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DIFFERENTIAL SOMATIC CELL COUNT IN MILK AS CRITERIA FOR ASSESSING COWS' UDDER HEALTH IN RELATION WITH MILK PRODUCTION AND COMPONENTS

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Abstract

The somatic cell count in cow's milk is used to control the inflammatory infection process and to assess the likelihood of subclinical and clinical mastitis. In the article, within the framework of experimental design of observations in a dairy cattle herd, the possibility in the cows' mammary gland monitoring status, based on the total somatic cell count determination and proportion for lymphocytes and polymorphonuclear neutrophils (PMN) in raw milk is shown for the first time in Russia. The obtained results confirm the relationship between somatic cell count (SCC) and daily milk yield for lactating animals. The aim of this work is to assess the relationship between the number of somatic cells in milk and their differentiation by species with milk production, milk component traits, and the risk of progressing subclinical and clinical mastitis in Holsteinized Black-and-White cows. The work was carried out from June 2020 to May 2021 (an experimental herd of Holsteinized Black-and-White cattle, PZ Ladozhsky — a branch of the Ernst Federal Research Center for Animal Husbandry, Krasnodar Territory). The total sample in a data set was 313 animals; the number of milk lactation records was 1931. The analysis of milk components was carried out using an automatic analyzer CombiFoss 7 DC (FOSS, Denmark) based on express methods of infrared spectroscopy and flow cytometry. The following milk traits were studied: daily milk yield, percentage of fat, protein, casein, lactose, dry matter, dry skimmed milk residue, traces of acetone and beta-hydroxybutyrate (BHB), freezing point and acidity, fatty acids (FA), SCC, DSCC (fraction of lymphocytes and PMN in the total amount of cells). In order to indirectly assess the mammary gland condition of cows, animals in the herd were conditionally divided into four groups: A — SCC $\leq$ 200 thousand cells per ml, DSCC $\leq$ 70 %; B — SCC $\leq$ 200 thousand cells per ml, DSCC > 70 %; C — SCC > 200 thousand cells per ml, DSCC > 70 %; D — SCC > 200 thousand cells per ml, DSCC $\leq$ 70 %. Also, the following animal gradation was applied regardless of the probability of mastitis: two groups with DSCC $\leq$ 70 and DSCC > 70 %; four subgroups with SCC $\leq$ 200, 201-500, 501-1000 and $\geq$ 1001 thousand cells per ml. We used logarithmic (normalized) SCC scores according to G.R. Wiggans et al. (1987) approach. The individual economic value of the daily milk yield of cows was determined. For assessing effects of environmental factors and their elimination on daily milk component traits, the equation of generalized linear models (GLM) was used. Estimates of phenotypic means for milk features were obtained by the GLM-equation using the least squares method. The pairwise comparison between means was performed using Tukey's test. Principal component analysis (PCA) was used to study the variability of milk composition

depending on their formation in the animal organism in order to determine the most significant parameters that determine the productivity of dairy cows. Healthy individuals and animals with suspected mastitis (predisposed to the onset of infection) (groups A and B) had desirable features of milk production, the daily milk yield was 25.7-27.7 kg, the average economic efficiency of milk production was 714-744 rubles per day per cow. Cows assigned to groups C (subclinical or clinical forms of active mastitis) and revealed as D (chronic mastitis) had the milk component traits superior to other groups with a relatively lower daily milk yield. Animals with high SCC values as well as with a chronic form of mastitis were most susceptible to metabolic disorders or ketosis, regardless of DSCC. An increase in the fat percentage in milk by 0.18-0.37 % ($p \leq 0.001$) for animals with SCC $\geq$ 1001 thousand cells per ml led to rise the share of saturated FA by 1.1-1.4 percentage points (p.p.), palmitic FA — by 0.4-1.2 p.p., medium-chain FA — by 1.0-1.4 p.p. An increase in the normalized scores of SCC by one point (limits from 1 to 10) led to a decrease in the daily milk yield by 0.6 kg, lactose percentage — by 0.062 p.p. and an increase in fat and protein by 0.090 and 0.055 p.p., respectively. Analysis of the main components revealed clear clusters for the protein and fat milk fractions, urea and fatty acids, acetone and BHB, freezing point and pH values, SCC and DSCC. A separate group included the daily milk yield and lactose percentage (together with ketone bodies) traits not related to other milk composition traits, thereby indicating the independent nature of the variability of these features. Further study of the relationship between the milk components synthesis in mammary gland and animal physiological status will make it possible to clarify the direction of selection in dairy cattle and define the genetic determination of milk production traits.

Keywords: milk, cow, somatic cell count, milk yield, fat, protein, fatty acids, acetone, BHB, mastitis, ketosis

The total somatic cell count (SCC) in the milk of dairy cows is crucial for detecting various mastitis, monitoring the inflammatory process, predicting subclinical and clinical mastitis, and assessing the health status of animals and milk quality. A more detailed analysis of the milk composition helps to develop ways to improve milk quality and milk yield [1, 2].

Despite of considerable progress in cow udder health over the past 40 years, mastitis continues to cause enormous economic damage to dairy cattle throughout the world. This is a complex pathology influenced by the environment, feeding, keeping conditions and genetic factors. In addition, pathogens that cause mastitis are constantly mutating, which requires adjustment of treatment regimens. Now dairy cattle are more productive than 20-30 years ago, which changes the type of herd management and breeding approaches [3].

Since the milk somatic cells are mostly lymphocytes, macrophages and polymorphonuclear neutrophils (PMN), for a more accurate characterization of the udder state and the prognosis of the occurrence of mastitis, in addition to evaluation of total SCC, it is advisable to differentiate somatic cells by their type [4-6]. Three major populations of cells found in milk play a key role in the mammary gland inflammation [7, 8]. Lymphocytes regulate the induction and suppression of immune responses. Macrophages recognize pathogens and initiate an immune response to invasion, resulting in a massive influx of PMN. Macrophages engulf bacteria, cellular debris, accumulated milk components and participate in tissue repairing. PMNs also protect the udder from bacterial entry in the event of mastitis [9].

In disease, the total number of SCCs and the composition of cells involved in the immune response usually change. In milk of healthy animals, the counts of somatic cells, mainly macrophages and lymphocytes, are low [10-13]. With any infection, the SCC increases significantly, and PMNs become predominant [14].

Flow cytometry coupled with infrared spectroscopy is a relatively inexpensive rapid method to assess SCCs and milk composition compared to fluorescence microscopy and arbitration methods [15, 16]. The method allows an accuracy of 0.839 to differentiate somatic cell types, 0.994 to detect SCCs, 0.820 to detect beta-hydroxymalic acid, and 0.800-0.950 for other milk components.

Many practitioners deem the number of somatic cells in milk to be insignificant for breeding, since the state of the mammary gland is more influenced by

the environment (including bacterial microbiota, especially staphylococci) than by genetic factors. However, population monitoring can contribute to breeding the most resistant and genetically adapted individuals. In Russia, in cows with SCC from 201 to 500 thousands per milliliter, milk production over lactation was 274 kg, or 4%, lower compared to animals with SCC not exceeding 200 thousands per milliliter. For SCC from 501 to 1000 thousands per milliliter, productivity was 348 kg (5%) lower, for SCC above 1001 thousand cells per ml408 kg (5.9%) lower. Note that the mass fraction of milk protein was 0.19% higher in animals with SCC of more than 1000 thousands per milliliter, which indirectly indicates physico-chemical changes in milk [17, 18].

At present, dairy cattle breeding for higher milk protein content is relevant because of general deficiency of protein in the human diet with constantly growing milk consumption. Also important is the traditional breeding for milk fat content to meet the market needs for butter, sour cream, cream, soft cheeses. However, without understanding the nature of the onset and course of the inflammation in the mammary gland, it is difficult to obtain high-quality products. It is obvious that an increase in productivity creates an additional physiological burden on the cow's body, which leads to metabolic stress, a decrease in resistance and a change in the composition of milk.

All these stimulate interest in search for putative biomarkers of the physiological state and productivity traits of dairy cows. Various types of somatic cells in milk can be such biomarkers.

For the first time, our observations in a herd of dairy cattle revealed that the total SCC in milk and the proportions of lymphocytes and PMN in SCC might be indicative of the state of cows' mammary gland. Our finding confirmed the relationship between the SCC score and daily milk production of lactating cows.

This work aimed to reveal the relationship of somatic cell counts (total and by species) with milk yielding, milk composition and the likelihood of occurrence of subclinical and clinical mastitis in Holsteinized Black-and-White cows.

Materials and methods. The work was carried out from June 2020 to May 2021 in an experimental herd of Holsteinized Black-and-White cows (PZ Ladoga — a branch of the Ernst FRC VIZH, Krasnodar Territory). The total number of measurements of daily milk indicators on 334 cows was 2023. After quality control for normal distribution and extreme values (outliers) for the main parameters, i.e., the mass fractions of fat (MFF), protein (MFP), lactose (MFL) and dry matter (DM), the sample size was 313 animals, with 1931 measurements in total. Control milking, individual sampling and conservation of milk samples using Microtabs tablets (USA) were carried out three times a day, in the morning (5.00-7.00), in the afternoon (12.00-13.30), and in the evening (18.00-20.00).

Milk component assay was performed (an automatic analyzer CombiFoss 7 DC, FOSS, Denmark; the analyzer consists of a MilkoScan for near infrared spectroscopy coupled with Fossomatic 7 DC for flow cytometry). All indicators were recorded automatically, the data were uploaded to the Microsoft Excel program for each sample. Before starting milk sample analysis, the readings for a standard milk sample and a synthetic medium containing somatic cells were recorded.

Each milk sample was analyzed individually; the values obtained were reduced to average daily values. The following parameters were determined: daily milk yield (DMY), MFF, MFP, mass fraction of casein (MFC), MFL, DM, dry skimmed milk residue (DSMR), traces of acetone and beta-hydroxybutyrate (BHB), freezing point (FP) and acidity (pH), fatty acids (FA) — $C_{14:0}$ (myristic), $C_{16:0}$ (palmitic), $C_{18:0}$ (stearic), $C_{18:1}$ (oleic), long-, medium- and short-chain fatty acids (LCFA, MCFA , SCFA), mono- and polyunsaturated FAs (MUFAs, PUFAs), saturated FAs (SFAs), transisomers (TFA), SCC, DSCC (differential

somatic cell count, DSCC as the proportion of lymphocytes and PMN in the total amount of cells). The device cannot record the proportion of macrophages, so it was calculated as the difference between the SCC taken as 100% and the DSCC percentage.

To indirectly assess the mammary gland state, all cows were conditionally grouped according to Schwarz [26] in our modification. Group A was healthy individuals (SCC ≤ 200 thousands per milliliter, DSCC ≤ 70%); group B was individuals with suspected mastitis (SCC ≤ 200 thousands per milliliter, DCC > 70%); C was individuals with subclinical/clinical mastitis, SCC > 200 thousands per milliliter, DSCC > 70%); D was individuals with chronic (persistent) mastitis (SCC > 200 thousands per milliliter, DSCC ≤ 70%). In addition, regardless of the likelihood of mastitis, animals were grouped by DSCC scores (two groups with DSCC ≤ 70 and > 70%) and by SCC scores (four subgroups with SCC ≤ 200, 201-500, 501-1000 and ≥ 1001 thousands per milliliter) (eight subgroups in total for the DSCC and SCC combination).

We used logarithmic (normalized) SCC estimates (SCCE) according to Wiggans [19]. The best animals in terms of SCC in milk corresponded to 1 point, the worst to 10 points, with a one-point step SCC:

$$SCCE = \log_2(SCC/100) + 3. \quad (1)$$

The individual estimated milk value (EMV) in phenotypic terms was determined based on the milk price (60% for MFP, 40% for MFF expressed for the basal contents of 3.0% protein and 3.4% fat) in terms of the physical mass of the chilled raw material. The economic value was specified by increasing and decreasing coefficients for raw material for processing which depend on the SCC value by (1.1 for SCC < 250 thousands per milliliter, 1.0 for SCC 250-400 thousands per milliliter, 0.9 for SCC = 400-1000 thousands per milliliter, and 0.5 for SCC > 1000 thousands per milliliter).

To assess the effect of environmental factors and their elimination on the daily indicators, the equation of generalized linear models (GLM) implemented in the STATISTICA 10 program [20] was used:

$$y_{ikm} = ML_i + DM_k + a_1 DIM + DSCC\_SCC_m + e_{ikm}, \quad [2]$$

where y is the estimated parameter for group m, month i, and milkmaid k; ML is the month of the productivity estimation (i = 10); DM is the fixed effect of the milkmaid (k = 6); DIM is a continuous effect of lactation days from calving (a_1 is regression coefficient); DSCC_SCC is the fixed effect of the group the animal is assigned to with regard to SCC and DSCC scores (m = 8); e is residual variance of the model.

Based on the GLM equation using the least squares method (Least-Squares, LS), estimates of phenotypic means for milk performance were obtained. Pairwise comparisons between means were made using Tukey's test. The principal component analysis (PCA) method was used for spatial visualization and analysis of the variability of milk composition depending on the individual characteristics of its formation to establish the most significant parameters that determine the dairy productivity. Descriptive statistics were calculated using the STATISTICA 10 program (StatSoft, Inc., USA) and Microsoft Excel 2013. The tables show the means (M), their standard errors (±SEM), and coefficients of variation (Cv, %). The differences were considered statistically significant at $p \leq 0.05$.

Results. The milk composition estimates provided by infrared spectroscopy or flow cytometry assay, serve as a kind of biomarker to control the productive traits through breeding and to manage animal health.

DSCC in milk from cows in our experiments ranged from 0 (no blood

cells detected) to 93.8%. I.e., the proportion of macrophages at the maximum DSCC was the smallest, which could indicate an active inflammatory process in the mammary gland.

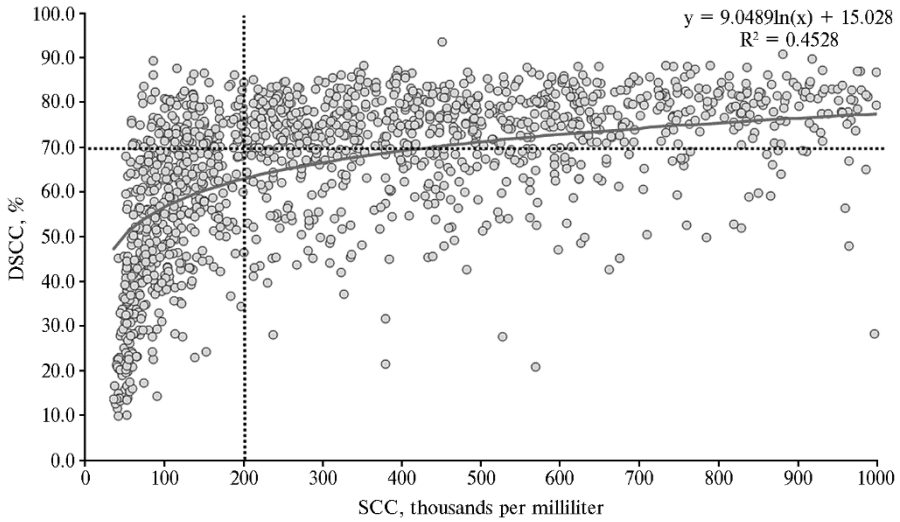

Fig. 1. Somatic cell differentiation (DSCC, lymphocytes and polymorphonuclear neutrophils) **in milk of Holsteinized Black-and-White cows (individual daily values) depending on somatic cell counts** (SCC) (PZ Ladoga — a branch of the Ernst FRC VIZH, Krasnodar Territory, 2020-2021). Dotted lines indicate the threshold values for DSCC and SCC.

Figure 1 shows the distribution of DSCC depending on the total number of somatic cell counts (with threshold values of 70% for DSCC and 200 thousand cells per mlSCC). With an increase in the SCC in milk, the DSCC index increased in a logarithmic function ($R^2 = 0.453$). The percentage of records with the lower threshold for SCC, regardless of the DSCC score, was 35.9%, with 13.4 and 50.7% excess for SCC < 70% and > 70%, respectively. These results draw to the conclusion that approximately two thirds of the herd have a subclinical pattern of mammary dysfunction, which often occur in high-yielding herds whose diet is high in concentrated feed. For 13.4% of animals, deviations in the functional properties of the udder were chronic.

The average daily milk yield in the studied animals was 25.4±0.2 kg with a mass fraction of fat and protein of 3.61±0.02 and 3.20±0.01%, respectively (Table 1). In our opinion, the low milk fat content is associated with the climatic conditions of the breeding area and the diet intended for high milk production. Values of metabolic biomarkers (ketone bodies or traces of acetone and BHB in milk) were within the physiological norm, the 0.30-0.35 and 0.15 mmol/l, respectively. In 1.1% of cases, the appearance of clinical (new-calving cows, from day 9 to day 78 after calving) and subclinical (second half of lactation, from day 167 to day 598 after calving) forms of ketosis occurred. Similar results for acetone and BHB in milk were obtained in the herds of the Black-and-White and Holstein breeds in the Tyumen region [21]. The relationship between the amount of acetone and BHB was linear ($R^2 = 0.631$). The concentration of urea in milk exceeded the optimal values (15.1-30.0 mg/100 ml) and amounted to 38.7 mg/100 ml. Earlier work on a dairy cattle population in the Moscow Province showed that an increase in the urea content in milk to 35.0-37.6 mg/100 ml led to a 0.27-0.55% decrease in MFF [22].

1. The impact of generalized linear model (GLM) **factors on milk productivity and composition of Holsteinized Black-and-White cows** (n = 1931, PZ Ladoga — a branch of the Ernst FRC VIZH, Krasnodar Territory, 2020-2021)

Parameter	M±SEM	Cv, %	Factor				R^2
			DL	MM	ML	DSCC/SCC	
Daily milk yield, kg	25.4±0.2	28.0	***	***	***	***	0.240
MFF, %	3.61±0.02	26.0	***	t	***	***	0.273
MFP, %	3.20±0.01	13.8	***	nr	***	***	0.315
MFC, %	2.69±0.01	13.8	***	nr	***	***	0.310
MFL, %	4.82±0.01	4.5	***	nr	**	***	0.285
Dry matter, %	12.72±0.028	9.5	***	nr	***	***	0.268
DSMR, %	9.08±0.01	5.3	***	nr	***	***	0.195
Acetone, mmol/l	0.055±0.002	160.1	***	***	***	**	0.171
BHB, mmol/l	0.013±0.001	326.4	*	***	***	***	0.256
Urea, mg/100 ml	38.7±0.1	16.8	nr	***	***	t	0.514
$C_{14:0}$, g/100 g	0.336±0.002	25.9	***	t	***	***	0.196
$C_{16:0}$, g/100 g	0.887±0.005	26.2	***	t	***	***	0.182
$C_{18:0}$, g/100 g	0.336±0.003	34.9	nr	nr	***	***	0.200
$C_{18:1}$, g/100 g	1.156±0.007	27.5	***	t	***	***	0.252
LCFA, g/100 g	1.426±0.010	30.7	**	*	***	**	0.263
MCFA, g/100 g	1.358±0.008	26.4	***	*	***	***	0.207
SCFA, g/100 g	0.479±0.003	31.0	***	nr	***	***	0.304
MUFAs, g/100 g	1.076±0.007	27.4	***	t	***	***	0.211
PUFAs, g/100 g	0.131±0.001	22.9	***	***	***	***	0.325
SFAs, g/100 g	2.373±0.015	27.7	***	t	***	***	0.275
SCC, thousand cells per ml	832±31	166.1	nr	nr	nr	***	0.526
DSCC, %	63.5±0.6	39.8	nr	t	***	***	0.685

N o t e. DL — lactation days, MM — milkmaid, ML — lactation month, DSCC/SCC — proportion of lymphocytes + polymorphonuclear neutrophils (DSCC) in total somatic cell counts (SCC) (8 subgroups), R^2 — determination coefficient; MFF — mass fraction of fat, MFP — mass fraction of protein, MFC — mass fraction of casein, MFL — mass fraction of lactose, DSMR — dry skimmed milk residue, BHB — beta-hydroxybutyrate, fatty acids $C_{14:0}$ — myristic, $C_{16:0}$ — palmitic, $C_{18:0}$ — stearic, $C_{18:1}$ — oleic, LCFA — long-chain fatty acids, MCFA — medium-chain fatty acids, SCFA — short-chain fatty acids, MUFAs — monounsaturated FAs, PUFAs — polyunsaturated FAs, SFAs — saturated FAs.
$^t p \le 0.1$, * $p \le 0.05$, ** $p \le 0.01$, *** $p \le 0.001$; nr — unreliable.

The milk fatty acid distribution was as follows: by saturation of the carbon chain 66.3% SFA, 30.1% MUFA, 3.7% PUFA; by the length of the carbon chain 39.8% LCFA, 37.9% MCFA, SCFA 13.4%, trans-isomers of fatty acids 2.7%; by the number of carbon atoms in the FA chain 32.3% oleic ($C_{18:1}$), 24.8% palmitic ($C_{16:0}$), 9.4% myristic ($C_{14:0}$) and stearic ($C_{18:0}$) each. The proportions of fatty acids in the population of Holsteinized cattle in the Moscow region was similar [23], however, the proportion of the milk fat fraction was higher (4.46%), while SFA accounted for 78.2%, MUFA for 21.8%, PUFA for 0.1% [23].

The detected milk SCC was consistent with an increased incidence of subclinical and clinical forms of mammary gland diseases in the herd, as well as persistent chronic mastitis. The coefficient of phenotypic variability was the highest for SCC (166.1%), acetone content (160.1%) and BHB (326.4%), which may indicate a significant influence of paratypical conditions (keeping technology, management, diet of animals). The minimum variability occurred for MDL (4.5%), DSMR (5.3%) and DM (9.5%). The variability of the main indicators of milk production ranged from 13.8% for protein to 26.0% for fat (including 22.9-34.9% FA) and 28.0% for daily milk yield. According to the model equation for a number of fixed and regression factors, the day of lactation and the stage of lactation had no effect on the of milk urea concentration, the content of stearic FA, SCC and DSCC.

The milkmaid factor turned out to be the least significant of all those considered and was not statistically significant for the content of protein, casein, lactose, dry matter and DSMR, stearic FA, short-chain FA and SCC. The milkmaid factor slightly affected ($p \le 0.1$) the MFF, the content of myristic and palmitic

FAs, MUFAs, SFAs and DCSCs In all likelihood, the effect of milking on the composition of cows' milk was eliminated by other more significant factors.

The month of lactation had a highly significant significance ($p \leq 0.01$-0.001) for all the studied parameters, except for SCC. The influence of factors from eight DSCC/SCC subgroups on the variability of milk parameters turned out to be highly significant ($p \leq 0.01$-0.001), with the exception of the concentration of urea ($p \leq 0.1$). The coefficient of determination of the model expectedly showed higher values for SCC ($R^2 = 0.526$), DSCC ($R^2 = 0.685$) and for urea ($R^2 = 0.514$). In other cases, the reliability of the model ranged from $R^2 = 0.171$ for acetone to $R^2 = 0.325$ for polyunsaturated fatty acids.

Table 2 submits the distribution of the least squares estimates for the trait of milk productivity, depending on the predicted state of the mammary gland, with the gradations of groups A, B, C and D. The highest daily milk yield (27.7 kg, $p \leq 0.001$) were characteristic for cows from group B, that is, individuals with suspected inflammatory processes in the mammary gland, however, SCC in group B was below 200 thousands per milliliter. This may be due to an increase in the number of PMN and leukocytes (78.3%, $p \leq 0.001$) at an early stage of bacterial infection and the subsequent immune response to phagocytosis. It is expected that after the absorption of the pathogen, a gradual increase in the activity of macrophages will occur followed by normalization of the udder function.

2. Parameters of milk productivity and composition of Holsteinized Black-and-White cows as influenced by the udder condition (*M*±SEM, PZ Ladoga — a branch of the Ernst FRC VIZH, Krasnodar Territory, 2020-2021)

Parameter	Group			
	A (n = 96)	B (n = 40)	C (n = 115)	D (n = 62)
Number of observations, %	29.5	6.4	50.7	13.4
Daily milk yield, kg	25.7±0.3***	27.7±0.6$^{**/***}$	25.5±0.3***	23.3±0.5
MFF, %	3.39±0.04	3.32±0.08	3.57±0.04***	3.69±0.06***
MFP, %	3.10±0.02	3.05±0.04	3.22±0.02***	3.34±0.03***
MFC, %	2.62±0.02	2.58±0.03	2.70±0.01***	2.80±0.02***
MFL, %	4.93±0.01***	4.92±0.02***	4.78±0.01***	4.74±0.01
Dry matter, %	12.51±0.06	12.37±0.10	12.66±0.05$^{*/**}$	12.89±0.08$^{**/***}$
DSMR, %	9.09±0.02	9.01±0.04	9.06±0.02	9.16±0.03***
Acetone, mmol/l	0.063±0.004*	0.064±0.008	0.052±0.004	0.065±0.006
BHB, mmol/l	0.010±0.002	0.012±0.004	0.012±0.002	0.023±0.003$^{*/***}$
Urea, mg/100 ml	37.9±0.3	37.8±0.4	37.4±0.2	37.8±0.3
$C_{14:0}$, g/100 g	0.318±0.004	0.315±0.008	0.337±0.004***	0.343±0.006***
$C_{16:0}$, g/100 g	0.823±0.012	0.811±0.021	0.897±0.010***	0.907±0.016***
$C_{18:0}$, g/100 g	0.320±0.006	0.308±0.010	0.327±0.005	0.342±0.008**
$C_{18:1}$, g/100 g	1.108±0.015	1.078±0.027	1.131±0.013	1.186±0.020$^{*/**}$
LCFA, g/100 g	1.375±0.021	1.330±0.037	1.384±0.018	1.447±0.028*
MCFA, g/100 g	1.256±0.018	1.236±0.032	1.372±0.015***	1.401±0.024***
SCFA, g/100 g	0.444±0.007	0.438±0.012	0.471±0.006**	0.492±0.009***
MUFAs, g/100 g	1.035±0.015	1.009±0.026	1.056±0.012	1.104±0.019$^{*/**}$
PUFAs, g/100 g	0.125±0.001	0.122±0.002	0.127±0.001	0.131±0.002**
SFAs, g/100 g	2.203±0.031	2.160±0.056	2.361±0.027$^{**/***}$	2.444±0.041***
SCC, thousand cells per ml	23.7±67.6	184.8±120.4	1315.9±57.5***	467.8±89.5***
DSCC, %	33.7±0.8	78.3±1.4***	80.2±0.7***	59.4±1.0***
EMV, rub. · day^{-1} · head^{-1}	714±10***	744±19***	516±9	585±14***

N o t e. DSCC/SCC — proportion of lymphocytes + polymorphonuclear neutrophils (DSCC) in total somatic cell counts (SCC), MFF — mass fraction of fat, MFP — mass fraction of protein, MFC — mass fraction of casein, MFL — mass fraction of lactose, DSMR — dry skimmed milk residue, BHB — beta-hydroxybutyrate, fatty acids $C_{14:0}$ — myristic, $C_{16:0}$ — palmitic, $C_{18:0}$ — stearic, $C_{18:1}$ — oleic, LCFA — long-chain fatty acids, MCFA — medium-chain fatty acids, SCFA — short-chain fatty acids, MUFAs — monounsaturated FAs, PUFAs — polyunsaturated FAs, SFAs — saturated FAs, EMV — estimated milk value. For a description of the groups, see the Materials and methods section. Sequential pairwise comparison is performed.
* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

Cows of group B were inferior to the rest in percentage of milk fat, protein, fatty acids, BHB ($p \leq 0.001$), except for lactose. However, in terms of EMV, these animals outperformed others by 30-228 rub. Cows of group A (healthy individuals) showed the best scores for SCC and DSCC, 23.7 thousand cells per ml and 33.7%,

respectively, with a higher daily milk yield (25.7 kg) compared to groups C and D. For the main milk components, these cows were inferior to those with high SCC value, except for MDL ($p \leq 0.001$) and traces of acetone ($p \leq 0.05$); the EMV values were close to group B, 714 rub. $\cdot$ day^{-1} $\cdot$ head^{-1}.

The cows of groups C (with subclinical or clinical active mastitis) and D (with chronic mastitis) exceeded other groups in the milk components, while have a relatively lower daily milk production. In animals from group C, MFF was 0.18 and 0.25% higher ($p \leq 0.001$) compared to groups A and B, in cows of group D 0.30 and 0.37% higher. MFP increased by 0.12 and 0.17%, respectively, and by 0.24 and 0.29%, respectively. The fatty acid content and casein also changed.

It should be noted that in cows of group D, the amount of milk ketone bodies increased, while for BHB, there was a highly significant difference, +0.011 (A/D, $p \leq 0.001$) and +0.013 mmol/l (B/D, $p \leq 0.05$), which is consistent with the results for the Holstein cattle population in Canada [24].

Despite higher MFF and MFP parameters, the economic efficiency of groups C and D was only 516 and 585 rub. $\cdot$ day^{-1} $\cdot$ head^{-1}. We believe that in our case, increased fat and protein secretion could be due to the active phase of the inflammation at the beginning (after calving and before the peak of daily milk yield) and the end of lactation (from day 210 after calving to the dry period), when there was a physiological maximum production of milk components. Schwarz et al. [25-27] noted similar patterns for populations of Holstein, Simmental and Brown Swiss breeds in Austria, China, Estonia, Germany and Spain.

We compared these parameters with respect to DSCC/SCC gradation for eight subgroups for a more detailed characterization of the changes in the daily productivity and milk composition (Table 3). It was found that the average daily milk yield of animals with SCC $\leq$ 200 thousand cells per/ml was 1.8-5.8 kg higher ($p \leq 0.001$) for DSCC $\leq$ 70% and 0.9-3.0 kg higher ($p \leq 0.001$) for DSCC > 70% compared to subgroups with other SCC scores. For the main milk component parameterss, e.g., MFF, MFP, and MFC, there was a significant superiority of cows' subgroups with CSC > 200 thousand cells per ml.

We concluded that an increased number of somatic cells in milk (in particular, > 1 million cells) changes the proportion of some FAs, regardless of the DSCC threshold. So, when comparing the contrasting subgroups with SCC $\leq$ 200 and $\geq$ 1001 thousand cells per ml, the greatest differences were found in the total fats, from +1.1 to +1.4 p.p. for SFAs, from +0.4 to +1.2 p.p. for palmitic FA, and from +1.0 to +1.4 p.p. for MCFAs. There also was a decrease in stearic and oleic fatty acids (by 0.6 and 1.0 p.p.), long-chain fatty acids (by 1.7-2.5 p.p.), monounsaturated fatty acids (0 .9-1.2 p.p.), and polyunsaturated fatty acids (by 0.2 p.p.). An increase in the fraction of saturated fatty acids with a simultaneous decrease in MUFA and PUFA leads to a decrease in the quality of processed products, since fatty acids with many double carbon bonds are recognized as the most useful for humans. Significantly higher values of EMV were in animals with SCC $\leq$ 200 thousand cells per ml (by 423-444 rubles compared to the cows with SCC $\leq$ 1001 thousand cells per ml).

Figure 2 shows the daily productivity vs. the normalized SCC estimates. In daily milk yield, cows with a SCC score of 1 point were statistically significantly ($p \leq 0.1$-0.001) superior by 1.5-6.0 kg to cows with 4 points and higher. The distribution of LS-estimates for the fat, protein and lactose fractions was similar to that for the DSCC/SCC control groups, that is, with an increase in the score, the MFF and MFP increased from 3.34 to 4.24% and from 3.02 to 3.57%. The dynamics of the MFL had an inverse pattern and averaged –0.062 p.p. for each point of the SCC score. In our opinion, this may be due to the more active use of milk sugar by the bacterial microbiota of the mammary gland.

3. Milk productivity and milk composition of Holsteinized Black-and-White cows depending on the SCC score gradations and DSCC values based on the LS-estimates and (*M*±SEM, PZ Ladoga — a branch of the Ernst FRC VIZH, Krasnodar Territory, 2020-2021)

Parameter	SCC, thousand cells per ml							
	DSCC ≤ 70 % (*n* = 829)				DSCC > 70 % (*n* = 1102)			
	≤ 200	201-500	501-1000	≥ 1001	≤ 200	201-500	501-1000	≥ 1001
Number of observations, %	29,5	7,8	4,3	1,4	6,4	15,3	14,5	20,9
Daily milk yield, kg	25,7±0,3***	23,9±0,6***	22,9±0,7*	19,9±1,2	27,7±0,6***	26,8±0,4***	25,2±0,4	24,7±0,4
MFF, %	3,39±0,04	3,71±0,07***	3,65±0,09*	3,95±0,16***	3,31±0,08	3,41±0,05	3,53±0,06*	3,72±0,05**/***
MFP, %	3,10±0,02	3,33±0,03***	3,37±0,04***	3,41±0,07***	3,05±0,04	3,13±0,02	3,24±0,03***	3,29±0,02***
MFC, %	2,62±0,02	2,80±0,03***	2,81±0,04***	2,85±0,06***	2,58±0,03	2,64±0,02	2,73±0,02***	2,74±0,02***
MFL, %	4,93±0,01***	4,80±0,02***	4,67±0,02***	4,55±0,04	4,93±0,02**/***	4,87±0,01***	4,81±0,01***	4,67±0,01
Dry matter, %	12,51±0,06	12,95±0,09***	12,80±0,12*	13,04±0,21**	12,36±0,10	12,50±0,07	12,68±0,07**	12,77±0,06***
DSMR, %	9,09±0,02	9,21±0,04***	9,11±0,05	9,05±0,09	9,01±0,04	9,05±0,03	9,11±0,03*	9,03±0,03
Acetone, mmol/l	0,063±0,004	0,056±0,007	0,071±0,009	0,097±0,016*/**	0,064±0,008*	0,049±0,005	0,046±0,006	0,060±0,005*
BHB, mmol/l	0,011±0,002	0,015±0,003	0,025±0,004**	0,068±0,008***	0,012±0,004	0,008±0,003	0,006±0,003	0,021±0,002***
Urea, mg/100 ml	37,9±0,3	37,8±0,4	37,4±0,5	38,5±0,9	37,9±0,4	38,0±0,3**	37,4±0,3	37,0±0,3
$C_{14:0}$, g/100 g	0,318±0,004	0,347±0,007***	0,331±0,009	0,366±0,016**	0,314±0,008	0,327±0,005	0,338±0,005**	0,344±0,005**
$C_{16:0}$, g/100 g	0,827±0,012	0,910±0,019***	0,897±0,025**	0,987±0,042***	0,808±0,021	0,844±0,014	0,879±0,015**	0,954±0,013***
$C_{18:0}$, g/100 g	0,320±0,006	0,345±0,009*	0,341±0,012	0,354±0,021	0,307±0,010	0,311±0,007	0,317±0,007	0,348±0,006**/***
$C_{18:1}$, g/100 g	1,108±0,015	1,184±0,025**	1,184±0,032*	1,263±0,055***	1,076±0,027	1,085±0,019	1,116±0,019	1,178±0,017**/***
LCFA, g/100 g	1,376±0,021	1,455±0,034*	1,429±0,044	1,519±0,075	1,327±0,037	1,336±0,025	1,368±0,026	1,434±0,023**
MCFA, g/100 g	1,256±0,018	1,404±0,029***	1,384±0,037**	1,516±0,063***	1,232±0,031	1,300±0,022	1,354±0,022**	1,441±0,020**/***
SCFA, g/100 g	0,444±0,007	0,498±0,011***	0,477±0,015*	0,532±0,025***	0,437±0,012	0,458±0,008	0,467±0,009*	0,483±0,008*/**
MUFAs, g/100 g	1,035±0,014	1,102±0,024**	1,102±0,031*	1,171±0,052**	1,006±0,026	1,009±0,018	1,042±0,018	1,102±0,016**/***
PUFAs, g/100 g	0,125±0,001	0,133±0,002**	0,127±0,003	0,138±0,005*	0,122±0,002	0,124±0,002	0,127±0,002	0,130±0,002**
SFAs, g/100 g	2,204±0,031	2,453±0,051***	2,409±0,066**	2,646±0,111***	2,154±0,055	2,249±0,038	2,329±0,039**	2,472±0,035**/***
SCC, thousand cells per ml	28±53	281±86*	586±112*/***	2039±189***	119±94	312±64	661±66***	2622±59***
DSCC, %	33,7±0,8	59,2±1,3***	60,4±1,7***	59,3±2,8***	78,1±1,4	78,2±1,0	79,7±1,0	82,3±0,9*/**
EMV, rub. · day^{-1} · $head^{-1}$	713±8***	636±14***	551±18***	269±30	753±15***	671±10***	593±11***	330±9

N o t e. DSCC/SCC — proportion of lymphocytes + polymorphonuclear neutrophils (DSCC) in total somatic cell counts (SCC), MFF — mass fraction of fat, MFP — mass fraction of protein, MFC — mass fraction of casein, MFL — mass fraction of lactose, DSMR — dry skimmed milk residue, BHB — beta-hydroxybutyrate, fatty acids $C_{14:0}$ — myristic, $C_{16:0}$ — palmitic, $C_{18:0}$ — stearic, $C_{18:1}$ — oleic, LCFA — long-chain fatty acids, MCFA — medium-chain fatty acids, SCFA — short-chain fatty acids, MUFAs — monounsaturated FAs, PUFAs — polyunsaturated FAs, SFAs — saturated FAs, EMV — estimated milk value. For a description of the groups, see the Materials and methods section. Sequential pairwise comparison is performed.
* $p \le 0.05$, * $p \le 0.01$, *** $p \le 0.001$.

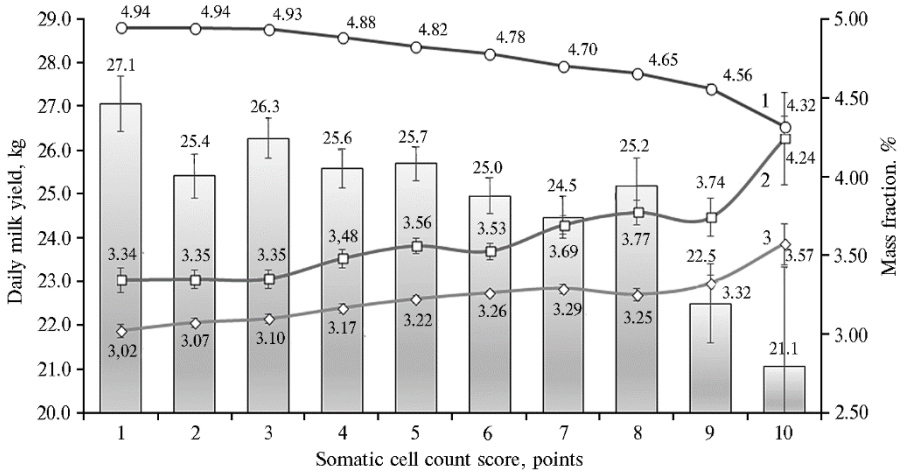

Fig. 2. Distribution of the least squares method-based LS-estimates for parameters of milk productivity and milk composition in Holsteinized Black-and-White cows depending on the somatic cell counts (SCC): diagram shows daily milk yield, 1 — mass fraction of lactose (MFL), 2 — mass fraction of fat (MFF), 3 — mass fraction of protein (MFP) (PZ Ladoga — a branch of the Ernst FRC VIZH, Krasnodar Territory, 2020-2021).

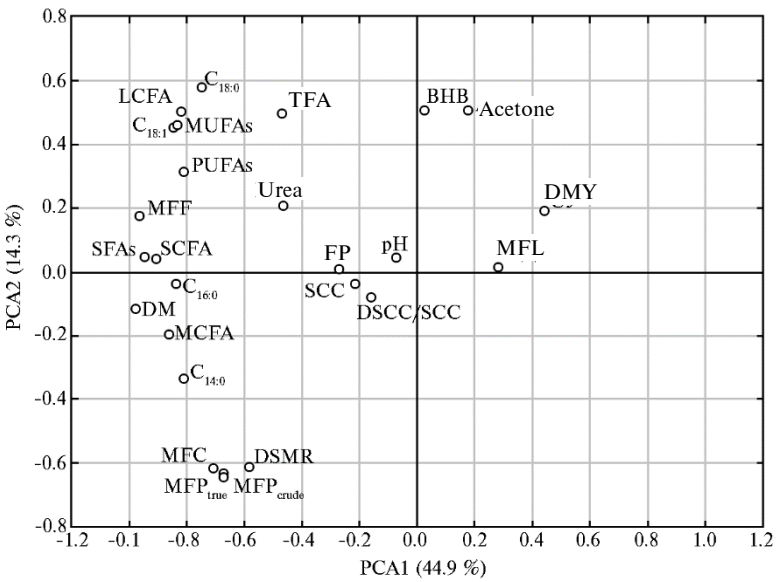

Fig. 3. Principal component analysis (PCA) **of milk parameter distribution in Holsteinized Black-and-White cows depending on variability along two factor axes:** DM — dry matter, DMY — daily milk yield, MFF — mass fraction of fat, MFP_{true} — mass fraction of true protein, MFP_{crude} — mass fraction of crude protein MFC — mass fraction of casein, MFL — mass fraction of lactose, DSMR — dry skimmed milk residue, BHB — beta-hydroxybutyrate, Acetone, Urea, fatty acids $C_{14:0}$ — myristic, $C_{16:0}$ — palmitic, $C_{18:0}$ — stearic, $C_{18:1}$ — oleic, LCFA — long-chain fatty acids, MCFA — medium-chain fatty acids, SCFA — short-chain fatty acids, MUFAs — monounsaturated FAs, PUFAs — polyunsaturated FAs, SFAs — saturated FAs, TFA — trans fatty acid isomers, sSCC — total somatic cell counts, FP — freezing point, DSCC — lymphocytes + polymorphonuclear neutrophils in SCC (PZ Ladoga — a branch of the Ernst FRC VIZH, Krasnodar Territory, 2020-2021).

We used principal component analysis (PCA) method to reveal co-variation of parameters characterizing milk composition and mechanisms underlying its formation (Fig. 3). The most significant variables turned out to be MFF, SFA and dry matter ($P \geq 0.99$), that is, their scatter of variables was minimized or evenly ordered, and the variance was maximized with respect to component 2. Using visualization based on two components of variability, it was shown that the protein

and fat fractions of milk formed their own clusters, while for a number of fatty acids, there was structural differentiation by the groups of medium- and long-chain fatty acids, mono- and polyunsaturated fatty acids. Urea as one of the factors of variability was closer to fatty acids. For biomarkers of metabolism (acetone and BHB), technological qualities (TQ and pH) and somatic cells (SCC, DSCC), their clusters were designated, not associated with other indicators of milk composition. The first component singled out the daily milk yield and the mass fraction of lactose (together with ketone bodies) into a separate group, thus denoting the independent (and at the same time complex) nature of the variability of these parameters.

The approach in which somatic cells are differentiated according to their types is of interest for accurate diagnosis of mammary gland physiology disorders, product quality control, and optimization of economic costs in the herd. Our findings have shown that the gradation of animals by groups based on DSCC and SCC makes it possible to distinguish individuals according to the likelihood of a subclinical form of mastitis. The obtained distribution of the milk productivity trait values and economic efficiency of cows indicated a significant influence of the studied factors. Schwarz et al. [26] reported similar data. On a sample of animals of different breeds from the dairy cattle populations of Austria, China, Estonia, Germany, and Spain, similar patterns were found in the composition of cow milk (percentage of fat, protein, lactose, traces of urea) and EMV. Previous studies [9, 25] have shown a close relationship between DSCC and SCC (both in combination and separately) with the presence of an infectious form of mastitis. With an increase in DCSC, the sensitivity of this biomarker in predicting the manifestation of infection increased.

It is also promising to study the structure of milk microbiota depending on milk composition, the number of somatic cells and their types. In Russia, an analysis of the association of *Staphylococcus aureus* isolates from milk with the manifestation of mastitis in cows has already been carried out [28]. We believe that the control over the change in the composition of cow's milk in the herd can be carried out using a complex of biomarkers, e.g., the amount of lactose, traces of acetone and BHB, which is currently used routinely in the farms of the Moscow region to predict the occurrence of mastitis and ketosis [22]. Principal component analysis has shown its effectiveness for determining the boundaries of interdependent variability of the component composition of milk in order to identify signs with independent variability.

Thus, the somatic cell counts in milk and the SCC differentiation by species can serve as additional criteria for predicting and monitoring the spread of mastitis. Our findings confirm the possibility of individual assessment of the mammary gland state based on the proportion of lymphocytes and polymorphonuclear neutrophils (differentiated somati cell count, DSCC) in the total amount of somatic cells in milk (SCC). Animals assigned to healthy individuals and individuals with suspected mastitis (predisposed to the onset of infection) had the desired indicators of milk production and economic efficiency. Animals with high SCC values, as well as with chronic mastitis were the most prone to metabolic disorders and ketosis, regardless of the SCC value. An increase in the percentage of fat in the milk of animals having SCC $\geq$ 1 million cells per ml led to a change in the ratio of fatty acids with an increase in the amount of saturated, medium chain and palmitic FAs. The normalized SCC estimates (SCCE) more clearly show the observed patterns in the change of cows' milk productivity parameters. An increase in SCCE by one point (limits from 1 to 10) led to a drop in daily milk yield by 0.6 kg of milk, in lactose by 0.062 p.p. and to an increase in the fat and protein levels by 0.090 and 0.055 p.p, respectively. Principal component analysis revealed structural clustering of milk composition parameter for fat and protein fractions, traces of metabolites (acetone, beta-

hydroxybutyrate), and somatic cell counts. Further study of the relationship between the synthesis of milk components in the mammary gland and the animal physiological status will clarify breeding parameters and genetic background of productivity traits. The development of methods for express diagnostics of animal health based on an expanded analysis of the milk component composition is one of the priorities for practical application of our research in the future.

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