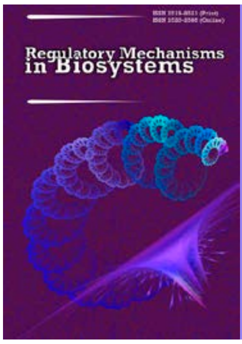

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Biochemical and hematological composition of blood of cattle fed with *Chlorella*

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We researched the influence of the additive *Chlorella* cultivated in a closed bioreactor under the influence of an electrostatistic field, on the biochemical and hematological parameters of blood in different age groups of cattle. The experiment was conducted on two groups of three month old heifers and two groups of lactating cows in the 7th month of lactation, with 15 individuals in each group; all animals in the groups were given the basic diet. During the 120-day experiment, the experimental group of heifers was fed on a suspension of *Chlorella* cultivated with the technology using an electrostatic field, in the following amounts: 1–30th day – 1.51 g, 31–60th day – 1.82 g, 61–90th day – 2.01 g, 91–120th day – 2.28 g of dry substance per individual daily; the group of lactating cows during 60 days was given the Chlorella additive to the amount of 6 g of dry substance per individual per day. Over the following 30 days the heifers and lactating cows were kept under observation to determine the "aftereffects" of the *Chlorella* suspension fodder additive. On the first day of the experiment and after every 30 days, blood was drawn from the animals' jugular veins for determination of the biochemical and hematological parameters of the blood During the experiment, the experimental groups of heifers and lactating cows were observed to have a strengthened antioxidant system due to increase in the activity of superoxide dismutase and catalase, and also to have a strengthened hematopoietic function and intensified metabolic and redox processes. Due to the stimulating effect of the suspension of *Chlorella* cultivated using the intense technology involving using an electrostatic field on the erythro- and leukopoiesis of different age groups of the cattle, we determined a strengthening of the cellular element of the animals' immune system. During the following 30 days, the heifers and lactating cows of the experimental groups were observed to exhibit "aftereffects" of the *Chlorella* suspension additive, namely increases in the values of biochemical, hematological and morphological indicators of the blood in comparison with these indicators for the animals in the control groups. As a result, we concluded that feeding different age groups of cattle with suspension of *Chlorella* cultivated in the conditions of a closed bioreactor under the influence of an electrostatic field, in the dosage of 155 ml per kg of dry substance of the animals' diet, contributes to the strengthening of non-specific defence of their organism, forming a potential for increasing the productive indicators and maintenance of the livestock.

Keywords: lactating cows; heifers; bioreactor; electrostatic field; fodder additive; leukogram

Introduction

A promising method of improving the efficiency of the indicators of productivity of agricultural livestock is improving their digestion of fodder, which is achieved through adding a broad spectrum of highly biologically active micro-nutrients. It is known that the natural food additives made of algae possess a broad spectrum of biologically active substances with high biological accessibility for the organism of cattle. One such additive is suspension of *Chlorella*. The latter is one of the most widely cultivated eukaryotic green microalgae and is grown to be used as an additive to the food of animals and humans, and is also used in the pharmaceutical and cosmetological industries all around the world, especially in Asian countries, such as Japan, Taiwan, and Korea (Ogbonna, 1997; Lee, 2001; Pulz & Gross, 2004; Becker, 2006; Spolaore, 2006; Bishop & Zubeck, 2012; Lum et al., 2013; Guccione, 2014; Costa et al., 2016).

In the conditions of optimum growth, the biomass of *Chlorella* consists 25–50% of protein, 5–35% of carbohydrates and 5–20% of fat which is present as non-saturated fatty acids, the greater part of which is stearic, oleic, arachidonic, linolenic and linoleic acids, 5–10% of mineral substances, mostly consisting of phosphorus, sulfur and magnesium, and also carotene, vitamins C and K and vitamins of group B (Panahi et al., 2015). The microalgae contains peptides, alkaloids, polysaccharides, which can be used as both antimicrobial and antibacterial substances, and likewise, *Chlorella* has antioxidant properties, for it contains antioxidant enzymes, such as superoxide dismutase and catalase (Shibata et al., 2003; Lee et al., 2010; Aliahmat et al., 2012; Zheng et al., 2012; Flerova & Bogdanova, 2014).

The most economically efficient biotechnological solution is cultivating *Chlorella* microalgae in devices of an open type, but in territories of mild climatic zones with constantly changing weather conditions, this method is impossible to implement (Borowitzka, 1999; Carvalho et al., 2006; Del Campo et al., 2007; Christaki et al., 2011; Huo, 2012). To solve this problem, a technology was developed to cultivate *Chlorella* in conditions of a closed type bioreactor using the stimulating effect of an electrostatic field, which allows a large amount of biomass to be obtained on small areas at low cost and with no loss of the algae's nutritional values and fodder qualities necessary for improving the productivity and maintenance of livestock. This technology allows a suspension of *Chlorella* to be obtained with a density of no less than 50 M cells per a ml every 48 hours (Flerova & Bogdanova, 2014; Schmigel et al., 2015a, 2015b).

The effect of suspension of *Chlorella* grown using the developed technology on the organism of cattle remains an open question. The study of this issue is impossible without analyzing the biochemical composition and hematological parameters of the blood, which characterize the functional condition of an organism.

The objective of this study was the analysis of biochemical and hematological blood parameters of different age groups of Yaroslavl breed cattle following addition of a suspension of *Chlorella* cultivated in an electrostatic field to their main diet.

Materials and methods

The studies were carried out on the premises of "Mologa" Ltd. in Yaroslavl Oblast on heifers and lactating cows of Yaroslavl breed of cattle. The animals for experiments were selected using the principle of analogous pairs in accordance with standard methodological recommendations, taking into account breed, age, average live body weight, origin, average daily growth among heifers, and average daily milk yield of lactating cows (Table 1) (Viktorov & Menkin, 1991). Throughout the experiment, the heifers and cows of the control and experimental groups received a daily food diet of nutritional value which equaled the norm for animals of a corresponding age (Makartsev, 2012).

Apart from the main daily diet, the animals of the experimental groups in "Mologa" Ltd. were fed with *Chlorella* suspension. These microalgae were cultivated in the conditions of a closed bioreactor under the influence of an electrostatic field of 15 kV pressure (Schmigel et al., 2015a; Schmigel et al., 2015b). This bioreactor is a device for cultivating *Chlorella*, which consists of 2 aquariums on a metal frame (in the upper aquarium, the water is held and a growth medium is prepared; in the lower aquarium, the *Chlorella* grows), two reservoirs, in this case barrels, were placed on a frame for maintenance of the prepared *Chlorella* suspension and a control unit for automizing the process of *Chlorella* suspension cultivation. The stimulation of *Chlorella* suspension by electrostatic field was carried out using a system of electrodes fixed on the parallel walls of the reservoirs. The electrodes were applied to the source of constant electric current, which was placed in the control unit (Fig. 1).

Table 1
Characteristics of experimental groups of Yaroslavl breed cows

Indicators	Heifers		Lactating cows	
	control	experimental	control	experimental
Number of animals, ind.	15	15	15	15
Average live body weight, kg	68.3 ± 3.5	69.7 ± 4.1	480 ± 21.7	499 ± 23.4
Average daily growth, g	567 ± 23.7	560 ± 25.1	–	–
Age group	heifers below one year		first heifers	
Origin	Dobry line		Dobry line	
Maintenance	1st to 6th month – group; 6th month – tie housing		tie housing	
Average daily milk yield of natural milk during 100 days of lactation, kg	–		24.38 ± 1.18	24.53 ± 0.92
Average daily yield of 4% milk during 100 days of lactation, kg	–		24.56 ± 1.19	24.86 ± 0.93

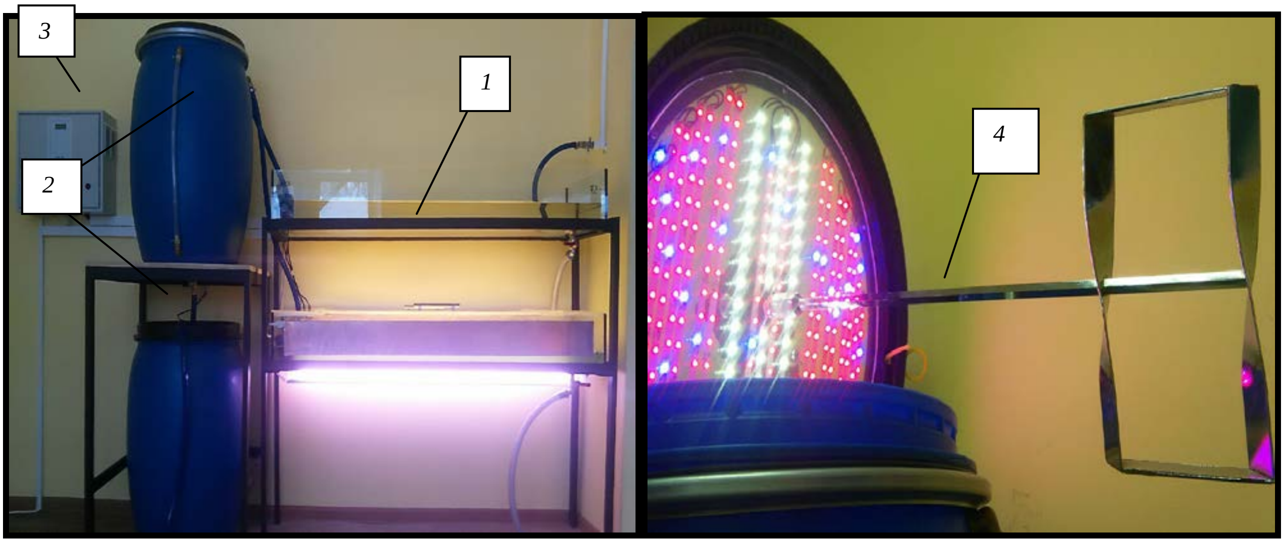

Fig. 1. Bioreactor for cultivating *Chlorella* in an electrostatic field: *1* – device for cultivating *Chlorella* in an electrostatic field; *2* – barrels for maintaining the prepared suspension of *Chlorella*; *3* – control unit with a source of constant electrostatic field and automatization of the process of cultivating *Chlorella* suspension; *4* – cover of the reservoir for maintaining *Chlorella* with a system of light-emitting diodes and an impeller mixer

Throughout the experiment, a new portion of *Chlorella* suspension with cellular density no less than 50 M/ml was obtained every 48 hours. The amount of *Chlorella* suspension for heifers and cows was calculated using the recommendations stating that the heifers which change their diet to roughage require 300–500 ml of *Chlorella* suspension per individual daily (Bogdanov, 2004). For determining the norm of feeding with *Chlorella* suspension, an experiment had been previously conducted on Yaroslavl breed heifers r. On the basis of this experiment, a recalculation for lactating cows was made, which determined that the norm of feeding with the *Chlorella* suspension per kg of dry substance of daily ration for one animal is 155 ml (Bogdanova & Flerova, 2016).

The heifers received *Chlorella* suspension in the following amount (ml/individual per days): 1–30th day of the experiment – 550, 31–60th – 660, 61–90th day – 730, 91–120th day – 830, which in recalculation to dry substance (g/individual per days) was: 1–30th day of the experiment – 1.51, 31–60th day – 1.82, 61–90 day – 2.01, 91–120th day – 2.28. The *Chlorella* suspension was fed to the heifers individually using special plastic bottles. During 60 days, the lactating cows were given 2,180 ml per individual a day, which in recalculation to dry substance was 6.02 g per individual a day.

Chlorella suspension was given individually after morning feeding: the heifers received it throughout 120 days, the cows received it during 60 days, and over the following 30 days were kept under observation to determine the "aftereffects" of the fodder additive. On the first day of the experiment and after every 30 days, before morning feeding, blood was drawn into a vacuum test tube from the jugular vein of the control and experimental animals.

The composition and ratio of leukocytes were analyzed using Giemsa stain. The identification of leukocytes used "The Atlas of

Blood Cells of Agricultural and Laboratory Animals" (Nikitin, 1949). Under the Mikmed-6 microscope, 200 cells were calculated on each blood smear, including lymphocytes, monocytes, neutrophils, eosinophils, basophils. The number of leukocytes was calculated using the standard method of a hemocytometer (Kondrahin, 2004). The number of erythrocytes and the amount of hemoglobin was determined using an Advia 60 automatic hematological analyzer with a Timepac box complex reagent (USA-Germany).

The activity of superoxide dismutase was determined in blood hemolysate obtained by adding 0.9 ml of water to 0.1 ml of blood, and centrifuged at 5,000 rotations per minute during 30 minutes. We used the spectrophotometric method based on recovering of nitrotetrazolium by superoxide radicals formed as a result of interaction between phenazinemetasulfate and recovered form (NAD). The activity of the enzyme was determined at 540 nm wave length and expressed in conventional units per mg of hemoglobin (Chevari et al., 1985). The catalase activity was determined in the blood serum using the spectrophotometric method after adding 0.1 ml of homogenate to 2 ml of 0.03% solution of hydrogen peroxide. Instead of homogenate, 0.1 ml of distilled water was added to a dummy tube. The reaction was stopped after 10 min by adding 1 ml of 4% solution of ammonium orthomolybdate, the activity of the enzyme was expressed in IU. The staining intensity was determined at 410 nm wave length (Korolyuk et al., 1988).

The parameters of the studied features were calculated using statistical methods. We determined the arithmetic mean (M), and the standard error (SE). For determining the differences between the average values, we tested the statistical hypotheses using Student's t-test ($P < 0.05$) after a preliminary calculation of normal distribution.

Results

Heifers. The results of the study showed that the proportion of different forms of leukocytes in the blood of heifers from the control and experimental groups was within the physiological norm throughout the experiment (Table 2).

Table 2
Proportion of different forms of leukocytes in the blood of heifers (M ± SE, n = 15)

The period of the experiment	Groups	Basophils, %	Eosinophils %	Neutrophils, %		Lymphocytes, %	Monocytes, %
				Band neutrophils	with segmented nuclei		
	Norm+	0–2	3–8	2–5	20–35	40–75	2–7
1st day	control	0	4.10 ± 0.33	3.10 ± 0.11	20.6 ± 1.1	68.4 ± 0.7	3.70 ± 0.49
	experimental	0	4.40 ± 0.59	3.20 ± 0.14	20.6 ± 0.4	69.4 ± 3.6	3.40 ± 0.33
30th day	control	0	4.50 ± 0.35	3.29 ± 0.33	20.5 ± 0.7	68.3 ± 1.5	2.79 ± 0.26
	experimental	0	4.71 ± 0.29	3.36 ± 0.23	20.5 ± 0.7	68.2 ± 1.6	2.86 ± 0.15
60th day	control	0	4.75 ± 0.42	3.20 ± 0.22	20.6 ± 0.4	68.2 ± 0.4	2.80 ± 0.22
	experimental	0	4.91 ± 0.54	3.30 ± 0.14	20.9 ± 0.7	68.3 ± 0.2	2.90 ± 0.21
90th day	control	0	4.46 ± 0.23	3.21 ± 0.24	20.1 ± 0.3	68.7 ± 1.0	3.21 ± 0.39
	experimental	0	4.65 ± 0.34	3.64 ± 0.23	20.9 ± 0.5	68.9 ± 0.8	3.29 ± 0.16
120th day	control	0	4.56 ± 0.34	3.28 ± 0.39	20.3 ± 0.5	69.1 ± 0.3	3.07 ± 0.21
	experimental	0	4.74 ± 0.23	3.71 ± 0.29	20.7 ± 0.4	69.6 ± 0.9	3.21 ± 0.35
150th day	control	0	4.59 ± 0.27	3.24 ± 0.25	20.6 ± 0.5	69.5 ± 0.2	3.18 ± 0.26
	experimental	0	4.64 ± 0.17	3.68 ± 0.33	20.9 ± 0.9	69.6 ± 0.7	3.25 ± 0.43

Note: * – $P < 0.05$ according to Student's t-test compared to the control; + – according to Nikitin (1949), Kondrahin (2004).

It should be mentioned that at all stages of the experiment, no basophils were found in the blood of heifers from the control and experimental groups (Table 2). The heifers of the experimental group were observed on the 90th day and before the end of the experiment to have some increase in the relative number of band neutrophils compared to the control values (Table 2).

Table 3
Hematological parameters of Yaroslavl heifers (M ± SE, n = 15)

Period of the experiment	Groups	Erythrocytes, 10^{12}/l	Hemoglobin, rgl	Leukocytes, 10^9/l
	norm+	5.0–7.5	99–129	4.5–12.0
1st day	control	5.74 ± 0.24	109.2 ± 5.3	6.87 ± 0.23
	experimental	5.72 ± 0.29	110.0 ± 5.0	7.16 ± 0.39
30th day	control	5.70 ± 0.21	105.2 ± 2.2	8.28 ± 0.41
	experimental	5.82 ± 0.56	109.8 ± 6.4	8.67 ± 0.51
60th day	control	5.76 ± 0.16	109.0 ± 3.0	9.57 ± 0.21
	experimental	5.88 ± 0.22	117.0 ± 2.0*	10.24 ± 0.49
90th day	control	5.91 ± 0.21	109.8 ± 3.9	10.30 ± 0.43
	experimental	6.38 ± 0.19*	114.2 ± 2.9	11.01 ± 0.38
120th day	control	5.93 ± 0.27	108.8 ± 3.8	10.50 ± 0.26
	experimental	6.46 ± 0.25*	113.5 ± 4.1	10.36 ± 0.06
150th day	control	5.98 ± 0.21	110.6 ± 2.7	10.23 ± 0.43
	experimental	6.41 ± 0.23*	114.2 ± 2.0	10.45 ± 0.18

Note: * – $P < 0.05$ according to t-criterion compared to the control; + – according Nikitin (1949), Kondrahin (2004).

During the study, we observed a statistically insignificant increase in the total number of leukocytes within the physiological norm in both groups of animals (Table 3). The number of erythrocytes among heifers of the experimental group was a little higher on the 30th and 60th days of feeding with *Chlorella* suspension compared to this parameter in the blood of animals from the control group. Then, this parameter reliably increased on the 90th and 120th days of the experiment in comparison to the same parameters for the control by 7.95% and 8.93% respectively, and this excess remained on the 30th day after the feeding with *Chlorella* suspension was over and equaled 7.20% (Table 3). At the same time, the hemoglobin content of animals from the experimental group exceeded this parameter in the control group, and by the 60th day, the difference between the content of hemoglobin in blood of animals from the control and experimental group was 7.30% and was statistically significant (Table 3). On the 90th and 120th days of the experiment, we observed some increase in the content of hemoglobin in blood of animals from experimental group compared to the control, and this tendency remained on the 30th day after the feeding with the suspension additive was over (Table 3).

Table 4
Activity of the enzymes of anti-oxidant system of Yaroslavl heifers at the stages of study (M ± SE, n = 15)

Period of the experiment	Groups	SOD, conv.units/mg of hemoglobin	CAT, IU
	Norm+	1.0–7.5	20–60
1st day	control	4.39 ± 0.33	39.40 ± 0.30
	experimental	4.97 ± 0.14	42.00 ± 2.91
30th day	control	4.13 ± 0.58	34.80 ± 1.52
	experimental	4.70 ± 0.76	36.00 ±2.72
60th day	control	4.86 ± 0.75	32.90 ± 1.25
	experimental	5.22 ± 0.37	36.00 ± 0.50*
90th day	control	4.68 ± 0.19	35.78 ± 2.78
	experimental	5.06 ± 0.28	40.20 ± 2.06*
120th day	control	4.74 ± 0.24	35.38 ± 1.44
	experimental	5.10 ± 0.19	39.40 ± 1.71*
150th day	control	4.81 ± 0.28	36.18 ± 1.74
	experimental	5.15 ± 0.34	39.42 ± 2.29*

Note: * – $P < 0.05$ according to t-criterion compared to the control, SOD – superoxide dismutase, CAT – catalase; + – according to Kondrahin (2004).

At the beginning of the experiment, the heifers of the experimenttal group were observed to have an increase in the activity of catalase in the blood serum; on 60th, 90th and 120th days it was significantly higher compared to the control, equaling 9.4%, 12.4% and 11.4% respectively ($P < 0.05$), on the 30th day after the end of feeding with *Chlorella* suspension, this increase remained statistically significant, equaling 8.9% (Table 4). It should be mentioned that throughout the experiment the activity of superoxide dismutase in the experimental group was a little higher than in the control group. However no statistically reliable increase in this parameter compared to the control was observed (Table 4).

Lactating cows. The proportion of separate forms of leukocytes in the blood of lactating cows of the control and experimental groups was also within the physiological norm (Table 5). No basophils were found in the blood of animals from either group. By the 30th day of the experiment, the relative number of band and neutrophils with segmented nuclei in the blood of lactating cows of the experimental group had slightly increased compared to the similar parameters of the control, and this tendency remained throughout the period of feeding with the additive.

It should be mentioned that on the 60th day, we observed a statistically significant increase in the number of eosinophils in the blood of lactating cows of the experimental group by 2.2% compared to the control value. On the 90th day, the number of monocytes in the blood of cows from the experimental group increased by 2% compared to the control (Table 4). Over the experiment, the hematological parameters of blood of lactating cows from the control and experimental groups were within the physiological norm (Table 5).

Table 5
The proportion of separate forms of leukocytes of lactating cows of Yaroslavl breed (M ± SE, n = 15)

Period of the experiment	Groups	Basophils, %	Eosinophils %	Neutrophils, %		Lymphocytes, %	Monocytes, %
				band neutrophils	with segmented nuclei		
	Norm +	0–2	3–8	2–5	20–35	40–75	2–7
1st day	control	0	4.00 ± 1.16	4.93 ± 0.70	20.6 ± 1.4	73.6 ± 2.5	2.57 ± 0.86
	experimental	0	3.71 ± 2.30	4.79 ± 1.26	20.1 ± 1.3	75.0 ± 3.1	2.86 ± 1.70
30th day	control	0	3.92 ± 0.88	4.4 3± 0.69	20.6 ± 1.3	69.4 ± 2.7	2.07 ± 0.25
	experimental	0	4.00 ± 0.54	4.64 ± 1.43	22.0 ± 1.2	73.7 ± 3.1	3.14 ± 0.85
60th day	control	0	4.29 ± 0.75	3.88 ± 1.88	20.6 ± 1.8	68.5 ± 2.6	3.23 ± 1.05
	experimental	0	6.50 ± 0.51*	4.14 ± 1.79	20.6 ± 1.4	70.9 ± 3.6	3.21 ± 1.24
90th day	control	0	6.64 ± 1.66	4.00 ± 2.08	20.1 ± 1.9	63.0 ± 3.8	2.36 ± 0.84
	experimental	0	6.71 ± 2.08	4.43 ± 1.87	21.9 ± 1.9	59.9 ± 4.4	4.36 ± 1.19*

Note: * – $P < 0.05$ according to t-criterion compared to the control; + – according to Nikitin (1949), Kondrahin (2004).

On the 30th day of the experiment, the number of erythrocytes of animals from the experimental group statistically significantly increased by 15.3% compared to the parameters of the control group, the observed tendency remained on the 60th and 90th days of the experiment and the excess of this parameter in the blood of animals from the experimental group was 5.1 and 4.5% respectively. At the same time, against the background of increase in the number of erythrocytes, an increase occurred in the content of hemoglobin in the blood of animals from the experimental group, which by the end of the 60th day equaled 8.4% ($P < 0.05$), this excess remained on the 90th day of the experiment, equaling 6.8%. On the 30th and 60th days of the experiment, the total number of leukocytes in the blood of lactating cows of the experimental group was slightly higher than it was in the blood of animals from the control group (Table 6).

Table 6
Hematological parameters of lactating cows of Yaroslavl breed (M ± m)

Period of the experiment	Groups, n = 15	Erythrocytes, 10^{12}/l	Hemoglobin, g/l	Leukocytes, 10^9/l
	Norm +	5.0–7.5	99–129	4.5–12.0
1st day	control	5.00 ± 0.23	99.2 ± 4.3	8.90 ± 1.31
	experimental	5.00 ± 0.34	100.0 ± 5.0	9.00 ± 1.49
30th day	control	5.11 ± 0.13	99.0 ± 1.6	8.65 ± 0.50
	experimental	5.89 ± 0.42*	102.0 ± 5.0	9.02 ± 0.58
60th day	control	5.06 ± 0.16	96.9 ± 2.8	7.72 ± 0.83
	experimental	5.32 ± 0.19*	105.0 ± 3.0*	7.90 ± 0.80
90th day	control	5.31 ± 0.12	103.0 ± 2.0	7.98 ± 0.25
	experimental	5.55 ± 0.22	110.0 ± 3.0*	7.97 ± 0.20

Note: * – $P < 0.05$ according to t-criterion compared to the control; + – according to Kondrahin (2004).

Throughout the experiment, the parameters of activity of catalase and superoxide dismutase among animals of the control and experimental groups were within the physiological norm. It should be mentioned that the parameters of the activity of superoxide dismutase and the activity of catalase of cows which received the *Chlorella* additive, were slightly higher than those of animals from the control group throughout the experiment (Table 7).

Discussion

Analysis of data from the literature showed that no experiment with usage of suspension of *Chlorella* stimulated by electrostatic field as a fodder additive for animals had been conducted before. However, there are detailed descriptions of the impact of the microalgae cultivated using other technologies, on the organism of both laboratory and agricultural animals. Use of concentration of *Chlorella* had a physiological and pharmaceutical effect on the organism of mice and rats, manifesting a stimulating effect on the lipid metabolism, immune modulating and antibacterial activity of the organism of laboratory animals (Hasegawa et al., 1997; Shibata et al., 2001). Also, this microalgae showed strong antioxidant properties which prevent the effect of oxidative stress, when it is used in treating mice with naphthalene intoxication, and also rats suffering from diabetic disorders and oxidative stress under the impact of cadmium (Shibata et al., 2003; Vijayavel et al., 2007, Kim et al., 2009). It has been demonstrated that *Chlorella* participates in modeling immune reactions in the organism of animals and increases the activity of hematopoietic cells which prevent the development of tumours (Tanaka et al., 1984; Tanaka et al., 1990; Ramos, 2010).

Table 7
Activity of enzymes of anti-oxidant system of lactating cows of the Yaroslavl breed at the stages of the study (M ± SE, n = 15)

Period of the experiment	Group	SOD, unit /mg of hemoglobin	CAT, µmol/l
	Norm +	1.0–7.5	20–60
1st day	control	3.59 ± 0.38	27.33 ± 2.39
	experimental	3.64 ± 0.33	27.17 ± 3.59
30st day	control	3.45 ± 0.96	31.69 ± 2.80
	experimental	4.15 ± 1.01	32.12 ± 4.48
60th day	control	2.36 ± 0.50	33.67 ± 5.39
	experimental	2.39 ± 0.37	34.76 ± 8.35
90th day	control	2.48 ± 0.24	29.45 ± 0.23
	experimental	2.67 ± 0.37	29.57 ± 0.29

Note: * – $P < 0.05$ according to the t-criterion in comparison with the control; SOD – superoxide dismutase, CAT – catalase; + – according to Kondrahin (2004).

Feeding different age groups of pigs and cattle with *Chlorella* suspension contributed to increase in hemoglobin in the blood, increase in the number of erythrocytes and some forms of leukocytes. Using *Chlorella* suspension as a fodder additive in diets of different age groups of pigs caused increase in the number of monocytes and decrease in the level of cholesterol in blood serum, and using the microalgae in the diet of young cattle caused normalization of the physiological parameters of the animals' blood serum, strengthened enzyme activity and increased content of γ – globuline fraction of protein (Kotrbáček et al., 1995; Doucha et al., 2009; Yan et al., 2012; Oshurkova et al., 2015; Panov et al., 2016; Lamminen et al., 2017).

The results of our experiments coincide with the data provided by other researchers and demonstrate that the biochemical composition and hematological parameters of the blood of heifers and lactating cows which received an additive of suspension of *Chlorella* cultivated in a closed bioreactor under stimulation by electrostatic field, in the amount of 155 ml per a kg of dry substance of the animals' diet were within the physiological norm regardless of the duration of feeding. We demonstrated the *Chlorella* suspension's stimulating effect on erythro- and leukopoiesis of different age groups of cattle, and also the shift of leukogram towards increase in the relative number of separate forms of leukocytes and increase in the hemoglobin count. Increase in hemoglobin when there is increase in the number of erythrocytes within the physiological norm according to the data on blood parameters of heifers and lactating cows of the experimental groups indicates the increase in the speed of redox reactions, contributes to increase in the oxygen capacity and provides the tissues with oxygen, thus causing strengthening in metabolic processes in the organism of animals (Nadarinskaya, 2004; Krisanov et al., 2014). At all stages of the experiment, we found no basophils in the blood of heifers and lactating cows of the experimental groups, which indicates absence of allergic reactions in the organism, such as allergic reactions to fodder elements, including *Chlorella* suspension (Kondrahin, 2004; Karasuyama et. al., 2011).

The animals which received the addition of *Chlorella* were observed to have an increase in parameters of catalase and superoxide dismutase throughout the experiment. We demonstrated that the increase in the activity of superoxide dismutase was followed by activation of catalase activity parameters both among heifers and cows of the experimental groups, which indicates absence of disbarlance in the antioxidant system (Nisticò et al., 1992). We determined that feeding different age groups of cattle with *Chlorella* additive stimulates the system of antioxidant protection of the organism of animals, thus increases the level of non-specific resistance (Kondrahin, 2004). As we know, the overall changes in the biochemical and hematological parameters analyzed in the study within the physiological norm also indicate the increase in non-specific immunity of animals (Krapivina et al., 2011). It should be mentioned that regardless of the age of animals which receivied the *Chlorella* additive, the organism's response occurred on the 30th day of the experiment, and also the "aftereffect" was observed for a period of 30 days after the feeding with the additive was stopped.

Conclusion

Therefore, we demonstrated the usefulness of including the suspension of *Chlorella* cultivated using the intensive technology using an electrostatic field, with cellular density of no less than 50 M per a ml in a dosage of 155 ml per kg of dry substance of the diet, in feeding different age groups of cattle, for it has a complex effect on increase in the biochemical, hematological and morphological blood parameters, strengthening metabolic processes and increasing the non-specific immunity of animals, which in its turn contributes to the formation of the potential for increase in productive parameters and maintenance of cattle. Therefore, we demonstrated that the suspension of *Chlorella* cultivated with intensive technology using an electrostatic field, with cellular density of no less than 50 m per a ml is useful in feeding cattle of different age groups in a dosage of 155 ml per kg of dry substance of the diet.

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