (number of lactations), and herd × influence of the sire.

Results. We have compared the animals by the years of birth to estimate breeding value (EBV) with regard to regional location and genetic grouping, including for the purpose of prediction of the selection effect and effectiveness of the selected sires from different generations. This comparison showed incremental growth of the genetic potential in bull groups regardless of the region of origin and the number of assessed offspring.

In Moscow region, average annual genetic trend estimates were +37 kg for milk, +1.7, and +0.8 kg for milk fat and protein, whereas in Leningrad region these values were more than 2 times higher, +84, +3.3 and +2.3 kg, respectively.

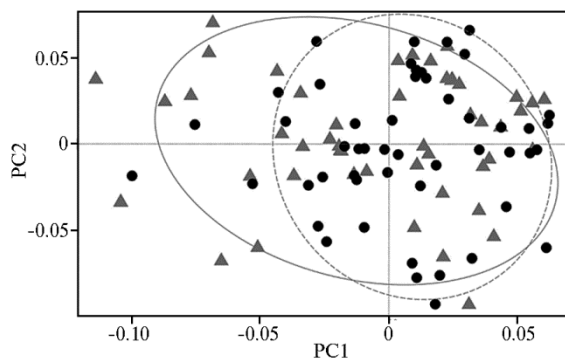


Fig. 1. Spatial distribution of bull sire populations of Russian Black-and-White improved by Holstein and Holstein breeds from households in Moscow (▲) and Leningrad (●) regions by principal components PC1 and PC2.

Allocation of linkage disequilibrium between SNP markers showed the polymorphism heritability at a distance from 0-5 to 60-70 kbp with $r^2 = 0.201-0.544$ for MP and $r^2 = 0.199-0.518$ for LP. Moderate relationship with the linkage from 0.151 (MP) to 0.153 (LP) had been observed at 110-120 kbp distances, which potentially indicates on possibility for equally effective QTL mapping in dairy cattle populations of different origin (Fig. 2). Minimum LDs were denoted at 1000 kbp distance between markers ($r^2 = 0.080$).

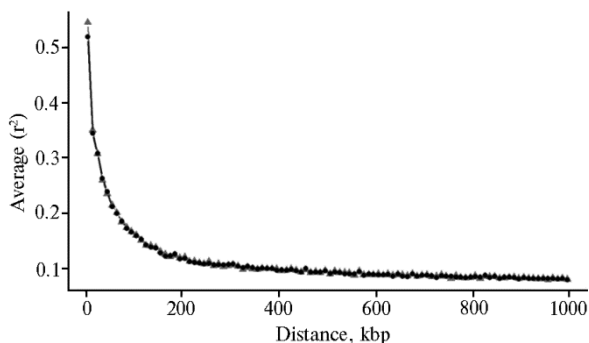


Fig. 2. Allocation of linkage disequilibrium between SNP markers at 1000 kbp distance for populations of bull sire populations of Russian Black-and-White improved by Holstein and Holstein breeds from households in Moscow (▲) and Leningrad regions (●).

components of the genetic variability by REML methods. Maximum precise values for genomic estimates have been obtained for milking (0.606), milk protein (0.535), weight fraction of protein (0.468), and milk fat (0.468). By fat content of milk, predictive estimates were minimal. In our opinion, this may be due to differences in the direction and intensity of selection of Russian Black-and-White improved by Holstein, and first of all, Holstein cattle in Moscow Province (for milking and percentage of fat and protein) and in Leningrad Province (for milking and total dairy products). One can speak about the effectiveness of predictive genomic estimates for clarification of the breeding value by parent averages (PA) and adjustment of the estimates by quality of offspring, provided insufficient number thereof. The obtained predictive estimates have allowed for short validation of the economically useful traits in heritability patterns of populations of Russian Black-and-White improved by Holstein and Holstein breeds from the central regions. For practical use of genomic prediction in breeding, it is important to develop selection criteria for reference population, to unify related cattle groups within an information system, to assess

We suppose that such growth was mainly due to massive and intensive use of pedigree Holsteins in both these regions. In-depth MDS analysis found that the regional cattle populations as a whole, through the example of sires, did not genetically differ from each other (Fig. 1). Here-with, fixation index (F_{st}) of 0.0025 completely confirmed the identity of animals by origin from the Black-and-White Holstein root.

Allocation of linkage disequilibrium between SNP markers showed the polymorphism heritability at a distance from 0-5 to 60-70 kbp with $r^2 = 0.201-0.544$ for MP and $r^2 = 0.199-0.518$ for LP. Moderate relationship with the linkage from 0.151 (MP) to 0.153 (LP) had been observed at 110-120 kbp distances, which potentially indicates on possibility for equally effective QTL mapping in dairy cattle populations of different origin (Fig. 2). Minimum LDs were denoted at 1000 kbp distance between markers ($r^2 = 0.080$).

We validated the estimated genomic breeding value as compared to other estimates (Table. 1) and found average repeatability of 0.343 for breeding record, 0.490 for genome, and 0.894 for offspring. This ensured excess of the direct genome value (DGV) as compared to PA estimates by +0.147 units, or by 14.7 %. Comparison for PA and DGV were made using EBV estimates by Spearman's correlation coefficient. EBV accuracy was calculated based on estimation of variance components

effectiveness of information obtained for a set of traits, to validate the estimates and to select young bulls with desirable genomic prediction for controlled mating.

1. Validation of the estimated breeding values of Russian Black-and-White improved by Holstein and Holstein breeds of servicing bulls (Moscow and Leningrad Regions) Validation of the estimated breeding values of bull sires of Russian Black-and-White improved by Holstein and Holstein breeds (Moscow and Leningrad provinces)

Trait	PA	DGV	EBV
Milk yielding for 305-day first lactation	0.495	0.606	0.900
Fat fraction (weight%)	0.272	0.371	0.897
Milk fat	0.208	0.468	0.899
Protein fraction (weight%)	0.403	0.468	0.896
Milk protein	0.338	0.535	0.879

Note. PA — parental averages, DGV — direct genome pedigree value, EBV — estimated breeding value. For PA and DGV, calculation of repeatability was made based on Spearman's correlation coefficient, for EBV calculation of the accuracy was made based on variation components by REML method.

In order to find additional parameters for quantitative and qualitative characterization of the milk composition, we have determined genetic inter-linkages and variability of a number of indicators (Table 2). Although some of them were due to paratypical factors, study of the genetic component may enable to identify complex regulation mechanisms of such traits both at population and at genome levels.

2. Genetic parameters and MLS assessment for additional indicators of milk composition in cows of Russian Black-and-White improved by Holstein and Holstein breeds (Moscow and Leningrad provinces)

Indicator	MLS	Indicator					
		lactose	DM	SNF	FP	SCS	Urea
Lactose	4.65±0.01	0.18 ^H	-0.07	0.24	0.09	-0.43	-0.07
DM	12.31±0.06	-0.08	0.10 ^H	0.24	-0.13	0.08	-0.19
SNF	8.99±0.02	-0.03	0.48	0.19 ^H	0.17	0.04	-0.23
FP	-0.586±0.003	-0.36	0.19	0.41	0.06 ^H	-0.03	-0.34
SCS	3.9±0.1	-0.73	0.07	-0.01	0.25	0.10 ^H	0.03
Urea	27.9±0.4	-0.16	-0.15	0.19	-0.53	-0.06	0.04 ^H

Note. DM — dry matter, SNF — solids-not-fat, FP — milk freezing point, SCS — somatic cell score; diagonally — heritability (^H), below diagonal — genetic correlations, above diagonal — phenotypical correlations.

Heritability of the traits in extended milk compositions significantly varied. Percentage of lactose (0.18), SNF (0.19) and SCS (0.10) ranges were close to standard values. DM and FP displayed low additive variability (0.10 and 0.06, respectively), with low urea content, which, at our opinion, was due to the lack of diet balance as per energy value (deficit) and protein (excess), and limited observations. Because of significant influence of paratypic factors, we failed to establish valid differences in inter-group variability between daughters of sires. Close genetic correlation was found between the lactose content in milk and the counts of somatic cells ($r_g = -0.73$), which may be due to use of lactose by bacteria. Moderate correlations were between dry matter and solids-not-fat ($r_g = 0.48$), as well as between milk freezing point and solids-not-fat ($r_g = 0.41$), concentration of urea ($r_g = -0.53$) and concentration of lactose ($r_g = -0.36$). When number of somatic cells in milk increases, its freezing point decreases ($r_g = 0.25$), which may evidence negative influence of pathogenic microflora on physical properties of milk. These studies would go on and would be aimed at extension of the database by control daily indicators for years 2013-2017.

Thus, estimated breeding values (EBV) in sires by single nucleotide polymorphism (SNP) are more effective than parent averages (PA). Population and genetic analysis had confirmed the validity of use of the unified reference sample for determination of EBV due to the identical origin of animals included in population core. The most tight linkage between the markers had been observed at a

distance of 5 to 120 kbp with minimum $r^2 = 0.15$, thus ensuring stable transfer of the genetic information for a number of quantitative trait loci at a breed level, regardless of the population affiliation. Additional indicators of milk composition may also be indicative of animal health and quality of products, which may, in its turn, extend the selection opportunities. Analysis of full-genome associations might allow for creation the map of metabolic pathways of milk biosynthesis in cows of Russian Black-and-White improved by Holstein and Holstein breeds.

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