International Journal of Psychosocial Rehabilitation, Vol. 24, Issue 03, 2020
ISSN: 1475-7192

Phagocytic Activity of Neutrophils in the Blood of Pigs that Received A "Microecological System" Alternative to Antibiotics

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Abstract--- *The study was aimed at the identification of a certain link of the nonspecific protection in pigs that enhances its activity during the application of different feeding plans with probiotic "EM-Vita", creates stable "microecological system", and provides high natural resistance. Weaned piglets aged 46-53 days were distributed into 3 groups by the method of analog pairs: Group I – control group, Group 2 and 3 – test groups that received even doses of the drug but at different intervals. It was established that in 2 months, pigs that received "EM-Vita" by both schemes had expressed increase in the level of nonspecific protection due to a general increase in the neutrophil count and their fractions capable of both oxygen-dependent microbicide activity and enhanced activity of this process. The application of the drug by one of the schemes provided the creation of the adaptive reserve of neutrophils that exert adsorptive activity, by the other scheme – the increase in the synthesis of cationic proteins. Thus, the periodicity of the application of probiotic "EM-Vita" significantly influences on the status of neutrophils by selective activation of different protective systems.*

Keywords--- *Blood, Neutrophils, Lactobacillus Plantarum, Antibiotic Replacement.*

I. INTRODUCTION

It is impossible to provide food supply security of a country without an effective upgrade of agro-industrial production (Petrovskiy et al., 2013). Antibiotics are often used in pig production as growth stimulators but they cause the reduction of the antimicrobial resistance to bacteria in humans and animals (Mevius, 2012; McKean, 2012). Stable "microecological system" that provides natural resistance, stimulates the synthesis of immune globulins, formation of immunological barrier towards pathogenic microbes not only in mammals but also in other living organisms, optimization of homeostasis, and acceleration of regenerative processes can become an alternative to the application of antibiotics (Dotta et al., 2011; Silva et al., 2018; Peixoto et al., 2018). Probiotic and prebiotic complexes effectively influence on the microcenosis providing the optimization of metabolic processes due to the synthesis of organic acids, biologically active substances, as well detoxication of harmful products (Gamko et al., 2010; Strom et al., 2002; Pavlov et al., 2011; Rechkalova, 2006; Sidorov, 2001; Ovsyannikov, 2009; Betin, 2016;

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DOI: 10.37200/IJPR/V24I3/PR2020340
Received: 24 Feb 2020 | Revised: 28 Feb 2020 | Accepted: 18 Mar 2020

Moshkutelo et al, 2010). Feeding probiotic "EM-Vita" contains microorganisms that are contained in natural habitat (Collado et al., 2007). It consists of a mixture of strains of *Lactobacillus plantarum* 376, *Lactobacillus casei* MDP-1, *Saccharomyces cerevisiae*, and molasses. In 1 cm^3 of the drug, not less than 10^7 CFU of lactic acid bacteria and 10^4 CFU of yeast are contained. Feeding supplement «EM-Vita» normalizes the microflora of gastrointestinal tract of animals contributing to the improvement of digestion. The microorganisms contained in the drug exert antagonistic effect against protozoan, pathogen microorganisms, and septic microflora, which leads to an increase in the activity of protective mechanisms and productivity. However, the studies conducted by different scientists showed that "despite numerous positive properties of probiotics in general, the properties of each drug cannot be automatically characteristic to other probiotics that are given to other animals grown in other conditions" (Uchasov, 2013). It is known that different forms of reactivity of neutrophils are primarily provided by or, at least, initiated by separate mechanisms and are often exerted independently from each other (Mayanskiy et al., 1989). At the same time, it was proved that different schemes of application of biologically active substances activated different protective mechanisms of the organism (Krapivina and Krivopushkin, 2009).

The aim of the experiment was to study the phagocyte activity of neutrophils in the blood of pigs that were given "EM-Vita" at different schemes.

II. Materials And Methods

The authors performed a scientific-production experiment at the facilities of a pig complex "Peretorgy" ("BMPC, Ltd.", Bryanskaya Oblast, Russia). Based on the breed, age and body weight, the animals were distributed into 3 groups by the method of analog pairs. Each group contained 10 weaned pigs aged 46-53 days with the weight of 11.18±0.01 kg. The pigs were obtained from sows (Duroc and Pietrain) inseminated by the sperm of a Large White boar. Group I contained control animals. Animals from Group II received 4 ml of feeding supplement "EM-Vita" 1 time per day for 2 months according to the following scheme: 7 days - 1 time per day, 7 days – no supplement. Animals from Group III also received "EM-Vita" supplement 4 ml per day for 2 months but the scheme was different: 7 days – 1 time per day, 14 days – no supplement. The pigs were contained in the facilities that complied with veterinary and zoo hygienic requirements and were fed according to the generally accepted norms (Kalashnikov et al., 2003). Blood samples were taken from 5 animals in each group from the jugular vein before feeding before the beginning of the experiment, in 1 and 2 months of the supplement intake, and in 1 month after the end of the supplement rearing. 6 days before the 3rd blood sampling, all the pigs were vaccinated against Aujeszky's Disease and swine erysipelas (Vetbiochim, Ltd.). Hemogram parameters were estimated by a hemanalyzer "Abacus junior vet 5" at the Bryansk SAU.

Phagocyte count (PC, %) was calculated as percent of neutrophils capable of absorption of latex beads, phagocytic index (PI, CU) - mean latex beads count absorbed by one active neutrophil, absolute blood phagocytosis (AO, 10^9/L) – total latex beads count absorbed by neutrophils in 1 liter of blood, phagocytic number (PN, CU) – mean latex beads count per 1 neutrophil (both active and inactive) (Chumacheiko et al., 1990). Functional metabolic activity of neutrophils was evaluated by the results of the reduction of nitro blue tetrazolium in NBT-positive neutrophils (+NBT, %) (Shubich, 1978).

DOI: 10.37200/IJPR/V24I3/PR2020340
Received: 24 Feb 2020 | Revised: 28 Feb 2020 | Accepted: 18 Mar 2020

Neutrophil activation index (NAI) was calculated as it was described in the manual "Reacomplex" to the NBT-test kit. Absorptive capacity of neutrophils (PC, %; PI, %; AP, 10^9/L; PN, CU) and the activity of their oxidase systems (+NBT, %, NAI) were evaluated in two conditions: basic (bas.) – freshly taken blood stabilized with heparin, and stimulated (stim.) – after the introduction of zymosan into the blood probes, which modeled the conditions of bacterial contamination and described adaptation reserve of absorptive and microbicide capacity of neutrophil granulocytes (Khaitov et al., 1995). Oxygen-independent microbicide capacity of neutrophils in peripheral blood was estimated by the content of cation proteins by the method of Zhibinov (1983) (Zhinov, 1983), calculating mean cytochemical coefficient (MCC) by the formula proposed by Makarevich (1988).

The obtained digital data was processed by the analysis of variance. Statistically significant differences were identified by Student's t-test by the method of Plokhinskiy (Plokhinskiy, 1961). The results were considered significant at $p<0.05$.

Physiological norms were within the intervals provided in the scientific literature (Chumachenko et al., 1990; Kondrakhin et al., 2004; Petrov et al., 2012).

III. RESULTS

The analysis of the morphological content of pig blood (Table 1) showed that the leucocyte count in blood in animals from all the groups before the experiment and during the experiment corresponded with the normal values without significant intergroup differences with a tendency to a decrease in pigs from Groups II and III in a month after the experiment.

Before the experiment, relative neutrophil count (sums of all the nuclear forms) in blood in animals from all the groups corresponded with normal values without significant intergroup differences. During the experiment, the level of neutrophils in blood in pigs from the control group did not change significantly with a tendency to the increase in 2 months of the test period.

In animals from Groups II and III, the neutrophil count was significantly higher than in 1 month of the test period by 5.90% and 8.84%, respectively.

The relative content of neutrophils in blood capable of absorption of foreign matter in basic conditions in pigs from all the groups before the test did not differ significantly and was higher than the norm, which indicated the factors that activated neutrophils and the capacity of the organism to react to them. In 1 month of the test period, the content of neutrophils in blood capable of absorption of foreign matter in basic conditions in pigs from Groups I, II and III did not differ significantly and significantly increased by 29.87, 31.38 and 36.22%, respectively. The relative content of neutrophils in blood capable of absorption of foreign matters in the conditions stimulated with zymosan in pigs from Groups I, II, and III did not differ significantly before the experiment and corresponded with the normal range. It was significantly higher than in the basic conditions by 17.78, 12.00, and 12.82%, respectively.

DOI: 10.37200/IJPR/V24I3/PR2020340
Received: 24 Feb 2020 | Revised: 28 Feb 2020 | Accepted: 18 Mar 2020

Table 1: The Influence of Rearing with Feed Supplement "EM Vita" on the Phagocyte Activity of Neutrophils in Pigs Blood

Parameters	Groups	Before rearing	1 month after the start	2 months after the start	1 month after finishing
Leucocytes, 10^9/L	1, n=5	9.08 ± 0.42	9.01 ± 0.43	8.89 ± 0.33	9.31 ± 0.44
	2, n=5	8.93 ± 0.15	9.02 ± 0.28	9.53 ± 0.40	8.90 ± 0.16
	3, n=5	9.34 ± 0.45	9.80 ± 0.53	9.34 ± 0.45	8.56 ± 0.17
Neutrophils, %	1, n=5	40.28 ± 0.50	40.82 ± 0.71	42.18 ± 1.04	41.1 ± 0.57
	2, n=5	40.5 ± 0.23	38.98 ± 0.78	41.28±0.31▲	40.66 ± 0.39
	3, n=5	39.92 ± 0.55	38.68 ± 1.05	42.10±0.40▲	40.4 ± 0.57
PI bas., %	1, n=5	59.60 ± 2.98	77.40 ± 4.53º	85.00 ± 0.55º	83.60 ± 0.40º
	2, n=5	65.00 ± 0.95	85.40 ± 1.29º	84.80 ± 1.46º	83.40 ± 0.87º
	3, n=5	62.40 ± 2.58	85.00 ± 1.14º	84.60 ± 0.93º	83.60 ± 0.60º
PI stim., %	1, n=5	70.20 ± 1.96^	85.60 ± 4.03º	92.20± 1.53º^	90.20 ± 0.73º^
	2, n=5	72.80 ± 2.4^	90.00 ± 1.45º	91.00± 1.14º^	91.40 ± 0.68º^
	3, n=5	70.40 ± 0.98^	92.00± 0.55º^	90.40± 1.12º^	91.00 ± 0.84º^
PI bas., CU	1, n=5	5.35 ± 0.21	3.58 ± 0.21	3.65 ± 0.11	3.90 ± 0.07
	2, n=5	5.40 ± 0.27	3.27 ± 0.09	3.67 ± 0.02	3.96 ± 0.05
	3, n=5	4.98 ± 0.08	3.40 ± 0.08	3.68 ± 0.09	3.93 ± 0.03
PI stim., CU	1, n=5	5.55 ± 0.16	4.28 ± 0.36	4.26 ± 0.10	4.31 ± 0.05
	2, n=5	5.78 ± 0.15	4.28 ± 0.36	4.18 ± 0.08	4.39 ± 0.05
	3, n=5	5.43 ± 0.09	3.75 ± 0.10	4.15 ± 0.14	4.37 ± 0.05
AP bas., 10^9/L	1, n=5	11.79 ± 0.29	10.10 ± 0.74	11.63 ± 0.64	12.50 ± 0.81
	2, n=5	10.26 ± 0.72	9.87 ± 0.66	12.26 ± 0.7	11.97 ± 0.34
	3, n=5	10.07 ± 0.58	11.00 ± 0.96	12.21 ± 0.39	11.38 ± 0.44
AP stim., 10^9/L	1, n=5	12.21 ± 1.19	12.19 ± 1.56	13.60 ± 0.82	13.80 ± 0.76
	2, n=5	13.57 ± 0.24	11.04 ± 0.86	13.96 ± 0.77	13.27 ± 0.40
	3, n=5	12.57 ± 0.47	12.12 ± 0.95	13.75 ± 0.50	12.66 ± 0.51
PC bas., CU	1, n=5	3.21 ± 0.27	2.74 ± 0.07	3.10 ± 0.11	3.26 ± 0.06
	2, n=5	3.51 ± 0.16	2.80 ± 0.11	3.11 ± 0.05	3.31 ± 0.07
	3, n=5	3.10 ± 0.13	2.89 ± 0.08	3.12 ± 0.08	3.28 ± 0.04
PC stim., CU	1, n=5	3.33 ± 0.25	3.27 ± 0.23	3.62 ± 0.10	3.60 ± 0.04
	2, n=5	3.76 ± 0.13	3.13 ± 0.15	3.55 ± 0.11	3.66 ± 0.07
	3, n=5	3.39 ± 0.15	3.19 ± 0.07	3.52 ± 0.15	3.65 ± 0.06
+NBT bas., %.	1, n=5	9.40 ± 0.68	11.00 ± 0.71	11.40 ± 0.51	12.20 ± 0.58 º
	2, n=5	9.60 ± 0.68	12.80 ± 1.07	13.00 ± 0.71 º	12.60 ± 0.24 º
	3, n=5	9.40 ± 0.68	12.00 ± 1	12.60 ± 0.51 º	13.00 ± 0.77 º
+NBT stim., %	1, n=5	24.80 ± 1.5^	35.80 ± 2.44º^	39.80 ± 0.80º^	45.20 ± 1.66º▲^
	2, n=5	24.40 ± 0.51^	36.80 ± 1.53º^	47.20 ± 0.97º▲^	50.60± 0.51*º▲^
	3, n=5	25.80 ± 1.20^	38.60 ± 0.40º^	45.60 ± 0.51º▲^	48.40 ± 0.68º▲^
NAI bas.	1, n=5	0.10 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.12 ± 0.01
	2, n=5	0.12 ± 0.01	0.18 ± 0.01*º	0.16 ± 0.01*º	0.15 ± 0.01*
	3, n=5	0.11 ± 0.01	0.17 ± 0.01*º	0.16 ± 0.01*º	0.15 ± 0.01*º
NAI stim.	1, n=5	0.38 ± 0.03^	0.59 ± 0.04º^	0.66 ± 0.02º^	0.81 ± 0.05º▲^
	2, n=5	0.39 ± 0.03^	0.62 ± 0.05º^	0.90 ± 0.04*º▲^	0.98 ± 0.03*º^
	3, n=5	0.41 ± 0.03^	0.65 ± 0.02º^	0.87 ± 0.03*º▲^	0.89 ± 0.04º^
MCC	1, n=5	1.32 ± 0.01	1.37 ± 0.04	1.40 ± 0.04	1.49 ± 0.03º
	2, n=5	1.35 ± 0.01	1.36 ± 0.04	1.31 ± 0.02	1.57 ± 0.01*º▲
	3, n=5	1.37 ± 0.01	1.31 ± 0.04	1.36 ± 0.01	1.52 ± 0.02º▲

Note: - * - $p<0.05$ in relation to Group I, ▲ – in relation to the previous period of the study, º - in relation to the beginning of the experiment, ^ - $p<0.05$ stim. in relation to bas., PI – phagocytic index, AP – activation of phagocytes, PC – phagocyte count, NBT - Nitro Blue Tetrazolium, NAI – neutrophil activation index, MCC - mean cytochemical coefficient, bas. – basic, stim. – stimulated.

DOI: 10.37200/IJPR/V24I3/PR2020340
Received: 24 Feb 2020 | Revised: 28 Feb 2020 | Accepted: 18 Mar 2020

In 1 month after the beginning of the experiment, the content of neutrophils in blood capable of absorption of foreign matter in the conditions stimulated with zymosan in pigs from Groups I, II, and III did not differ significantly and significantly increased by 21.94, 23.63, and 30.68%, respectively, in comparison with the basic. Adaptive reserve of neutrophils in blood capable of absorption of foreign matter was observed only in animals from Group III (by 8.33% higher in the stimulated conditions in comparison with the basic). During the other periods of the study, the content of neutrophils in blood capable of absorption of foreign matter in the conditions stimulated with zymosan in pigs from all the groups did not change significantly and did not have intergroup differences, and was significantly higher than in the basic conditions.

Phagocytic index in the basic conditions (PI bas.,) in pigs from all the groups corresponded with the highest values of the physiological norm without significant intergroup differences. In 1 month of the experimental period, PI bas. in pigs from Groups I, II, and III decreased by 33.08, 39.44, and 31.73% ($p<0.05$), respectively. In further periods, there were no significant changes in this parameter. PI in the stimulated condition (PI stim.) in pigs from all the groups corresponded with the lowest values of the physiological norm without significant intergroup differences. In 1 month of the experimental period, PI stim. in pigs from Groups I, II, and III decreased by 32.88, 25.95, and 30.94% ($p<0.05$), respectively. In further periods, there were no significant changes in this parameter.

Adaptive reserve of the intensity of absorptive capacity of neutrophils in blood in 1 month of the experimental period was observed in pigs from Group II and III (by 30.889 and 10.29%, respectively, $p<0.05$). It was higher in the stimulated conditions in comparison with the basic. In the rest experimental periods, it was higher in animals from all the groups ($p<0.05$).

Absolute phagocytosis value, as well as phagocyte number in the basic and stimulated conditions in pigs from all the groups before the experiment, did not have significant intergroup differences when there was no adaptive reserve pf this mechanism of protection. In 1 month of the experimental period, there was a tendency to a decrease in the phagocyte number in the basic and stimulated conditions in pigs from all the groups with a further tendency to an increase in this parameter values. In 1 month of the experimental period, in pigs from Group III, adaptive reserve of the phagocyte number was observed (by 10.38%, $p<0.05$ higher in the stimulated conditions in comparison with the basic). In animals from Groups I and II, it was not observed during this period.

Relative number of NBT-positive neutrophils in blood in the basic conditions in pigs from all the groups before the experiment did not have intergroup differences and corresponded with the norm values with the increased level of reactivity of these cells in further experimental periods: in pigs from Group I – by 29.79% ($p<0.05$) in 1 month after the end of supplement application, and in pigs from Groups II and III – by 35.42 and 34.04% ($p<0.05$), respectively, in 2 months of the experimental period in comparison with the initial period.

Relative number of NBT-positive neutrophils in blood in the conditions stimulated with zymosan in pigs from all the groups before the experiment did not have intergroup differences, was lower than the norm values and increased in pigs from Groups I, II, and III by 44.35, 50.82, and 49.61% ($p<0.05$), respectively, already in 1 month of the experimental period in comparison with the initial period. Further increase in the number of NBT-positive neutrophils in blood in the conditions stimulated with zymosan was observed only in 1 month after the end of the

DOI: 10.37200/IJPR/V24I3/PR2020340
Received: 24 Feb 2020 | Revised: 28 Feb 2020 | Accepted: 18 Mar 2020

supplement application (by 13.57% in comparison with the previous period of the study, $p<0.05$), and in animals from Group II and III – already in 2 months of the experimental period (by 28.26 and 18.13%, respectively, in comparison with the previous period of the study, $p<0.05$).

Neutrophil activation index in blood in the basic conditions (NAI bas.) in pigs from all the groups before the experiment did not have intergroup differences and corresponded with the normal values. In pigs from Group I, NAI bas. did not differ significantly during all the experimental period, in pigs from Group II in 1 month and in 2 months, it was higher by 50.00 and 33.33%, $p<0.05$ in comparison with the initial period, respectively. In pigs from Group III, NAI was higher by 54.55, 45.45, and 36.36%, $p<0.05$ in comparison with the initial period in 1 month and 2 months of the experimental period and 1 month after the end of the supplement application, respectively. During all the periods of the study, except for the initial, the pigs that received the drug had NAI significantly higher than in animals from the control group.

NAI in the conditions stimulated with zymosan (NAI stim.) in pigs from all the groups before the experiment did not have intergroup differences and corresponded with the normal values. In 1 month of the experimental period, it was established that there was a significant increase in this parameter in pigs from Groups I, II, and III by 55.26, 58.97, and 58.54%, respectively. In 2 months of the experimental period, the increase in this parameter values in comparison with the previous study was established only in pigs from Groups II and III by 45.16 and 33.85%, $p<0.05$, respectively. Besides, NAI stim. in pigs from Groups II and III during this period was higher than in pigs from Group I by 36.36 and 31.82%, $p<0.05$, respectively.

Relative number of NBT-positive neutrophils in blood in the conditions stimulated with zymosan in pigs from all the groups during all the periods of the study was significantly higher than the analog parameters in the basic conditions by 154.16….301.58 and 225….575.00%, respectively.

The content of cationic proteins in neutrophils of blood in pigs from all the groups before the experiment did not have intergroup differences and corresponded with the normal values. In 1 and 2 months of the experimental period, mean cytochemical coefficient (MCC) did not change significantly, and in 1 month after the end of the supplement application in pigs from groups II and III, a significant increase in this parameter values was observed by 13.85 and 11.76%, respectively, in comparison with the previous period of the study. MCC was higher in pigs from Group II than in pigs from Group I by 5.37%, $p<0.05$.

IV. DISCUSSION

There are data that on the 21st day after the vaccination, the level of leucocytes significantly decrease in cow blood (Maximov et al., 2014). In pigs from Groups I and III there was a tendency towards the decease of leucocytes in 6 days after the vaccination, and in pigs from Groups II and III – in 36 days after the vaccination, which could be associated with a more expressed process of the formation of the immune response in pigs that received the drug by the following scheme: 7 days of receiving and 14 days of interruption.

Neutrophils were the first to react to the arising stress-factors, including the appearance of antigens in the organism (Garkavi et al., 1990; Marinin et al., 1993). In the norm, the exchange of neutrophils between the bone

DOI: 10.37200/IJPR/V24I3/PR2020340
Received: 24 Feb 2020 | Revised: 28 Feb 2020 | Accepted: 18 Mar 2020

marrow, blood and tissues is a well-balanced process that is maintained at a constant level (Mayanskiy and Galliulin, 1984). It shifts when antigens appear and signal for enhances migration of neutrophils (Karland et al., 1978; Dolgushin and Bukharin, 2001). Thus, a significantly higher number of neutrophils in blood of animals from Group II and III in 6 days after the vaccination in comparison with the previous period of the study (by 5.90 and 8.84%, respectively) indicated a more expressed increase level of nonspecific protection that developed after the vaccination in pigs that received "EM-Vita" by both schemes. The tendency to a decrease in the content of these cells that was observed in 1 month after the end of supplement application was more expressed in pigs from Group III, which indicated the transition of neutrophil phase of the organism response to the antigen and formation of the lymphocytic immune response (Babayan et al., 1988; Lebedev and Ponyakina, 1990).

The guidelines for doctors "Inflammation" (1995) say that in the norm, the majority of neutrophils in the peripheral blood exist in the inert condition. Only the appearance of circulating immune complexes creates the conditions for their activation (Serova, 1995). Ermolina et al. in their study showed that sick animals had low activity of phagocytosis (Ermolina et al., 2008). The fact that a relative number of neutrophils in blood capable of absorption of foreign matter in the basic conditions in pigs from all the groups before the experiment was higher than the normal values indicated that there were factors that activated neutrophils and the capacity of the organism to react to them. The increase in the number of neutrophils in blood capable of absorption of foreign matters in the basic conditions in pigs from Groups I, II, and III in 1 month by 29.87, 31.38, and 36.22%, ($p<0.05$), respectively, could be associated with the influence of numerous stress-factors in the post-weaning period (Vodyannikov and Shkalenko, 2017; Gorlov et al., 2019). There are data that with age, phagocytic activity increases. Thus, the lowest values of phagocytosis are observed at 20 days of age and highest – by the 4^{th} month of age (Pavlyukh, 1989).

Any destabilization of humoral homeostasis provokes the excitement of neutrophils [17] and the introduction of zymosan, a component of a bacterial wall, into the blood samples reveals a possible functional activity of neutrophils in blood. The difference between the functional activity of neutrophils in blood in the conditions stimulated with zymosan (induced) and basic (spontaneous) conditions is an adaptive reserve. The fact that adaptive reserve of neutrophils in blood capable of absorption of foreign matter in 1 month of the experimental period was observed only in pigs from Group III (by 8.33%, $p<0.05$, higher than in the stimulated conditions in comparison with the basic conditions) indicates the ability of "EM-Vita" (7 days of the drug application and 14 days of interruption) to form adaptive reserve of neutrophils capable of absorption of foreign matters.

A decrease in the phagocytic index, that characterizes the intensity of the absorption of latex beads, in the stimulated and basic conditions in pigs from all the groups in 1 month of the experimental period with a simultaneous increase in the phagocyte number indicated the extensive pathway of this type of protection of neutrophils without a significant influence of the indicated drug on this process. It should be noted that the presence of adaptive reserve of the intensity of absorptive capacity of neutrophils in blood in 1 month of the experimental period ($p<0.05$) in pigs from Groups II and III (by 30.89 and 10.29% higher in the stimulated conditions in comparison with the basic conditions, respectively) and the absence of this reserve in animals from the control group indicated the capability of the drug to take part in the formation of the adaptive reserve of the intensity of the absorptive capacity of neutrophils in blood.

DOI: 10.37200/IJPR/V24I3/PR2020340
Received: 24 Feb 2020 | Revised: 28 Feb 2020 | Accepted: 18 Mar 2020

Such integral parameters as absolute phagocytosis and phagocyte number in the basic and stimulated conditions in pigs from all the groups had a tendency towards the increase in 2 months of the experimental period, which was, probably, associated with a vaccination performed 6 days prior. In 1 month of the experimental period, pigs from Group III had an adaptive reserve ($p<0.05$) of the phagocyte number (by 10.38% higher in the stimulated conditions than in the basic) and pigs from Groups I and II did not have it, which indicated a positive influence of the drug used by the scheme 7 days of application and 14 days of interruption on the formation of an adaptive reserve of the neutrophil capacity to absorb foreign matters. The increase in the phagocytic activity and phagocytic number after probiotic consumption was also observed by other researchers (Fedyukh et al., 2016; Wojcik, 2014).

The evaluation of the readiness to the reactions that restore homeostasis can be performed by the NBT-test, which is based on the oxygen-dependent microbicide activity, which is a way of utilization of oxygen by neutrophils with the formation of free radicals and hydrogen peroxide. This can be done without additional stimulation (spontaneous or basic NBT-test) or with stimulation of neutrophils *in vitro* (induced or stimulated NBT-test) (Mazurikh and Mikhailov, 1999). In healthy animals, both spontaneous and induced NBT activity in neutrophils is significantly higher than in the diseased (Ermolina et al., 2008).

An increase in the number of NBT-positive neutrophils in the basic and stimulated conditions in pigs from Group I by 29.79 and 86.01% ($p<0.05$), respectively, in 1 month after the end of the drug application in comparison with the initial period and an earlier increase in such cells in blood of pigs from Groups II and III (in 2 months of the experimental period by 35.42 and 34.04%, $p<0.05$, respectively, in the basic conditions in comparison with the previous period, and in the stimulated conditions - by 28.26 and 18.13%, $p<0.05$, respectively, in comparison with the previous period) indicated a higher reactivity of neutrophils in blood in terms of the expression of oxygen-dependent microbicide activity in animals that received the drugs by both schemes.

Indication of the drug by both schemes led to a higher intensity of the functioning of oxidase mechanisms of neutrophils, which is indicated by a higher index of activation of neutrophils (NAI) in pigs from Groups II and III in the basic conditions by 50.00 and 41.67%, respectively, already in 1 month of the experimental period, and in the stimulated conditions – by 36.36 and 31.82%, respectively, in 2 months of the experimental period, than in the pigs from the control group.

Relative number of NBT-positive neutrophils and NAI in the conditions stimulated by zymosan in pigs from all the experimental groups during all the periods of the study were significantly higher than the analog parameter values in the basic conditions by 154.16….301.58 and 225….575.00%, which indicated both the presence of adaptive reserve of this protective mechanism and its intensive character (due to a more intensive increase in NAI in the stimulated conditions in comparison with the basic conditions).

The level of oxygen-independent microbicide activity of neutrophils in blood was evaluated by the mean cytochemical coefficient (MCC) that is characterized by the content of cationic proteins. These proteins are synthesized at the stage of myeloblasts in azurophilic granules. The level of MCC does not depend on the stimulation of the cell and is defined by the amount of the substance synthesized in the process of granulocytosis. Thus, in cows with microelement insufficiency, MCC was 0.86 – 0.95, and in healthy animals – from 1.29 to 1.48

DOI: 10.37200/IJPR/V24I3/PR2020340
Received: 24 Feb 2020 | Revised: 28 Feb 2020 | Accepted: 18 Mar 2020

[44], MCC in neutrophils of healthy dogs – from 0.99±0.09, and in patients (with the 5th degree of chronic renal insufficiency) – 0.15±0.05 (Voronina et al., 2017). The content of cationic proteins in neutrophils decreases during disease (Nagaev et al., 1997), and an increase in the cationic proteins is often observed as a compensatory reaction in the conditions of the oxidase system insufficiency (Khaitov, 1995).

A significantly higher MCC (by 5.37%) in pigs from Group I indicate on the capacity of the drug to enhance the synthesis of cation proteins in neutrophils during the process of granulocytopoesis used by the scheme 7 days of application and 7 days of interruption.

V. CONCLUSION

Pigs from Groups II and III that received "EM-Vita" had an increase in the nonspecific protection in 2 months of the experimental period, which was indicated by a significantly higher number of neutrophils in animals from Groups II and III by 5.90 and 8.84%, increase in neutrophil count capable of oxygen-dependent microbicide activity in the basic and stimulated conditions, and the intensification of the oxidase systems of neutrophils in the conditions stimulated with zymosane by 130.77 and 112.20%, respectively, and the presence of adaptive reserve of the intensity of the absorption of foreign matter in 1 month of the experimental period.

The use of the feeding supplement "EM-Vita" for 2 months by the scheme 7 days of application and 7 days of interruption led to the enhancement of the synthesis of cation proteins in neutrophils during the process of granulocytosis.

The use of feeding supplement "EM-Vita" for 1 month by the scheme 7 days of application and 14 days of interruption led to the development of the adaptive reserve of neutrophils capable of the absorption of foreign matters.

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