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EFFECT OF FENBENZYL AND FENBENDAZOLE ON THE ANTIOXIDANT STATUS IN DOGS DURING EXPERIMENTAL INFECTION WITH CAUSATIVE AGENT OF TOXOCARIASIS

***V. V. STYBEL*[1]**, *Doctor of Veterinary Sciences, Professor, Department of Parasitology and Ichtyopathology*
https://orcid.org/0000-0002-0285-6182
***B. V. GUTYJ*[1]**, *Doctor of Veterinary Sciences, Professor, Department of Pharmacology and Toxicology*
https://orcid.org/0000-0002-5971-8776
***W. S. SAID*[1]**, *Graduate Student, Department of Pharmacology and Toxicology*
http://orcid.org/0000-0002-3273-4143
***K. KUBIAK*[2]**, *Doctor Habilitatus, Professor, Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats*
https://orcid.org/0000-0001-9640-2597
***M. JANKOWSKI*[2]**, *Doctor Habilitatus, Professor, Department of Internal Medicine and Clinic of Diseases of Horses, Dogs and Cats*
https://orcid.org/0000-0002-2356-2511
***I. A. MAKSYMOVYCH*[1]**, *Candidate of Veterinary Sciences, Associate Professor, Department of Internal Animal Diseases and Clinical Diagnostics*
http://orcid.org/0000-0002-9012-0262
***Z. A. GUTA*[1]**, *Candidate of Veterinary Sciences*
https://orcid.org/0000-0002-5652-6939
***T. V. MARTYSHUK*[1]**, *Candidate of Agricultural Sciences*
https://orcid.org/0000-0002-8445-1794
***V. I. KARPOVSKYI*[3]**, *Doctor of Veterinary Sciences, Professor, Department of Animal Biochemistry and Physiology named after Academician M. F. Gulyi*
https://orcid.org/0000-0003-3858-0111
[1]Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, 50 Pekarska st., Lviv 79010, Ukraine
[2]Wrocław University of Environmental and Life Sciences, 25 Norwida st., Wrocław 50-375, Poland
[3]National University of Life and Environmental Sciences of Ukraine, 15 Heroyiv Oborony st., Kyiv 03041, Ukraine
E-mail: bvh@ukr.net

Abstract. *The article presents the results of studies of the effect of fenbenzyl and fenbendazole on the antioxidant status in dogs under the experimental infection with a causative agent of toxocariasis. Experiments were performed on 18 dogs, two and four months old. Three groups of six animals in each were formed: control and two experimental. Puppies in all groups were experimentally infected with causative agent of toxocariasis at a dose of 5.000 embryonated Toxocara canis eggs per kg of body weight. The control group of dogs was as untreated control. Puppies of the first experimental group were fed with the drug fenbendazole at a dose of 150 mg per 3 kg of body weight once a day for three days in a single dose. Puppies of the second experimental group were fed with the drug fenbenzyl at a dose of 350 mg per 3 kg of body weight once a day for three days. While studying the activity of the enzymatic part of the antioxidant defense system, namely catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase, as well as a non-enzymatic part: the level of reduced glutathione, an increase in the activity of these indicators was found in animals of experimental groups. It should be noted that the application of the drug fenbenzyl in infected dogs contributed to a better normalization of the antioxidant system in animals than the use of the drug fenbendazole. The use of fenbenzyl in infected dogs contributed to a more likely increase in the antioxidant status in dogs of the second experimental group, as this drug includes milk thistle that exhibits antioxidant properties due to the presence of silymarin, which restores damaged liver cells. Studies confirm the effectiveness of the use of milk thistle in drug fenbenzyl in dogs with the development of toxocariasis infection to activate the protective systems of their body.*

Keywords: *pollodoxin, doxycycline hyclate, broiler chickens, ornithobacteriosis, Ornithobacterium rhinotracheale, pharmacokinetic parameters*

Introduction

The results of research and generalization of the literature indicate that among the parasitic diseases of dogs the most common in our country and abroad are gastrointestinal helminthiases, among which the leading place has toxocariasis – nematode infection from the order *Ascaridida* (Pryima, 2010; Bodnia, 2016; Ozlati et al., 2016; Zibaei & Sadjjadi, 2017).

It is known that the intestinal form of the disease is caused by mature Toxocara, and the larvae – visceral. In the process of migration, larvae, as well as their metabolites, can cause severe multiorgan damage up to death (Moisieieva et al., 2017; Said et al., 2018; Stybel et al., 2021). However, some mechanisms of activation of lipid-free radical oxidation processes in the development of toxocariasis in dogs and their relationship with the body's defense systems, especially the immune system, which is closely related to the antioxidant defense system in animals, remain unclear.

Toxocara plays a significant role in stimulating the formation of free radical oxidation and imbalance between oxidant and antioxidant content with the subsequent development in animals of so-called oxidative stress, including blood leukocytes, which are among the first to respond to changes in the environment under the influence of Toxocara metabolites (Robertson & Thomson, 2002; Vidal et al., 2003; Svirzhevska, 2011; Macuhova et al., 2013). An important role in the

development of oxidative stress in infected animals is the balance of prooxidant synthesis and antioxidant protection, the shift of this balance towards prooxidants causes compensatory activation of the antioxidant system from the damaging effects of free radicals and peroxide compounds (Martyshuk et al., 2016; Gutyj et al., 2017; Grymak et al., 2020).

The antioxidant defense system is a system responsible for regulating the intensity of radical formation and neutralization of peroxidation products (Martyshuk & Hutyi, 2021; Varkholiak et al., 2021). The main mechanism of these reactions control is related to the chain of reversible redox reactions of metal ions, ascorbate, tocopherol, glutathione, and other substances (Martyshuk et al., 2021). In addition, the importance of these methods is especially important for the preservation of long-standing macromolecules of nucleic acids and proteins, some components of membranes (Holovakha et al., 2018).

As a result of the application of anthelmintic drugs for treating animals with toxocariasis, side effects caused by the reaction of dogs to the death of Toxocara can occur, because the destruction of Toxocara also leads to the release of toxins (Rubinsky-Elefant et al., 2011; Zakharchuk & Harazdiuk, 2014; Noor et al., 2019; Said et al., 2020).

A wide range of anthelmintic drugs is used to treat dogs with toxocariasis. As a result of the destruction of parasites' bodies after the application of anthelmintics, somatic poisons and metabolites are released in the body of the host that cause intoxication and contribute to the reduction of its defense systems. The inclusion of milk thistle in the treatment of patients with toxocariasis allows to protect their body from the effects of parasite toxins and strengthen the immune and antioxidant potential.

Some authors have found a stimulating effect of milk thistle on the activity of antioxidant and hepatoprotective effects in animals (Toklu et al., 2008; Martyshuk & Gutyj, 2019; Martyshuk et al., 2021). However, the complex application of milk thistle and fenbendazole on the function of the liver and the protective systems of the dog's body is currently insufficiently covered in the scientific literature.

That is why the **purpose** of the study was to investigate the effect of fenbenzyl and fenbendazole on the antioxidant status in dogs during experimental infection with the causative agent of toxocariasis.

Materials and methods of researches

The work was performed during 2017–2020 at the Department of Parasitology and Ichthyopathology, Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv. The experiments were performed on 18 dogs, two to four months of age, and three groups of six animals were formed in each: control and two experimental groups. Puppies of all groups were experimentally infected with the causative agent of toxocariasis at a dose of 5.000 embryonated *T. canis* eggs per kg of body weight. The control group of dogs was as untreated control. Puppies of the first experimental group were fed with the drug fenbendazole drug at a dose of 150 mg per 3 kg of body weight once a day for three days in a single dose. Puppies of the second experimental group were fed with the drug Ffenbenzyl drug (TC U 00492990-027:2020 "The drug Fenbenzyl") at a dose of 350 mg per 3 kg of body weight once a day for three days.

The drug fFenbenzyl drug was developed at the Department of Pharmacology and Toxicology and the Department of Parasitology and Ich-

thyopathology of the Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies Lviv, which contains fenbendazole and milk thistle.

The state of the antioxidant defense system was assessed by the activity of catalase, superoxide dismutase, and indicators of the glutathione system in the blood. The activity of superoxide dismutase (SOD, EC 1.15.1.1) was determined by the method of Dubinina et al. (1983), catalase (CAT; EC 1.11.1.6) – by the method of Korolyuk (1988), glutathione peroxidase (GPx) (EC 1.11.1.9.) and glutathione reductase (GR) (EC 1.6.4.2.) – by the method of Lemeshko et al. (1985); the content of reduced glutathione (RG) – by the method of Butler (1963) (Vlizlo et al., 2012).

All animal manipulations were performed in accordance with the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986).

The analysis of research results was performed using the software package Statistica 6.0. The probability of differences was assessed by Student's t-test. The results of the mean values were considered statistically significant at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (ANOVA).

Results of the research and their discussion

It is established that with the development of toxocariasis in dogs, the activity of the antioxidant defense system is suppressed, which is indicated by a decrease in the activity of its enzymatic and non-enzymatic parts. On the basis of the conducted researches it is established that during experimental toxocariasis in dogs on days 20 and 25 of the experiment, catalase activity in blood of animals of the control group decreased by 35.3% compared towith the initial values. The lowest catalase activity was in blood of infected dogs on day 30 of the experiment, where it was 0.09 ± 0.06 mg H_2O_2, which was 47.1% lower than the initial values (Table 1).

It was found that catalase activity in animals of the first experimental group on the 5th day of the experiment increased to 0.17 ± 0.03 mg of H_2O_2 when fenbendazole was used in infected dogs. Subsequently, a decrease in the activity of this enzyme to 0.11 ± 0.04 mg of H_2O_2 was observed. It should be noted that on the 20th and 25th day of the experiment, catalase activity in blood of dogs of the first experimental group was higher by 27.3% compared with the control.

When using the drug Ffenbenzyl drug in infected dogs, an increase in catalase activity in their blood was found throughout the experiment. Thus, on the 15th and 20th days of the experiment, an increase in the activity of this enzyme by 23.1 and 63.6%, relatively, was found compared with dogs of the control group. On day 25 of the experiment, a slight decrease in catalase activity was observed in blood of dogs of the second experimental group compared with the previous day, but on day 30 of the experiment, again a high catalase activity was found in blood of dogs treated with fenbenzyl, where it increased almost 2 times compared with animals in the control group.

A decrease in superoxide dismutase activity in blood was also found with the development of toxocariasis in dogs, which at 20 and 25 days of the experiment decreased by 19.9 and 28.2% compared with the initial values taken before the infection with the causative agent of toxocariasis. On day 30 of the experiment, the activity of superoxide dismutase in blood of dogs of the control group was the lowest – 10.3 ± 0.64 IU/mg protein (Table 2).

1. The effect of fenbenzyl and fenbendazole on catalase activity in blood of dogs infected with toxocariasis (M ± m, n = 6)

Blood test time	Catalase activity, mg H_2O_2		
	group of animals		
	control	experimental 1	experimental 2
Before treatment	0.17 ± 0.05	0.15 ± 0.04	0.16 ± 0.05
Day 5	0.20 ± 0.04	0.17 ± 0.03	0.18 ± 0.05
Day 10	0.14 ± 0.06	0.15 ± 0.03	0.17 ± 0.04
Day 15	0,13 ± 0.05	0.15 ± 0.04	0.16 ± 0.05*
Day 20	0.11 ± 0.03	0.14 ± 0.06*	0.18 ± 0.04**
Day 25	0.11 ± 0.05	0.14 ± 0.03*	0.16 ± 0.02***
Day 30	0.09 ± 0.06	0.11 ± 0.04*	0.18 ± 0.05***

Note: statistically significant differences were considered compared with the control group: * P < 0.05; ** P < 0.01; *** P < 0.001.

The use of drugs fenbendazole and fFenbenzyl in dogs of the experimental groups contributed to the activation of superoxide dismutase activity in their blood, so on the 20th day of the experiment, the enzyme activity in dogs of the first experimental group increased by 17.6% and in the second experimental group – by 29.6% compared with the control. The highest superoxide dismutase activity was in blood of animals of the second experimental group on days 20 and 25 of the experiment, where it was 16.2 ± 1.10 and 16.0 ± 1.56 UI/mg protein, respectively, which is 42.9% higher than the values in dogs of the control group. On the 30th day of the experiment, it was found that in blood of dogs of the second experimental group, the enzyme activity increased by 54.4%, while in the first experimental group – by 37.9%.

As a result of research, it is established that the development of toxocariasis in dogs of the control group causes

2. The effect of fenbenzyl and fenbendazole on superoxide dismutase activity in blood of dogs infected with the causative agent of toxocariasis (M ± m, n = 6)

Blood test time	Superoxide dismutase, IU/mg protein		
	group of animals		
	control	experimental 1	experimental 2
Before treatment	15.6 ± 0.70	15.9 ± 0.65	15.7 ± 0.68
Day 5	17.1 ± 0.95	16.5 ± 0.59	16.9 ± 0.87
Day 10	15.2 ± 0.54	15.7 ± 0.60	16.0 ± 0.94
Day 15	14.4 ± 0.82	15.4 ± 0.47	15.9 ± 0,99*
Day 20	12.5 ± 0.86	14.7 ± 0.90*	16.2 ± 1.10**
Day 25	11.2 ± 0.64	14.4 ± 1.05**	16.0 ± 1.56***
Day 30	10.3 ± 0.64	14.2 ± 1.23**	15.9 ± 1.10***

Note: statistically significant differences were considered compared with the control group: * P < 0.05; ** P < 0.01; *** P < 0.001

a decrease in the level of reduced glutathione, one of the main indicators of the non-enzymatic part of the glutathione system of antioxidant protection. Thus, on the 15th and 20th days of the experiment, the level of reduced glutathione decreased by 17.4 and 21.7% compared with the initial values. On days 25 and 30 of the experiment in blood of animals of the control group, the level of the studied indicator continued to decrease to 0.31 ± 0.04 mmol/L (Table 3).

It should be noted that reduced glutathione is the main antioxidant for red blood cells, which plays the role of a coenzyme for methemoglobin reduction to functionally active hemoglobin. In addition, it involves the detoxification of several toxic compounds, as well as hydrogen peroxide and lipid hydroperoxides that are formed in the reactions of interaction of reactive oxygen species with unsaturated fatty acids of red blood cell membranes. Thus, reduced glutathione plays an important role in the maintaining functional characteristics of red blood cell membranes in dogs with the development of toxocariasis.

While studying the effect of fenbendazole and fenbenzyl on the level of reduced glutathione in blood of dogs infected with toxocariasis, it was found that its level in blood of the first experimental group on day 10 of the experiment was 0.43 ± 0.04, and the second experimental group – 0.45 ± 0.05 mmol/L, whereas in the control group this figure was significantly lower – 0.40 ± 0.04 mmol/L. On the 25th and 30th day of the experiment, we note an increase in the level of reduced glutathione in dogs of experimental groups, so in the first experimental group this figure increased by 18.2 and 29.0%, and in the second – by 42.4 and 45.2% compared with the control.

An equally important enzyme of the glutathione system of antioxidant protection is glutathione peroxidase, which catalyzes the reduction of hydrogen peroxide or organic hydroperoxides and consequently protects cells from the action of reactive oxygen species. The results of the studies showed that glutathione peroxidase activity decreased in blood of animals with the development of toxocariasis throughout the experiment. The lowest glutathione peroxidase activity was in blood of dogs of the control group on days 25 and 30 of the experiment, where it decreased by 23.9 and 26.7%, respectively, compared with baseline values (Table 4).

3. The effect of fenbenzyl and fenbendazole on the level of reduced glutathione in blood of dogs infected with toxocariasis (M ± m, n = 6)

Blood test time	Reduced glutathione, mmol/L		
	group of animals		
	control	experimental 1	experimental 2
Before treatment	0.46 ± 0.03	0.44 ± 0.04	0.47 ± 0.04
Day 5	0.42 ± 0.04	0.45 ± 0.05	0.46 ± 0.03
Day 10	0.40 ± 0.04	0.43 ± 0.04	0.45 ± 0.05
Day 15	0.38 ± 0.02	0.41 ± 0.03	0.45 ± 0.02*
Day 20	0.36 ± 0.03	0.40 ± 0.05*	0.46 ± 0.03**
Day 25	0.33 ± 0.05	0.39 ± 0.04*	0.47 ± 0.02**
Day 30	0.31 ± 0.04	0.40 ± 0.06**	0.45 ± 0.03***

Note: statistically significant differences were considered compared with the control group: * P < 0.05; ** P < 0.01; *** P < 0.001.

4. The effect of fenbenzyl and fenbendazole on glutathione peroxidase activity in blood of dogs infected with the causative agent of toxocariasis (M ± m, n = 6)

Blood test time	Glutathione peroxidase, μmol $NADPH_2$h/mg protein		
	group of animals		
	control	experimental 1	experimental 2
Before treatment	17.6 ± 2.47	17.8 ± 3.10	18.0 ± 2.85
Day 5	17.9 ± 2.99	18.0 ± 3.15	18.1 ± 2.89
Day 10	16.9 ± 2.85	17.5 ± 3.00	17.8 ± 2.75
Day 15	15.2 ± 3.15	16.0 ± 2.56	16.6 ± 3.05*
Day 20	14.6 ± 3.65	16.3 ± 2.68*	17.0 ± 3.15**
Day 25	13.4 ± 3.55	16.0 ± 3.62*	17.7 ± 2.45***
Day 30	12.9 ± 3.10	16.2 ± 2.85**	18.2 ± 2.59***

Note: statistically significant differences were considered compared with the control group: * P < 0.05; ** P < 0.01; *** P < 0.001.

During the treatment of animals with the development of toxocariasis with drugs fenbendazole and Ffenbenzyl, it was found that glutathione peroxidase activity on the 15th day of the experiment increased by 5.3 and 9.2% compared with the control. On the 20th day of the experiment, glutathione peroxidase activity in blood of dogs of the first experimental group increased by 11.6%, and the second experimental group – by 16.4% compared with the control. On the 25th day of the experiment, the enzyme activity in dogs of the experimental groups ranged from 16.0 ± 3.62 to 17.7 ± 2.45 μmol $NADPH_2$h/mg protein, while in the control this figure was 13.4 ± 3.55 μmol $NADPH_2$h/mg protein. On day 30 of the experiment, glutathione peroxidase activity was the highest in dogs of the second experimental group treated with Ffenbenzyl.

In the study of glutathione reductase activity, it was found that in dogs of the control group, its activity decreased by 21.1% on the 30th day of the experiment compared with the initial values (Table 5).

When using the drug fenbendazole in dogs of the first experimental group, on the 15th day of the experiment, an increase in glutathione reductase activity by 5.5% was found, on the 20th day of the experiment – by 9.3%, on the 25th day of the experiment – by 16.1% and on the 30th day of the experiment – by 12.4% compared with the control.

When treating dogs of the second experimental group with the drug fFenbenzyl, a more likely increase in glutathione reductase activity was found in comparison with the first experimental group. Thus, on the 10th and 15th day of the experiment, the activity of the enzyme in blood of dogs of the second experimental group increased by 4.0 and 8.8% in comparison with the control. Subsequently, the glutathione reductase activity continued to increase and, accordingly, on the 20th day of the experiment was 6.24 ± 1.17 μmol $NADPH_2$h/mg protein, while in the control this figure was 5.47 ± 1.21 μmol $NADPH_2$h/mg protein. On days 25 and 30 of the experiment, the enzyme activity was the highest in the second experimental group, where it increased by 20.9 and 27.3%, respectively, compared with the control.

Thus, the drug Ffenbenzyl after use in dogs with the development of experimental toxocariasis activated the antioxidant defense system, as indicated by the high content of reduced glutathione and the activity of enzymes of the antioxidant

5. The effect of fenbenzyl and fenbendazole on glutathione reductase activity in blood of dogs infected with toxocariasis (M ± m, n = 6)

Blood test time (days)	Glutathione reductase, μmol NADPH,h/mg protein		
	group of animals		
	control	experimental 1	experimental 2
Before treatment	6.37 ± 1.12	6.38 ± 1.08	6.40 ± 1.15
Day 5	6.15 ± 1.20	6.24 ± 1.23	6.30 ± 1.12
Day 10	6.01 ± 0.85	6.11 ± 1.10	6.25 ± 1.00
Day 15	5.82 ± 0.96	6.14 ± 1.20	6.33 ± 1.09
Day 20	5.47 ± 1.21	5.98 ± 1.11	6.24 ± 1.17
Day 25	5.21 ± 1.30	6.05 ± 1.22*	6.30 ± 0.98**
Day 30	5.02 ± 0.57	5.64 ± 0.98*	6.39 ± 1.10**

Note: statistically significant differences were considered compared with the control group: * P < 0.05; ** P < 0.01; *** P < 0.001.

system: catalase, superoxide dismutase, glutathione peroxidase, and glutathione reductase. This may be due to the fact that the drug contains milk thistle, which has antioxidant properties, as it contains vitamins B, A, E, K, precursors of vitamin D, carotenoids, macronutrients – Calcium, Potassium, Magnesium, Iron and trace elements – Zinc, Copper, Manganese, Iodine.

Conclusions and future perspectives

The positive effect of the drugs fenbendazole and fFenbenzyl on the indicators of the antioxidant defense system in blood of dogs experimentally infected with the with causative agent of toxocariasis was revealed. It should be noted that the use of the drug fFenbenzyl in infected dogs contributed to a better normalization of the antioxidant system than the use of the drug fenbendazole.

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Анотація. *У статті наведенні результати досліджень впливу фенбенсилу та фенбендазолу на антиоксидантний статус організму собак за експериментального інвазування збудником токсокарозу. Досліди проведено на 18 собаках, дво-чотиримісячного віку. Було сформовано три групи із шести тварин у кожній: контрольну та дві дослідні групи. Цуценят усіх груп експериментально заражали збудником токсокарозу в дозі 5000 інвазійних яєць T. canis на кг маси тіла. Контрольна група собак була в якості нелікованого контролю. Цуценятам першої дослідної групи згодовували препарат «Фенбендазол» у дозі 150 мг на 3 кг маси тварини один раз на добу впродовж трьох діб в одноразовій. Цуценятам другої дослідної групи згодовували препарат «Фенбенсил» у дозі 350 мг на 3 кг маси тварини один раз на добу впродовж трьох діб. Під час вивчення активності ензимної ланки системи антиоксидантного захисту, а саме каталази, супероксиддисмутази, глутатіонпереоксидази, глутатіонредуктази, а також неензимної ланки: рівня відновленого глутатіону, у тварин дослідних груп встановлено підвищення активності цих показників. Варто зазначити, що застосування препарату «Фенбенсил» інвазованим собакам сприяло кращій нормалізації показників антиоксидантної системи у тварин, ніж застосування препарату «Фенбендазол». Застосування інвазованим собакам препарату «Фенбенсил» сприяло вірогіднішому підвищенню антиоксидантного статусу організму собак другої дослідної групи, оскільки до цього препарату входить розторопша плямиста, яка проявляє антиоксидантні властивості завдяки наявності у своєму складі речовини силімарину, який відновлює пошкоджені клітини печінки. Проведені дослідження підтверджують ефективність застосування розторопші плямистої в складі препарату «Фенбенсил» собакам за розвитку токсокарозної інвазії для активізації захисних систем їхнього організму.*

Ключові слова: *паразитологія, фармакологія, собаки, антиоксидантна система, розторопша плямиста*

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MEAT QUALITY UNDER USE OF NATURAL FEED ADDITIVES IN YOUNG PIG FEEDING

S. A. TKACHUK*[1]*, *Doctor of Veterinary Sciences, Professor,*
Department of Veterinary Hygiene named after Professor A. K. Skorokhodko
https://orcid.org/0000-0002-6923-1793
ZBIGNIEW SOBEK*[2]*, *Doctor Habilitatus, Professor, Department of Genetics and Animal Breeding*
https://orcid.org/0000-0003-4115-4527
I. V. YATSENKO*[3]*, *Doctor of Veterinary Sciences, Professor,*
Department of Veterinary Sanitary Examination and Veterinary Forensic Medicine
https://orcid.org/0000-0001-8903-2129
L. V. TKACHYK*[1]*, *degree seeker, Department of Veterinary Hygiene named after Professor A. K. Skorokhodko*
https://orcid.org/0000-0002-9136-2925
L. B. SAVCHUK*[4]*, *Candidate of Agricultural Sciences, Associate Professor, Department of Normal and Pathological Physiology and Morphology*
https://orcid.org/0000-0002-5720-3533
[1]National University of Life and Environmental Sciences of Ukraine, 15 Heroyiv Oborony st., Kyiv 03041, Ukraine
[2]Poznan University of Life Sciences, Wojska Polskiego 28, 60-624 Poznań, Poland
[3]Kharkiv State Zooveterinary Academy, 1 Academic st., Mala Danylivka 62341, Kharkiv Oblast, Ukraine
[4]State Agrarian and Engineering University in Podilia, 13 Shevchenko st., Kamianets-Podilskyi 32316, Khmelnytsky region, Ukraine
E-mail: ohdin@ukr.net, yacenko-1971@ukr.net, tkluz78@gmail.com, lyuba.savchuk.2015@ukr.net

Abstract. *The total tasting score of meat (23.83 ± 0.31 points) and broth (23.90 ± 0.24 points) from the experimental group of pigs fed with Lg-max feed additive at a dose of 2.0 g/day was higher than in the control (22.33 ± 0.38 points) and meat taste in this group was significantly higher by 13.95% (P < 0.01) compared with the control. In contrast, in the meat of pigs fed with Lg-max feed additive at a dose of 4.0 g/day, the indicator of appearance, smell and taste was significantly lower by 17.39% (P < 0.01), 23.40 (P < 0.01) and 13.9% (P < 0.05), respectively, compared with the control. The appearance of meat from pigs fed Lg-max 2.0 g/day with Sel-Plex was significantly lower by 10.87% (P < 0.05) compared to the control.*

According to the indicators of the tasting evaluation of the broth from the meat of pigs in the experimental groups, no statistically significant difference was found with those in the

control, which may indicate a positive effect of the studied feed additives on the organoleptic characteristics of pork. However, the odor of the broth (in the meat of pigs fed with Lg-max feed additive at a dose of 4.0 g/day) was significantly lower by 20.8% (P < 0.01), and the taste (meat from pigs fed with Lg-max feed additive at a dose of 2.0 g/day together with Sel-Plex) – by 21.74% (P < 0.05) compared with the control.

According to microscopic and biochemical parameters, pork from pigs of experimental and control groups met the requirements of current regulations for fresh meat obtained from healthy animals.

Keywords: *pork, microscopic and biochemical parameters, tasting, Lg-max and Sel-Plex feed additives*

Introduction

Clarification of problematic issues in modern pig farming is necessary to establish the compliance of keeping technologies with the biological characteristics of animals and the quality of food products. In addition, the obtained food products must meet the strict requirements of European legislation, and therefore it is necessary to study the quality of meat and fat products, which are produced by traditional technologies by domestic producers (Remizova, 2016).

Ensuring quality control of meat is possible through an integrated approach to this problem solving, with a comprehensive study that should be aimed at identifying organoleptic and physicochemical parameters in the food product. All indicators are defined by normative documents focused on international and European standards (Havrylenko et al., 2017).

Analysis of recent researches and publications

Pork quality is assessed by the following indicators: appearance, color, pH, water-holding capacity, tasting parameters (Boles et al., 1998). These indicators depend on various factors: breed, feeding ration, which in turn affected the chemical composition of pork, microscopic, biochemical parameters, and sensory evaluation (Moeller et al., 2010; Hoa et al., 2019).

At the same time, the feeding ration is one of the main factors influencing the quality of pork. In recent years, various feed additives have been used in pork production (Han et al., 2000; Chae et al., 2002).

Currently, the main purpose of the use of various feed additives is to obtain maximum productivity, increase feed efficiency and ensure a high level of balanced feeding and improve food consumption. According to the analysis of the scientific literature, it follows that modern producers use different types of feed additives in pig feeding. Many of them are produced in the USA, England, France, and other countries and they belong to flavoring and aromatic substances, enzyme preparations, probiotics, etc.

One of the most important parameters of the nutritional value of feed is its energy level, which should not only provide physiological processes to maintain the body's vital functions, but also animal growth and development. It is known that the main energy sources are starch from cereals and fats from oilseeds. The choice of fat source for pigs is determined by the price of this raw material and in most cases very rarely pays attention to the composition of the fat source, namely, which fatty acids make up the oil or fat used in the diet. After all, fat is primarily perceived as

a source of energy (Schönfeld et al., 2016; Zheng et al., 2021).

At the same time, preference is given to natural feed additives, in particular, research conducted over the past 5–10 years to address the issue of the optimal ratio of omega-6 to omega-3 polyunsaturated fatty acids, which is important for maintaining homeostasis of biological processes and metabolism in animals and humans and affects meat quality. Research results are in improving meat chemical composition, extension its storage time, and reducing the ratio of omega-3 to omega-6 polyunsaturated fatty acids. It is known that the optimal ratio of Omega-3 to Omega-6 polyunsaturated fatty acids from various sources is from 1:2 to 1:4. While in the diet of most modern people this ratio is about 1:20 to 1:30 (Jaturasitha et al., 2001; Yefremov et al., 2012).

Among the essential polyunsaturated fatty acids, in particular, the family of Omega-6 or Omega-3, have the ability to counteract cardiovascular, neurodegenerative diseases, and metabolic disorders, contribute to reducing blood cholesterol. Omega-3 polyunsaturated fatty acid derivatives can act as signaling molecules by modulating the anti-inflammatory response and controlling cellular processes involved in programmed cell death (apoptosis), lymphocyte proliferation, inhibition of inflammatory cytokines, and phagocytosis. Because omega-6 polyunsaturated fatty acids are involved in the regulation of eicosanoid synthesis, they control the activity of the immune system (Gjerlaug-Enger et al., 2015; Shvediuk et al., 2017; Midyk et al., 2018).

In this scientific study, Lg-max and Sel-Plex feed additives were used in the feeding of young pigs. The Lg-max feed additive contains algae *Schizochytrium limacinum* and *Rosmarinus officinalis* rosemary extract and is a source for replenishment of animal omega-3 polyunsaturated fatty acids, namely docosahexaenoic acid, currently used for dogs and cats. Therefore, for the first time, our research proved the need to use Lg-max feed additive in different amounts in the main diet of fattening pigs.

In addition, the Sel-Plex feed additive, as a source for organic form of selenium, is widely used in pig feeding but mostly to improve reproductive quality. The organic form of selenium in comparison with inorganic (sodium selenite) has several significant advantages. Sel-Plex contains 1000 mg/kg of selenium, more than 98% of which is represented by selenomethionine and selenocysteine, which are biologically active forms of this trace element found in nature (wheat, soybeans, etc.). It has a higher availability, especially under stress, and is not an oxidant, unlike selenite.

Purpose – to determine the sensory, microscopic and tasting parameters of pork after adding Lg-max and Sel-Plex feed additives to the pig diet.

Materials and methods of research

The material for the study was young pigs of meat and fat breed (Landrace × Large White) and muscle tissue samples from the longest back muscle (*m. longissimus dorsi*) in pigs, taken at the level of 10–12 thoracic vertebrae at slaughter at the end of the experimental period.

The experimental groups were formed from young castrated male pigs.

To conduct the experiment, after a 15-day equalization period, 4 groups of analogs by origin, age, and live weight were formed (5 animals in the control and experimental groups). There were 5 pigs in the pen (Table 1). The following periods of pig breeding are used in the experimental farm: suckling period – 28 days; growing period – 30–90 days; fattening – 90–180 days.

Feed additives for animals of the experimental group were administered as

1. Scheme of scientific experiment

Group of animals	Number of animals	Experimental period		
		equalizing	growing	fattening
Control	5	Main diet (MD)	MD	MD
1st experimental (E_1)	5		MD + 2.0 g Lg-max	MD + 2.0 g Lg-max
2nd experimental (E_2)	5		MD + 4.0 g Lg-max	MD + 4.0 g Lg-max
3rd experimental (E_3)	5		MD + 2.0 g Lg-max and Sel-Plex (0.5 mg/kg).	MD + 2.0 g Lg-max and Sel-Plex (0.5 mg/kg).

part of a premix to feed, taking into account the needs of animals in Omega-3 polyunsaturated fatty acids (daily requirement is 672 mg). Experimental feed additive contains 353 mg of Omega per 1 g.

During the entire study period, the animals were fed twice a day with dry combined fodder and water ad libitum. Feed was weighed at each feeding of the pigs, and their actual consumption was taken into account on a daily basis.

The sensory evaluation of pork was performed according to the following indicators: color, flavor, texture, condition of tendons and fat (DSTU 4823.2:2007).

The pH value of meat was determined by the potentiometric method using a pH meter-150 according to (DSTU ISO 2917-2001) 24 hours after the slaughter of animals.

The content of amino-ammonia nitrogen in mg per 10 cm^3 of meat-water extract in pork was determined according to Sofronov. The content of primary degradation products of proteins in the broth was determined by the reaction with copper sulfate in the broth and the determination of volatile fatty acids by (GOST 23392-2016).

Results of the research and their discussion

Evaluation of pork quality began after slaughter by sensory evaluation of pig carcasses. The pigs were Landrace × Large White (meat and fat). Thus, the sensory evaluation confirmed the fresh degree of meat and that it was obtained from healthy animals, namely: the smell on the surface of the carcasses was pleasant, specific, the color of the meat was pale pink, the carcasses were well bled. The cut of the meat was dense, elastic; the hole formed when pressed with a spatula was quickly leveled. The muscles were slightly moist, leaving no wet spots on the filter paper. The fat was white, the texture was soft and elastic. The tendons were elastic, dense, the surface of the joints smooth.

Post-mortem inspection and trichinoscopy of the meat confirmed that the carcasses of pigs were obtained from healthy animals.

Subsequently, in order to confirm compliance with the requirements for pre-slaughter aging, the slaughter of animals and the fact that the meat is fresh and obtained from healthy pigs, laboratory studies were performed: microscopy of pork smears to determine the number of microbial cells in the field of view of the microscope ulcer tissue; reaction with copper sulfate in the broth, the essence of which is the precipitation of proteins by heating, the formation in the filtrate of complexes of copper sulfate with the products of primary decomposition of proteins

that precipitate; the content of volatile fatty acids by a method based on the isolation of volatile fatty acids that may accumulate in the meat of sick animals, and determining their amount by titration with a solution of sodium hydroxide obtained distillate; amino-ammonia nitrogen content, which is the most characteristic and constant sign of spoilage of meat and the presence of possible diseases of pigs.

According to the research results presented in Table 2, it follows that all these indicators correspond to the regulatory documents. For some of the indicators obtained, a statistically significant difference is observed.

Microscopy of smears-imprints obtained from meat should not reveal microbial cells or there are up to 10 of them in the field of view of the microscope; by reaction with copper sulfate, the broth should be clear; content of volatile fatty acids – up to 4.0 mg KOH; amino-ammonia nitrogen content – up to 1.26 mg; The pH of fresh meat and that obtained from healthy animals should be in the range of 5.6 to 6.2; reaction to peroxidase – positive.

According to the results of Kravchuk's research, the reaction with copper sulfate should be used as an additional method in the comprehensive assessment of meat quality, and the determination of volatile fatty acids – as arbitration, together with bacterioscopy of smears (Kravchuk, 2009).

Thus, microscopy of the smears did not show any traces of muscle tissue breakdown, the smears stained poorly. However, the number of microbial cells in the field of view of the microscope in E_2 and E_3 groups was significantly higher by 83.3 ($P < 0.01$) and 80.4% ($P < 0.05$), respectively, compared with the control. In the E_1 group, a tendency to increase the number of microbial cells in the field of view of the microscope was found compared with the control. Thus, the pork samples from pigs in the experimental groups corresponded to fresh meat obtained from healthy animals. Although, in E_2 and E_3 groups, there was a significant increase in microbial cells compared to the control.

Table 2 shows that the content of volatile fatty acids in pork from pigs of the E_2 and E_3 groups significantly increased by 23.2 ($P < 0.05$) and 16.8% ($P < 0.01$), respectively, compared with the control. In E_1 group found a tendency

2. Microscopic and biochemical parameters of pork (M ± m, n = 5)

Indicator	Control	Experimental group		
		E_1	E_2	E_3
Smear microscopy, the number of microbial cells	2.40 ± 0.51	2.60 ± 0.40	4.40 ± 0.51**	4.33 ± 0.42*
The content of volatile fatty acids, mg KOH	1.25 ± 0.01	1.31 ± 0.03	1.54 ± 0.11*	1.46 ± 0.05**
The content of amino-ammonia nitrogen, mg	0.80 ± 0.07	0.89 ± 0.06	1.00 ± 0.04*	0.90 ± 0.06
PH value	5.76 ± 0.07	5.78 ± 0.08	5.94 ± 0.04	5.80 ± 0.05
Reaction with copper sulfate	the broth is transparent	the broth is transparent	the broth is transparent	the broth is transparent
Reaction to peroxidase	positive (+)	positive (+)	positive (+)	positive (+)

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with the control of the corresponding age.

to increase the number of microbial cells in the field of view of the microscope compared with the control. A slight increase in volatile fatty acids in the meat from pigs of the E_2 and E_3 may be due to an increase in microbial contamination of the carcasses, as well as an increase in the amount of subcutaneous fat.

It is known that volatile fatty acids can be formed from both lactic acid and amino acids by deamination. In addition, volatile fatty acids are formed during the breakdown of lipoids. Researchers have studied the role of volatile fatty acids in various physiological and pathophysiological conditions but some of them indicate the possibility of using volatile fatty acids as biochemical markers for diagnosing several diseases (Nezghoda et al., 2019).

One of the chemical reactions that indicates the formation of amino compounds and ammonia bases is a determination of amino-ammonia nitrogen content in pork. Thus, in the E_2 group, the content of amino-ammonia nitrogen was significantly higher by 25.0% ($P < 0.05$) compared with the control. In the E_1 and E_3 groups, a tendency to increase volatile fatty acids was found compared with the control. It can be assumed that the amount of amino-ammonia nitrogen in pork from pigs of the experimental group that received 4.0 g/day of Lg-max feed additive is related to the content of volatile fatty acids and the number of microbial cells.

One of the most important factors characterizing the quality of pork is acidity (pH). Because the concentration of hydrogen ions in meat depends on the content of glycogen and lactic acid in the muscles at the time of slaughter and, as a consequence, is a derivative of the physiological state in animals before slaughter, and reflects the course of postmortem processes in the carcass. Closely related to this indicator are color, water-holding capacity, tenderness, and other meat quality indicators. Due to the processes of post-mortem glycolysis, the pH shifts to the acidic side until a final value specific to each type of meat. In pork, the final pH value is reached after 24 hours and is from 5.6 to 6.4 under normal conditions. The pH value is influenced by stressful situations that occur in animals before slaughter (predominance, transportation, physical, mental stress, high temperatures, etc.). All these factors cause stress in animals that induces increased adrenaline secretion and promotes the breakdown of glycogen in the liver (Teuteberg et al., 2021).

In addition, Kasyanchuk & Bogatko (2017) noted that meat obtained from animals with dystrophic changes in the liver or kidneys has higher pH (6.2 ± 0.1) than meat obtained from healthy animals (5.6–5.7). At pH values up to 5.4 and below, conditions are created for the survival and reproduction of gram-negative bacteria, most strains of yeast and fungi (Bohatko, 2017).

According to the reaction on determination the acidity of pork in the control and experimental groups, the pH value ranged from 5.76 to 5.80, which is within the normal range for fresh meat, matured (after slaughter 24 hours) and obtained from healthy animals.

In reaction with copper sulfate, the obtained broth was transparent, indicating that when the minced meat was heated in a boiling water bath, no primary breakdown products of meat proteins (peptones, polypeptides) were formed.

The reaction on the presence of peroxidase (an enzyme of protein nature) was positive, which indicates a sufficiently high activity of this enzyme. Confirmation of a positive reaction is

3. Tasting evaluation of pork (cooking test), points (M ± m; n = 5)

Indicator	Control	Experimental group		
		E_1	E_2	E_3
Appearance	4.60 ± 0.19	4.40 ± 0.24	3.80 ± 0.12**	4.10 ± 0.10*
Color	4.30 ± 0.20	4.50 ± 0.22	4.30 ± 0.12	4.80 ± 0.12
Flavor	4.70 ± 0.20	4.80 ± 0.13	3.60 ± 0.10**	4.30 ± 0.20
Taste	4.30 ± 0.12	4.90 ± 0.10**	3.70 ± 0.20*	4.50 ± 0.16
Consistence	4.60 ± 0.19	4.80 ± 0.20	4.70 ± 0.20	4.20 ± 0.12
Succulence	4.30 ± 0.25	4.70 ± 0.20	4.90 ± 0.10	4.20 ± 0.12
Total score	22.33 ± 0.38	23.83 ± 0.31	20.83 ± 1.12	21.75 ± 0.53

Note: * P < 0.05; ** P < 0.01; *** P < 0.001 compared with the control of the corresponding age.

the appearance of blue-green color, which turns brown. This indicates that the peroxidase decomposes oxygen peroxide with the release of oxygen, which oxidizes benzidine. This produces para-quinone diamide, which with underoxidized benzidine gives a blue-green compound that turns brown.

Thus, the qualitative reactions can be used to state that pig slaughtering technology is followed, as well as that the meat is obtained from healthy animals.

At the same time, we conducted a tasting evaluation of meat, which is an important indicator of its quality. In several scientific studies, meat tasting is used to possibly identify differences between species, lines and crosses, particularly in poultry (Kucheruk, 2018) and in pigs (Birta et al., 2010; Novgorodskaya, 2014).

Researchers point to differences in the sensory and tasting characteristics of pig meat after slaughter, which depend on different stress sensitivities. Meat obtained from stress-sensitive pigs has low consumer properties and autolytic processes are slower (Lykhach et al., 2016). In addition, the smallest share of stress-sensitive animals was in the group of landrace animals (Vashchenko, 2017).

Tasting evaluation of meat (from the longest back muscle) in pigs, as well as meat broth, was performed by a 5-point scale. The meat samples taken for tasting had the same size and temperature according to the current regulations (Table 3).

According to the results shown in Table 3, it follows that the appearance of pig meat from the E_2 group was significantly lower by 17.39% (P < 0.01), and E_3 – by 10.87% (P < 0.05), compared with the indicator in the control.

At the same time, in the E_2 group, the flavor and taste indicators were significantly lower by 23.4 (P < 0.01) and 13.9% (P < 0.05), respectively, compared to the control.

However, the meat taste index in the E_1 group was significantly higher by 13.95% (P < 0.01) compared to the control. However, for the rest of the tasting indicators of pork (in E_1 group), there is a tendency to increase compared to the control.

Table 4 presents the results of the tasting evaluation of the broth. Thus, the flavor of broth in the E_2 group was significantly lower by 20.8% (P < 0.01), and the taste in the E_3 group – by 21.74% (P < 0.05) compared with the control.

However, most of the parameters by the tasting evaluation of the broth from the meat of the experimental pigs did not have a statistically significant difference with the control, which may indicate a

4. Tasting evaluation of pork broth, points (M ± m; n = 5)

Indicators	Control	Experimental group		
		E_1	E_2	E_3
Appearance	4.20 ± 0.24	4.60 ± 0.24	4.80 ± 0.20	4.20 ±0.37
Flavor	4.80 ± 0.20	4.80 ± 0.20	3.80 ± 0.21**	4.00 ± 0.32
Taste	4.60 ± 0.24	4.90 ± 0.10	4.60 ± 0.25	3.60 ± 0.24*
Transparency	4.20 ± 0.37	4.80 ± 0.20	4.80 ± 0.20	4.40 ± 0.24
Richness	4.80 ± 0.20	4.80 ± 0.12	4.20 ± 0.37	4.60 ± 0.24
Total score	22.60 ± 0.87	23.90 ± 0.24	21.40 ± 1.12	20.80 ± 0.86

Note: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ compared with the control of the corresponding age.

positive effect of the studied feed additives on the sensory characteristics of pork.

No statistically significant difference was found between the indicators of the total score, but in the E_1 group this indicator was slightly higher than in the other experimental groups.

Therefore, according to the results of the tasting analysis, it can be stated that the tasting values of meat and broth from pigs in the E_1 group were higher compared to control samples, apparently due to increased content of extractives and free amino acids in it under the influence of feed additives in different doses on nitrogen and lipid metabolism in the body of pigs.

Thus, according to the tasting evaluation of pork and broth, the best quality meat is obtained from pigs fed Lg-max at a dose of 2.0 g/day.

Conclusions and future perspectives

The total tasting score of meat (23.83 ± 0.31 points) and broth (23.90 ± 0.24 points) from pigs of the experimental group fed with Lg-max feed additive at a dose of 2.0g/day was higher than in the control (22.33 ± 0.38 points) and the meat taste in this group was significantly higher by 13.95% ($P < 0.01$) compared with the control. In contrast, in the meat of pigs fed with Lg-max feed additive at a dose of 4.0 g/day, the indicator of appearance, smell and taste was significantly lower by 17.39% ($P < 0.01$), by 23.40 ($P < 0.01$) and 13.9% ($P < 0.05$), respectively, compared with the control. The appearance of meat from pigs fed with Lg-max 2.0 g/day with Sel-Plex was significantly lower by 10.87% ($P < 0.05$) compared to the control.

According to the indicators of the tasting evaluation of the broth from the meat of pigs in the experimental groups no statistically significant difference was found with those in the control, which may indicate a positive effect of the studied feed additives on the organoleptic characteristics of pork. However, the favor of the broth (in the meat of pigs fed with Lg-max feed additive at a dose of 4.0 g/day) was significantly lower by 20.8% ($P < 0.01$), and the taste (meat from pigs fed with Lg-max feed additive at a dose of 2.0 g/day together with Sel-Plex) – by 21.74% ($P < 0.05$) compared with the control.

According to microscopic and biochemical parameters, pork from pigs in the experimental and control groups met the requirements of current regulations for fresh meat obtained from healthy animals.

Further research concerns the histological examination of the liver in young pigs after the use of different amounts of feed additives.

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Анотація. *Загальна дегустаційна оцінка м'яса (23,83 ± 0,31 балів) і бульйону (23,90 ± 0,24 балів) від свиней дослідної групи, яким згодовували кормову добавку Lg-max 2,0 г/добу, була вище, ніж у контролі (22,33±0,38 балів) і показник смаку м'яса цієї групи був достовірно більшим на 13,95% (P<0,01), порівнюючи з показником у контролі. Натомість, у м'ясі свиней, яким згодовували кормову добавку Lg-max 4,0 г/добу, показник зовнішнього вигляду, запаху та смаку був достовірно меншим відповідно на 17,39% (P < 0,01), 23,40% (P < 0,01) і 13,9% (P < 0,05), порівнюючи з контролем. Показник зовнішнього вигляду м'яса свиней, яким згодовували кормову добавку Lg-max 2,0 г/добу разом із Сел-Плексом був достовірно меншим на 10,87% (P < 0,05), ніж у контролі.*

За показниками дегустаційної оцінки бульйону з м'яса свиней дослідних груп не встановили статистично значущої різниці із такими в контролі, що може свідчити про позитивний вплив досліджуваних кормових добавок на органолептичні показники свинини. Однак, показник запаху бульйону (у м'ясі групи свиней, яким згодовували кормову добавку Lg-max 4,0 г/добу) був достовірно меншим на 20,8% (P < 0,01), а смаку (у м'ясі свиней, яким згодовували кормову добавку Lg-max 2,0 г/добу разом із Сел-Плекс) – на 21,74% (P < 0,05), порівнюючи з показниками в контролі.

За мікроскопічними й біохімічними показниками свинина від свиней дослідних і контрольної груп відповідала вимогам чинних нормативних документів щодо свіжого м'яса, отриманого від здорових тварин.

Ключові слова: *свинина, мікроскопічні та біохімічні показники, дегустація, кормові добавки Lg-max і Сел-Плекс*

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PHYSICOCHEMICAL AND MICROBIOLOGICAL EXAMINATION OF RAW MILK

O. M. YAKUBCHAK, *Doctor of Veterinary Sciences, Professor, Department of Veterinary Hygiene named after Professor A. K. Skorokhodko*
https://orcid.org/0000-0002-9390-6578
T. V. TARAN[1], *Candidate of Veterinary Sciences, Associated Professor, Department of Veterinary Hygiene named after Professor A. K. Skorokhodko*
https://orcid.org/0000-0002-9370-8539
V. O. USHKALOV, *Doctor of Veterinary Sciences, Professor, Ukrainian Laboratory of Quality and Safety of Agricultural Products*
http://orcid.org/0000-0001-5694-632X
S. V. MIDYK, *Candidate of Veterinary Sciences, Research Department for Monitoring the Safety of Agricultural Products, Ukrainian Laboratory of Quality and Safety of Agricultural Products*
http://orcid.org/0000-0002-2682-2884
K. O. BERLOUS, *Undergraduate*
http://orcid.org/0000-0002-9846-0202
National University of Life and Environmental Sciences of Ukraine, 15 Heroyiv Oborony st., Kyiv 03041, Ukraine
E-mail: olga.yakubchak@gmail.com; ttaran@ukr.net; ushkalov63@gmail.com; svit.mid@gmail.com

Abstract. *Ukraine is actively implementing safety legislation and certain indicators of food quality, in particular, raw milk, to the requirements of the European Union. Modern requirements for raw milk require careful analysis of hygienic indicators. Raw milk materials supplied to "Bila Tserkva Dairy Plant" LLC and dairy plant in PJSC "Vita" of Kyiv Region were studied. The count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM) and the species composition of milk microflora, in particular, bacteria of the genus Salmonella, Staphylococcus aureus, Listeria monocytogenes, bacteria of Escherichia coli group, psychrotrophic and spore-forming microorganisms were determined by microbiological methods. Physicochemical methods were used to determine: density, mass fraction of dry matter, somatic cell content, acidity, purity group, mass fractions of protein and fat. According to research results, the quality of farm milk is in an order of magnitude better than milk obtained from private households, in particular, by MAFAM count. The technology of obtaining farm milk ensures its production of extra and first grades, while milk obtained in the conditions of private households – the first grade and non-grade. According to physicochemical parameters, milk obtained under different conditions did not differ significantly. Microbiological parameters differed significantly. The average count of MAFAM in the milk from*

private households was 4361.25 ± 241.15, which is 12.6 times higher than MAFAM count even in milk of the first grade produced by a dairy farm. Irrespective of the season and conditions of raw milk production, all tested samples met the requirements of the current DSTU for the absence of bacteria of the genus Salmonella in 25 cm³, Staphylococcus aureus in 0.1 cm³, and Listeria monocytogenes in 25 cm³. Bacteria of the Escherichia coli group were not detected in farm milk during the year, in contrast to milk from private households, where they were detected in spring and autumn (20% of cases). Both in farm and milk from private households, a group of mesophilic microorganisms prevailed over spore-forming and psychrotrophic ones. However, their number was different. Prospects for further research are to determine the sources of entering various types of microorganisms in raw milk and to develop procedures to eliminate the possibility of milk contamination with foreign microflora.

Keywords: *raw milk, farm milk, milk from private households, milk microflora, quality parameters*

Introduction

Ukraine is currently undergoing the necessary reforms to approximate regulations on food safety and quality, in particular milk and dairy products, in accordance with the Association Agreement with the European Union. Ensuring the proper quality and safety of raw milk and dairy products is especially important for the domestic consumer, as well as for the further promotion of Ukrainian food products to the European Union market ([1]Kondrasii et al., 2016; Ministry of Agrarian Policy and Food, 2019). Of course, a rational and logical way to ensure the safety and quality of raw milk, and in the future – dairy products is to prevent their contamination by foreign substances and microorganisms on farms (CAC/RCP 57, 2004).

It should be noted that microorganisms that enter the milk differ in type, multiplication activity, and metabolism and are a factor that limits milk shelf life (Oliveira et al., 2011; Bohnlein et al., 2021). This phenomenon in the dairy industry also limits the export of dairy products (Chaharovskyi, 2020; Ukrinform, 2020).

Analysis of recent researches and publications

In 2018, a new national standard DSTU 2662:2018 "Raw cow's milk. Technical conditions" was developed, it was enacted on September 1, 2019 (2018). However, currently, it is almost impossible to meet the requirements of the standard for microbiological parameters, especially on farms that use outdated raw milk production technologies and do not always comply with modern hygiene requirements and private farms, and the share of such raw milk is significant for some facilities (Pronko et al., 2020). The new standard aims to increase milk quality requirements and regulate the use of low-grade milk for certain purposes, namely, for the production of non-food products, such as animal feed or technical casein (Ministry of Agrarian Policy and Food, 2019). However, such products can be produced by a very small share of market operators, and therefore often raw milk that does not meet the requirements is processed into dairy products, which increases the risk not only of defects but also diseases in consumers. Requirements

of current regulations apply to operators only and are not related to individuals who produce milk for their consumption. As a result, according to the current requirements, producers must introduce good practices in the production, processing, and marketing of milk and dairy products ([2]Kondrasii et al., 2016; Ministry of Agrarian Policy and Food, 2019).

It should be noted that the count of mesophilic aerobic and facultative anaerobic microorganisms (MAFAM) in milk is one of the most important indicators not only of its quality but also safety. This indicator also determines the sanitary conditions for obtaining and primary processing raw milk, suitability for the manufacture of dairy products. The number of MAFAM in milk is considered the most critical indicator due to the fact that, in Ukraine, a significant share of raw milk for the dairy industry is milk obtained from farms that do not have proper hygiene and production practices (GHP/GMP) and private farms (Ministry of Agrarian Policy and Food, 2019; Pronko et al., 2020).

However, its cooling is mostly insufficient and occurs prematurely (Pronko et al., 2021). Such milk no longer has bactericidal properties during delivery to milk processing facilities and, in addition, with increasing temperature during transportation to the milk processing facilities, the microflora begins to multiply actively. The development of microflora in milk causes several changes that complicate technological processes and worsen the quality of dairy products. The influence of this factor depends on the season and ambient temperature (Bohnlein et al., 2021).

According to various authors, the maximum number of anaerobic spores is found in milk in late winter and spring, which may be due to reduced feed quality and poor sanitation on farms during this period. The maximum number of heat-resistant bacteria was found in the summer months. In the autumn, their number decreased by two or more times (Burke et al., 2021).

The presence of heat-resistant bacteria in raw milk indicates that the milk is not cooled or cooled insufficiently immediately after milking because only psychrotrophic microorganisms, most of which have low heat resistance and inactivate at low temperatures, can develop in milk cooled to a temperature of 3–5 °C. A large number of heat-resistant bacteria in milk can also arise due to violation in hygienic conditions of its production, as a result of which microorganisms from equipment, dairy utensils, etc. enter it (Ledo et al., 2020).

In the process of obtaining raw milk with proper quality, attention should be paid to water hygiene. The quality of water on the farm (for washing udder, washing and disinfecting milking equipment) must meet the requirements of the current standard for drinking water. High microbial contamination of water occurred when from 438 to 589 thousand psychrotrophic microorganisms were found in 1 cm^3 of the experimental sample (Ledo et al., 2020).

In the presence of inflammatory processes in the udder, the number of microflora increases significantly, in particular, during the latent form of mastitis – up to tens of thousands, and in the case of clinical course – millions of bacteria in 1 cm^3 of milk. Failure to comply with sanitary and hygienic requirements for milk production, animal diseases, especially subclinical mastitis, lead not only to a decrease in the nutritional value of milk but also to the fact that it becomes dangerous for human health (Moradi et al., 2021; Rios-Muniz et al., 2019).

Thus, the safety and quality of raw milk are closely associated with the organization of hygiene requirements and compliance with sanitary measures on the dairy farm during its obtaining, primary processing, cooling, storage, and transportation to milk processing facilities. ([1]Kondrasii et al., 2016; [2]Kondrasii et al., 2016; Jans et al., 2016; Willis et al., 2018; Zulauf et al., 2018).

The purpose of the work was to analyze the physicochemical and microbiological parameters of raw milk supplied to milk processing facilities.

Materials and methods of research

The work was performed in the period from December 2019 to November 2020. Samples of raw milk for laboratory tests were taken at "Bila Tserkva Dairy Plant" LLC and the dairy plant of PJSC "Vita" (Kyiv region) during its reception at the appropriate facilities.

The study of changes in the species composition of the microflora in all raw milk, which was received by the milk processing facilities, was performed depending on the season and the conditions of its production.

Determination of subclinical mastitis and organoleptic evaluation were performed at the sampling site. Physicochemical and bacteriological analyses were conducted in the Ukrainian Laboratory of Quality and Safety of Agricultural Products.

A sampling of milk was performed according to DSTU 8553:2015 "Raw milk and raw cream. Rules for acceptance, sampling and preparing samples for control" (2015).

The milk microflora concerning the contamination with microorganisms of different groups was studied using the following methods: the total count of bacteria was determined according to DSTU 7357:2013 "Milk and dairy products. Methods of microbiological control", the number of psychrotrophic microorganisms, cultures and colony counts were determined similarly but incubated in a thermostat at a temperature of 7.0 ± 1.0 ºC for 7–10 days. Detected microorganisms were identified using the "Bergey's manual of systematic bacteriology" (2007); spore-forming bacteria were determined by seeding the 4th, 5th, and 6th of six ten-fold dilutions of milk heated to 85 ºC for 10 minutes. Pasteurized milk of selected dilutions was added to Petri dishes, filled with IPA and kept in a thermostat at a temperature of 30 °C for 3 days, after which the number of microorganism colonies was counted; detection of *Listeria monocytogenes* was performed according to DSTU ISO 11290-2:2000 "Microbiology of food and animal feed – horizontal method of detection of *Listeria monocytogenes*"; detection of bacteria of the genus *Salmonella* was performed according to DSTU IDF 93A:2003 "Milk and dairy products. Determination of *Salmonella"* (IDF 93A:1985, IDT).

The number of somatic cells was determined according to GOST 23453-90 "Milk. Methods for determining the number of somatic cells", milk density – according to DSTU 6082:2009 "Milk and dairy products. Methods for determining the density", acidity – according to GOST 3624-92 "Milk and dairy products. Titrometric methods for determining acidity", fat content – according to DSTU ISO 1211:2002 "Milk. Gravimetric method for determining the fat content (control method)" (ISO 1211:1999, IDT), protein content – by the method of formal titration GOST 25179-90 "Milk. Meth-

ods for protein determination"; dry matter content – according to DSTU ISO 6731:2007 "Milk, cream and condensed milk". Determination of total dry matter content (control method) – according to ISO 6731:1989, IDT.

The obtained research results were processed statistically using MS Excel. We calculated the mean values (M), the error of the mean values (m). The difference was considered probable for $P < 0.05$.

Results of the research and their discussion

We conducted an analysis of microbiological and physicochemical parameters of milk supplied to milk processing facilities from December 2019 to November 2020. In this case, we considered the origin of the milk (milk obtained under conditions of farms or from cows in private households) and the season.

During the study period, "Bila Tserkva Dairy Plant" LLC received 30% of premium milk and 70% of the first grade, however, according to some indicators, the milk also corresponded to extra grade. But the dairy plant did not exhibit extra milk, because the hygienic indicators (the content of somatic cells and microorganisms) did not correspond to this.

The research results of microbiological and physicochemical parameters of milk received for processing in "Bila Tserkva Dairy Plant" LLC are given in Table 1.

According to Table 1, fluctuations in performance depending on the season are noted. In terms of density, milk corresponded in most cases to extra grade in summer and autumn, as evidenced by the average density of milk received for processing from dairy farms in these seasons. In winter and autumn, the milk density corresponded to extra and first grades. Similar results were for the dry matter content. As for the number of somatic cells, milk corresponded to extra and higher grades only in summer, while in other times of the year, corresponded to the first grade. Moreover, the lowest number of somatic cells was in summer, the highest – in spring and winter due to the increase in the number of cows with mastitis in the cold season. The average number of somatic cells was 421.70 ± 15.26, which met the requirements for the first grade.

Milk acidity complied with current regulations during the year but was lowest in winter. The protein content was the highest in summer and autumn and slightly higher than the baseline (baseline – 3%), lower – in winter and spring but within the current requirements for raw milk. The average was 3.27% during the year. The fat content in milk obtained from farms was the lowest in spring, and it was stable and slightly exceeded the baseline (baseline for Ukraine – 3.4%) in other seasons. The average fat content, in this case, was 3.6%. According to the purity group, the milk obtained from farms complied with the current DSTU 3662:2018 and was the first group throughout the year.

The data given in Table 1 shows that in farm and chilled milk in winter and spring the average MAFAM count was <100 thousand CFU/cm^3, which corresponds to the extra grade, but the number of somatic cells corresponded to the first grade in these seasons. In summer, this figure was more than 2 times higher and according to DSTU 3662:2018 corresponded to the extra grade. The average value of MAFAM count was 132.43 ± 2.7. Thus, we note the influence of the season on physicochemical parameters and MAFAM count in raw milk obtained from farms. Taking into account all indicators used to determine the grade, high-grade milk was delivered to the

1. Physicochemical and microbiological parameter of farm milk depending on the season ("Bila Tserkva Dairy Plant" LLC) (M ± m, n = 20)

Parameter	Season			
	winter (03/12/2019–28/02/2020)	spring (02/03/2020–29/05/2020)	summer (01/06/2020–31/08/2020)	autumn (01/09/2020–19/11/2020)
Density, kg/m^3	27.99 ± 0.15	27.91 ± 0.19	28.18 ± 0.09	28.08 ± 0.22
Mass fraction of dry matter, %	11.99 ± 0.03	11.89 ± 0.01	12.36 ± 0.01	12.39 ± 0.02
Purity group, not lower than	I	I	I	I
Acidity, °T	16.22 ± 0.09	16.51 ± 0.04	17.09 ± 0.05	16.46 ± 0.03
Mass fraction of protein, %	3.28 ± 0.02	3.19 ± 0.01	3.29 ± 0.02	3.31 ± 0.02
Mass fraction of fat, %	3.61 ± 0.03	3.5 8 ± 0.0 2	3.63 ± 0.01	3.64 ± 0.02
Number of somatic cells, thousand/cm^3	436.22 ± 25.01	441.23 ± 26.02	381.21 ± 3.01	428.12 ± 7.01
MAFAM count, thousand CFU/cm^3	96.21 ± 2.06	99.22 ± 4.7	231.27 ± 1.23	103.03 ± 2.68

milk processing facility only in summer and partially in autumn, while in spring and winter milk corresponded to the first grade, in particular, due to increased somatic cell content and low density.

In PJSC "Vita", raw milk comes from farms (41%) and private households (59%). During the period under study, raw milk was of the first grade and non-grade. Quality parameters in milk obtained by PJSC "Vita" are given in Table 2.

Analysis of the data given in Table 2, with regard to physicochemical parameters of raw milk received by PJSC "Vita" from private households, it should be noted that in some respects it differed significantly from milk received from farms. In particular, this applies to sanitary and hygienic indicators – the number of somatic cells and MAFAM count.

Milk from private households contained the least somatic cells in summer, as well as milk obtained from the farm, but the number of somatic cells in the first case was on average 278.25 ± 17.84, which met the requirements for extra and higher grades. However, if we compare the average values of the number of somatic cells in milk obtained from farms and private households, their number was 1.8 times higher in milk from farms. According to this indicator, milk from farms in winter and autumn was low-grade, and only in summer, it corresponded to the first grade.

According to MAFAM count, all milk received by PJSC "Vita" was accepted only in the second grade. Although according to the current regulations, non-grade milk for dairies was to be accepted only for technical purposes from January 1, 2020. The highest MAFAM count in milk from private households was in summer, as well as milk from farms, but this figure was 11 times higher in milk obtained from private households. Similar excesses were observed in other seasons, probably due to poor quality and insufficiently rapid cooling of milk in the private households. The average MAFAM count in milk from private households was at the level of 4358.61 ± 286.15, which is 4.9 times higher than

MAFAM count in milk obtained on the farm (892.10 ± 75.58).

Farm milk in terms of density in some seasons corresponded to the extra grade, and from private households, the density index ranged from 27.51 ± 0.01 to 27.81 ± 0.20 kg/m^3, which corresponds to extra and first grades. Dependencies on the seasons were not noted. In terms of dry matter, milk from farms and private households did not differ much.

In terms of acidity, raw milk supplied to PJSC "Vita" met the normative values (16–18 °T) and averaged 17.27 °T. Milk from farms had an average acidity of 16.57 °T, which is 0.7 °T higher because milk obtained from private households is not always able to cool quickly and efficiently immediately after milking.

The average protein content in milk from private households was 2.97% and thus was slightly lower than the baseline (3%), except for milk obtained in autumn. In farm milk, this figure was on average at baseline. During the calendar

2. Microbiological and physicochemical parameters of milk received for processing by PJSC "Vita" from farms and private households (M ± m, n = 20)

Parameter	Season			
	winter (03/12/2019–28/02/2020)	spring (02/03/2020–29/05/2020)	summer (01/06/2020–01/08/2020)	autumn (01/09/2020–19/11/2020)
Chilled milk from farms				
Density, kg/m^3	27.5 ± 0.34	27.30 ± 0.26	28.03 ± 0.36	28.04 ± 0.24
Mass fraction of dry matter, %	11.51 ± 0.16	11.57 ± 0.08	11.74 ± 0.12	11.69 ± 0.05
Purity group, not lower than	I	I	I	I
Acidity, ºT	16.88 ± 1.25	17.01 ± 1.14	18.03 ± 1.16	17.22 ± 1.24
Mass fraction of protein, %	3.03 ± 0.01	2.99 ± 0.03	3.01 ± 0.04	3.06 ± 0.01
Mass fraction of fat, %	3.47 ± 0.11	3.38 ± 0.02	3.48 ± 0.13	3.62 ± 0.04
Number of somatic cells, thousand/cm^3	629.14 ± 23.01	480.70 ± 34.42	398.04 ± 45.01	496.53 ± 26.43
MAFAM count, thousand CFU/cm^3	933.42 ± 89.97	1239.18 ± 69.83	1291.27 ± 65.01	1382.88 ± 74,00
Chilled milk from private households				
Density, kg/m^3	27.69 ± 0.05	27.81 ± 0,20	27.52 ± 0.06	27.51 ± 0.01
Mass fraction of dry matter, %	11.69 ± 0.04	11.52 ± 0.05	11.99 ± 0.12	11.57 ± 0.03
Purity group, not lower than	I	I	I	I
Acidity, ºT	17.07 ± 0.06	17.27 ± 0.14	17.89 ± 0.07	17.33 ± 0.29
Mass fraction of protein, %	2.98 ± 0.01	2.89 ± 0.07	2.99 ± 0.03	3.02 ± 0.04
Mass fraction of fat, %	3.49 ± 0.09	3.48 ± 0.04	3.45 ± 0.2	3.58 ± 0.03
Number of somatic cells, thousand/cm^3	297.16 ± 21.64	271.16 ± 18.25	266.36 ± 13.24	279.75 ± 18.23
MAFAM count, thousand CFU/cm^3	1412.58 ± 219.03	1427.92 ± 217.43	14579.58 ± 316.09	1437.54 ± 212.05

year, the protein content in all samples of raw milk from farms and private households corresponded to the norm (not less than 2.8%).

The fat content in milk obtained in private households was on average 3.5%, which was 0.1% higher than the basic fat content and 0.1% lower than that in farm milk, so there is no reliable difference. Regarding the fat content in milk, depending on the season, this figure was the lowest in summer and the highest in autumn, which coincides with the trend observed for farm milk.

In terms of purity, milk obtained from private households did not comply with the current DSTU 3662:2018, as during the year it was assigned to the second purity group, which does not meet the requirements of the current standard and can be accepted at the milk processing facilities only as non-grade for technical purposes.

In addition, we studied the species composition of the microflora in milk obtained from farms and private households, depending on the season (Table 3).

3. The species composition of microorganisms in milk (M ± m, n = 20)

Microorganism	Season			
	winter (03/12/2019–28/02/2020)	spring (02/03/2020–29/05/2020)	summer (01/06/2020–31/08/2020)	autumn (01/09/2020–19/11/2020)
Chilled farm milk				
Bacteria of the genus *Salmonella*, in 25 cm^3	-	-	-	-
Staphylococcus aureus, in 0.1 cm^3	-	-	-	-
Listeria monocytogenes, in 25 cm^3	-	-	-	-
Bacteria of *Escherichia coli* group, number of cases, %	-	-	-	-
Spore-forming m/o, thousand/cm^3	12.09 ± 0.07	16.01 ± 0.04	19.37 ± 0.07	11.02 ± 0.03
Mesophilic m/o, thousand/cm^3	60.11 ± 0.06	55.18 ± 1.24	173.58 ± 1.09	62.92 ± 0.74
Psychrotrophic m/o, thousand/cm^3	24.01 ± 0.03	28.03 ± 0.07	38.32 ± 0.79	29.09 ± 0.12
Milk from private households				
Bacteria of the genus *Salmonella*, in 25 cm^3	-	-	-	-
Staphylococcus aureus, in 0.1 cm^3	-	-	-	-
Listeria monocytogenes, in 25 cm^3	-	-	-	-
Bacteria of *Escherichia coli* group, number of cases, %	0	20	0	20
Spore-forming m/o, thousand/cm^3	19.44 ± 0.13	16.12 ± 0,03	67.18 ± 0.02	33.78 ± 0.02
Mesophilic m/o, thousand/cm^3	317.97 ± 1.01	341.84 ± 0.59	449.60 ± 1.26	342.74 ± 1.92
Psychrotrophic m/o, thousand/cm^3	75.17 ± 0.09	69.96 ± 0.74	29.98 ± 0.75	61.02 ± 0.03

The results of the study are given in Table 3, indicate that regardless of the season and conditions, all tested samples of raw milk met the requirements of the current DSTU for the absence of bacteria of the genus *Salmonella* in 25 cm^3, *Staphylococcus aureus* in 0.1 cm^3 and *Listeria monocytogenes* in 25 cm^3.

Bacteria of *Escherichia coli* group were not detected in farm milk during the year, in contrast to milk from private households, where this group of bacteria was detected in spring and autumn (20% of cases).

As for the other microflora, both in farm and in milk from private households, a group of mesophilic microorganisms prevailed over spore-forming and psychrotrophic ones. However, their number was different, because in general, the average MAFAM count was 3.31 times higher in milk obtained in the conditions of private households (Tables 1, 2).

The number of spore-forming microorganisms in farm milk is 5.5 times lower in summer and 3 times lower in autumn than in milk obtained from private households. In other seasons of the year, the number of spore-forming microorganisms in milk produced by economic entities with different forms of ownership did not differ significantly.

As for the mesophilic microflora, milk contamination in all seasons of the year was higher in the milk of cows from private households, although the smallest difference between the indicators was in summer. In particular, in winter – 5.3, in spring – 6.2, in summer – 2.6, in autumn – 5.4 times.

The number of psychrotrophic microorganisms in farm milk, compared to the milk of cows from private households, differed the most in winter and was 3 times lower. In spring and autumn, it was lower – 2.5 and 2.1 times, respectively, and in summer it differed the least and was only 1.3 times lower.

This difference in total bacterial contamination and individual groups of microorganisms in farm milk and milk obtained from private households, apparently, can be explained by the fact that the latter technology involves mixing several small batches of milk in one container, and cow owners give milk as a rule once a day, thus milk from evening milking (cooled) and morning (warm) can be mixed that activates growth and multiplication of microflora. In addition, the sanitary and hygienic conditions for obtaining, primary processing of milk from private households, its storage, and transportation do not meet modern requirements for the production and circulation of raw milk.

Conclusions and future perspectives

Quality parameters of raw milk depend on the conditions of its production and the season. Milk of extra and first grades comes from farms, and private households – only non-grade. In addition, in summer and autumn more premium milk is received. Problematic indicators that do not allow to obtain milk of extra grade in farm conditions are hygienic: MAFAM count and the number of somatic cells.

Raw milk obtained in the conditions of private households does not meet the requirements of the current DSTU 3662:2018 in terms of purity and MAFAM count, so now it remains relevant to cooperate with the owners and their use of appropriate milking and refrigeration equipment.

All tested samples of raw milk, regardless of the season and milk production conditions, met the requirements of the current standard for the absence

of bacteria of the genus *Salmonella* in 25 cm^3, *Staphylococcus aureus* in 0.1 cm^3, and *Listeria monocytogenes* in 25 cm^3.

Bacteria of *Escherichia coli* group were not detected in farm milk during the year, while in milk from private households they were recorded in spring and autumn (20% of cases).

In farm milk and milk from private households, a group of mesophilic microorganisms prevailed over spore-forming and psychrotrophic ones.

Prospects for further research are to determine the sources of entering various types of microorganisms in milk and to develop procedures to eliminate the possibility of milk contamination with foreign microflora, especially in private households.

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Анотація. *В Україні активно відбувається імплементація законодавства з безпечності та окремих показників якості харчових продуктів, зокрема, молока-сировини до вимог Європейського союзу. Сучасні вимоги до молока-сировини вимагають ретельного його аналізу за гігієнічними показниками. Досліджували сире молоко-сировину, що надходило на ТОВ «Білоцерківський молочний комбінат» та молокозавод ПАО «Віта» Київської області. Мікробіологічними методом визначали кількість мезофільних аеробних та факультативно анаеробних мікроорганізмів (КМАФАМ) та видовий склад мікрофлори молока, зокрема, бактерії роду Salmonella, Staphylococcus aureus, Listeria monocytogenes, бактерії групи кишкових паличок, спороутворюючі та психротрофні мікроорганізми. Фізико-хімічними методами визначали: густину, масову частку сухих речовин, вміст соматичних клітин, кислотність, групу чистоти, масову частку білка та жиру. За результатами досліджень якість фермерського молока є на порядок кращою, порівнюючи з молоком, отриманим в умовах особистих селянських господарств, зокрема, за КМАФАМ. Технологія отримання фермерського молока забезпечує його виробництво вищого й першого ґатунків, у той час, як молоко, отримане в умовах особистих селянських господарств – першого ґатунку та негатункове. За фізико-хімічними показниками молоко,*

отримане за різних умов, достовірно не відрізнялося. Суттєво відрізнялися мікробіологічні показники. Середній показник КМАФАМ молока з особистих селянських господарств був 4361,25 ± 241,15, що у 12,6 раза перевищує КМАФАМ, навіть молока першого ґатунку, отриманого в умовах молочнотоварної ферми. Незалежно від пори року й умов отримання молока-сировини всі досліджені проби відповідали вимогам чинного ДСТУ щодо відсутності бактерій роду Salmonella у 25 см^3, Staphylococcus aureus, у 0,1 см^3 та Listeria monocytogenes, у 25 см^3. У фермерському молоці не виявляли бактерій групи кишкових паличок упродовж року, на відміну від молока з особистих селянських господарств, де їх виявляли навесні і восени (по 20% випадків). Як у фермерському, так і в молоці з особистих селянських господарств переважала група мезофільних мікроорганізмів над спороутворюючими і психротрофними. Проте їхня кількість була різною. Перспективи подальших досліджень полягають у визначенні джерел потрапляння різних видів мікроорганізмів у молоко-сировину та розробленні процедур усунення можливості обсіменіння молока сторонньою мікрофлорою.

Ключові слова: *молоко-сировина, фермерське молоко, молоко з особистих селянських господарств, мікрофлора молока, показники якості*

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ROLE OF THE AUTONOMIC NERVOUS SYSTEM IN THE REGULATION OF PHOSPHORUS AND CALCIUM METABOLISM IN COWS

O. V. ZHURENKO, *Doctor of Veterinary Sciences, Associate Professor, Department of Animal Biochemistry and Physiology named after Academician M. F. Gulyi*
https://orcid.org/0000-0002-4933-0372
V. I. KARPOVSKYI, *Doctor of Veterinary Sciences, Professor, Department of Animal Biochemistry and Physiology named after Academician M. F. Gulyi*
https://orcid.org/0000-0003-3858-0111
V. V. ZHURENKO, *Candidate of Veterinary Sciences, Department of Animal Biochemistry and Physiology named after Academician M. F. Gulyi*
https://orcid.org/0000-0003-2097-9212
National University of Life and Environmental Sciences of Ukraine, 15 Heroiv Oborony st., Kyiv 03041, Ukraine
E-mail: Zhurenko-lena@ukr.net

Abstract. *The leading role in the mobilization of the organism adaptive capabilities is played by neuro-humoral mechanisms, primarily the activity of the central nervous system. The full-fledged mineral nutrition of cattle depends on the animal supply with macroelements, which are included in the structure of many enzymes or are their activators, taking a leading role in redox reactions. The studies were carried out on cows of the Ukrainian black-spotted dairy breed of 2–3rd lactation. By the results of studying the autonomic nervous system tone, 3 experimental groups were formed: 1 – normotonics, 2 – vagotonics, 3 – sympathicotonics. Blood from cattle was collected twice a year in summer and winter. Whole blood was stabilized with heparin, blood serum was obtained by sedimentation method, and blood cells – by centrifugation of heparinized blood, plasma collection and after triple washing of cells in the cold isotonic solution, followed by centrifugation. The tone of the autonomic nervous system in cows in summer is inversely related to the content of phosphorus in whole blood ($r = -0.73$; $P < 0.05$) and blood cells ($r = -0.87$; $P < 0.001$). However, in blood serum, these relationships are unreliable both in summer and winter ($r = -0.02–0.24$). In winter, the tone of the autonomic nervous system in cows is inversely reliably associated only with phosphorus content in whole blood ($r = -0.81$; $P < 0.01$). Unlike the tone of the autonomic nervous system, the season has a significant effect on calcium content in whole blood ($F = 8.94 > FU = 8.41$; $P < 0.01$), while the content of this element in serum and blood cells of cows did not depend from the season. So, the tone of the autonomic nervous system and the season affect the content of calcium and phosphorus in blood of cows.*

Keywords: *cows, blood, nervous processes, the tone of the autonomic nervous system, calcium, phosphorus*

Introduction

The current state of the agricultural industry is due to the global impact of modernization, which is accompanied by an increase in the technogenic load on animals. The leading role in the mobilization of the adaptive capabilities of the organism is played by neuro-humoral mechanisms, primarily the activity of the central nervous system (Pogorlov, 2010). Studies on the influence of the higher nervous activity type in cows on the mineral status of the animal's organism are of scientific and practical interest. The full-fledged mineral nutrition in cattle depends on the animal supply with macroelements, which are included in the structure of many enzymes or are their activators, taking a leading role in redox reactions (Guyot et al., 2012). Macroelements, despite their low content in the body, play a significant biological role (Byts, 2010; Gromova et al., 2010). In addition to the general positive effect on the processes of growth and development, the specific effect of several macroelements on the most important physiological processes has been established; their significance is also explained by their interaction with biologically active substances – hormones and vitamins (Zakharenko, 2004). The optimal content and ratio of essential trace elements in the animal's organism determine the normal course of their physiological functions, high resistance, and productivity (Kalyn, 2011). The absence or deficiency of individual mineral elements, as well as their suboptimal ratio in the diets, leads to a decrease in the efficiency of feed nutrients and, as a consequence, to a decrease in livestock productivity.

Analysis of recent researches and publications

It is known that metabolic processes at the cellular and subcellular levels are provided by the functioning of about 2000 enzymes, each of which catalyzes a corresponding chemical reaction (Gapon, 2005). In turn, the catalytic activity of enzymes is provided by coenzymes of non-protein origin – organic compounds or inorganic elements (metal ions – macro- and microelements). Despite the fact that minerals have no energy value, like proteins, fats, and carbohydrates, many enzymatic processes in the body are impossible without certain elements. Macroelements play a key role in maintaining acid-base balance, osmotic pressure, membrane potential, and transmission of nerve stimuli. The conducted research has established a reliable influence of the main characteristics of cortical processes on the content of macroelements in bovine blood (Avtsyn, 1991). The macroelement content in the body is quite stable, even relatively large deviations from the norm are compatible with the vital activity of the organism. The main process of absorption of macro- and microelements occurs in the upper part of the small intestine, namely in the duodenum. Regulation is carried out under the effect of the central and autonomic nervous system, and the endocrine system. However, insufficient attention is paid to the study of the individual characteristics of mineral homeostasis in the body of productive cows in an intact and stressed state (McDowell, 2003; Zinko, 2017). Determination of individual characteristics of higher nervous activity in animals provides a deeper understanding of the cortical mechanisms in the regulation of various physiological functions, creating the prerequisites for a targeted impact on them (Skalny, 2004; Danchuk et al., 2017).

Materials and methods of research

The studies were carried out on cows of the Ukrainian black-spotted dairy breed of the 2–3rd lactation. By the results of

studying the tone of the autonomic nervous system, 3 experimental groups were formed (5 animals in each): 1 – normotonic, 2 – vagotonic, 3 – sympathicotonic cows. Research material was blood samples from cattle taken from the jugular vein in the morning before feeding. Blood was collected twice a year in summer and winter. Whole blood was stabilized with heparin, blood serum was obtained by sedimentation method, and blood cells – by centrifugation of heparinized blood, plasma collection and after triple washing of cells in the cold isotonic solution, followed by centrifugation (Vlizlo et al., 2012). The obtained digital data were processed statistically using the applied software package "Microsoft Office Excel 2013". The arithmetic mean value (M) and its error (m) were determined. The probability of differences in mean values was determined by the Student's test. Parameters changes were considered significant at $P < 0.05$ (including $P < 0.01$ and $P < 0.001$).

Research results and their discussion

The studies showed that in animals with a different tone of the autonomic nervous system the phosphorus content in various blood fractions did not exceed physiological limits (Table 1).

In particular, the phosphorus content in bovine whole blood depending on the vegetative status and season was 20.5–25.0 mg/100 mL, in blood serum – 10.9–12.1 mg/100 mL, and in blood cells – 47.1–52.5 mg/100 mL. It was found that both in warm and cold seasons the phosphorus content did not differ significantly in different blood fractions of normotonics and vagotonics.

In sympathicotonic animals, in the warm season, the phosphorus content was lower in whole blood by 11.8% ($P < 0.001$), and in blood cells – by 8.2% ($P < 0.001$) in comparison with indicators in normotonic cows.

Whereas in the cold season, the phosphorus content was significantly lower in whole blood by 16.0% ($P < 0.001$) and in blood cells – by 6.4% ($P < 0.001$) compared with indicators in normotonic cows. It should be noted that time of the year affects the phosphorus content in various fractions of bovine blood.

So, regardless of the vegetative status in cows, in the cold season, the phosphorus content in blood serum is 7.2–9.4% lower ($P < 0.05$–0.01) compared with the indicator of these animals in summer. In addition, in sympathicotonic cows, the content of this metal in whole blood in winter is significantly lower (by 6.8%; $P < 0.05$) compared with the indicator in these animals in summer. But the phosphorus content in blood cells of these animals in warm and cold seasons does not differ significantly.

Studies showed that in animals with a different tone of the autonomic nervous system, the content of inorganic phosphorus in the blood serum did not exceed physiological levels and, depending on the vegetative status of animals and season, was 4.6–5.1 mg/100 mL. It was found that both in warm and cold seasons in different blood fractions of normotonics and vagotonics, the content of inorganic phosphorus in blood serum did not differ significantly. However, in the warm season, the content of inorganic phosphorus in the blood serum in sympathicotonic animals was lower by 5.7% ($P < 0.05$) and in the cold season – by 5.6% ($P < 0.05$) compared with indicators in normotonic cows. It should be noted that the season affects inorganic phosphorus content in bovine serum. Thus, in normotonic, vagotonic, and sympathicotonic cows in the cold season, this indicator was 6.0–7.7% ($P < 0.05$) lower than in these animals in the warm season.

1. Phosphorus content in blood of cows with different vegetative status depending on the season, mg/100 mL (M ± m, n = 5)

Substrate	Vegetative status		
	normotonic	vagotonic	sympathicotonic
Summer			
Blood serum	24.98 ± 0.23	24.30 ± 0.35	22.03 ± 0.26***
Whole blood	12.08 ± 0.19	11.98 ± 0.17	11.78 ± 0.33
Blood cells	51.15 ± 0.60	52.45 ± 0.26	46.98 ± 0.89***
Winter			
Blood serum	24.43 ± 0.46	23.58 ± 0.60	20.53 ± 0.30***
Whole blood	11.20 ± 0.34	10.85 ± 0,40	10.88 ± 0.37
Blood cells	50.30 ± 0.60	50.70 ± 0.95	47.08 ± 0.28***

Note: differences are significant in comparison with normotonics: * P < 0.05; ** P < 0.01; *** P < 0.001.

The studies have established the influence of the vegetative status in cows on the phosphorus content in blood depending on the season.

The predominance of the influence of the parasympathetic division of the autonomic nervous system on the heart, both in summer and in winter, does not affect phosphorus content in whole blood, serum, and blood cells in cows (η^2_χ = 0.02–0.40). The predominance of the sympathetic part of the autonomic nervous system influence on the work of the heart in summer affects the phosphorus content in bovine whole blood – η^2_χ= 0.92 (P < 0.001) and blood cells – η^2_χ = 0.72 (P < 0.01). In winter, the influence of the tone of the autonomic nervous system in sympathicotonic cows on the phosphorus content in whole blood and blood cells stayed significant (η^2_χ = 0.80–0.89 (P < 0.01–0.001). The relationship of the autonomic nervous system in cows with phosphorus content in their blood depending on the season has been established.

Thus, the tone of the autonomic nervous system in cows in summer is inversely reliable related to phosphorus content in whole blood (r = -0.73; P < 0.05) and its cells (r = -0.87; P < 0.001). However, in blood serum, these relationships are unreliable both in summer and winter (r = -0.02–0.24). In winter, the tone of the autonomic nervous system in cows is inversely reliable related only to phosphorus content in whole blood (r = -0.81; P < 0.01). Thus, regardless of the season, the changes in heart rate difference according to the results of the trigeminovagal test in cows by one unit, the phosphorus content in whole blood changes in the opposite way by 0.09–0.12 mg/100 mL (P < 0.05–0.01). Moreover, up to 53% (P < 0.01) in summer and up to 48% (P < 0.05) variations in the content of this metal in whole blood of cows in winter can be caused by the tone of the autonomic nervous system of animals.

Regardless of the season, the changes in heart rate difference according to the results of the trigeminovagal test in cows by one unit, the phosphorus content in the blood cells changes in the opposite way by 0.15–0.21 mg/100 mL (P < 0.001). In addition, up to 75% (P < 0.001) in summer and up to 66% (P < 0.001) variations in the content of this metal in blood cells in winter can be caused by the tone of the autonomic nervous system of animals. It should be

noted that regression analysis did not show a reliable dependence of phosphorus content in blood serum on the vegetative status of animals. It was found that the tone of the autonomic nervous system and phosphorus content in blood serum of cows did not shown a significant relationship ($F = 0.53 < FU = 3.55$; $P < 0.05$), while the content of this element in whole blood ($F = 43.0 > FU = 3.55$; $P < 0.001$) and blood cells ($F = 27.5 > FU = 3.55$; $P < 0.001$) depends on the vegetative status. Unlike the tone of the autonomic nervous system, the season has no significant effect on phosphorus content in blood cells of cows ($F = 2.44 < FU = 4.41$; $P < 0.05$), while its effect on phosphorus content in whole blood ($F = 8.54 > FU = 4.41$; $P < 0.01$) and its serum ($F = 14.4 > FU = 4.41$; $P < 0.001$) is significant. It should be noted that analysis of phosphorus content in whole blood, serum, and blood cells of cows showed no reliable interaction between the vegetative status and season of the year ($F = 0.10–1.00 < FU = 3.55$; $P < 0.05$).

The studies showed that in animals with a different tone of the autonomic nervous system, the calcium content in various blood fractions did not exceed the physiological limits (Table 2).

In particular, the calcium content in whole blood of cows, depending on the vegetative status and season, was 5.0–5.6 mg/100 mL, in blood serum – 7.7–8.5 mg/100 mL, and in blood cells – 2.3–2.6 mg/100 mL.

It was found that in the warm season, the calcium content in blood cells of vagotonics is significantly higher by 10.4% ($P < 0.05$) in comparison with the indicators in normotonic animals. In addition, in summer, the content of ionized calcium in vagotonics is significantly lower by 4.6% ($P < 0.05$) compared to the indicators of normotonic animals. However, the content of this macroelement in whole blood and serum did not differ significantly.

Unlike the indicators in vagotonic animals, in blood serum of sympathicotonic cows, the content of ionized calcium in summer was 7.3% higher ($P < 0.05$) than that in cows with normotonia, and calcium content in blood cells was higher by 12.4% ($P < 0.05$) than in normotonics. It should be noted that the ratio of ionized calcium content to total one in blood of sympathicotonic cows in summer is 6.3% ($P < 0.05$) higher than that in normotonic cows.

This indicator did not differ significantly from that one in normotonic cows in the cold season. Besides, in sympathicotonic cows, the only calcium content in blood cells significantly differs from the indicators of normotonic animals in the cold season (higher by 8.3%, $P < 0.05$).

It should be noted that the time of the year affects calcium content in whole blood of cows. Thus, calcium content in whole blood of vagotonic and sympathicotonic cows in the cold season is lower by 9.6% ($P < 0.05$) and 7.1% ($P < 0.05$), respectively, than in these animals in summer. But calcium content in other blood fractions in these animals in warm and cold seasons does not differ significantly.

The influence of the vegetative state of cows on blood calcium content depending on the season has been established.

The predominance of the influence of both the sympathetic and the parasympathetic divisions of the autonomic nervous system on the work of the heart in summer affects calcium content in blood cells of cows – $\eta^2_\chi = 0.58–0.65$ ($P < 0.05$). Along with this, the effect of the autonomic nervous system tone on the content of ionized calcium in blood serum of cows was established – $\eta^2_\chi = 0.65–0.70$ ($P < 0.05–0.01$).

Thus, the tone of the autonomic nervous system in cows in summer is significantly directly related to the content of ionized calcium in blood serum ($r = 0.87$;

2. Calcium content in blood of cows with different vegetative status depending on the season, mg/100 mL (M ± m, n = 5)

Substrate	Vegetative status		
	normotonic	vagotonic	sympathicotonic
Summer			
Whole blood	5.27 ± 0.12	4.98 ± 0.18	5.26 ± 0.12
Blood serum	8.10 ± 0.11	7.69 ± 0.28	8.17 ± 0.20
Blood cells	2.30 ± 0.07	2.54 ± 0.05*	2.58 ± 0.05*
Ionized Ca^{++}	3.46 ± 0.04	3.3 ± 0.03*	3.71 ± 0.06*
Ionized Ca/total Ca	0.43 ± 0.01	0.43 ± 0.01	0.45 ± 0.01*
Winter			
Blood serum	5.43 ± 0.16	5.46 ± 0.16	5.63 ± 0.03
Whole blood	8.34 ± 0.35	8.27 ± 0.32	8.51 ± 0.03
Blood cells	2.36 ± 0.05	2.40 ± 0.05	2.56 ± 0.03**
Ionized Ca^{++}	3.60 ± 0.17	3.42 ± 0.14	3.68 ± 0.03
Ionized Ca/total Ca	0.43 ± 0.01	0.41 ± 0.01	0.43 ± 0.01

Note: differences are significant in comparison with normotonics: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

$P < 0.001$) and the ratio of ionized calcium to total one in blood serum ($r = 0.57$, $P < 0.05$). In winter, the tone of the autonomic nervous system in cows has no significant correlations with calcium content in various blood fractions ($r = 0.24–0.51$).

There is a significant dependence of calcium content in bovine blood on the tone of the autonomic nervous system in summer, while in winter, the tone of the autonomic nervous system does not reliably limit the content of this element in blood of animals (Table 3).

However, in summer, if the heart rate differences according to the results of the trigeminovagal test in cows changes by one unit, the content of ionized calcium in whole blood changes in the same way by 0.02 conditional units ($P < 0.001$), and the ratio of ionized to total calcium in blood serum by 0.01 conditional units ($P < 0.05$). Moreover, in summer, up to 75% ($P<0.001$) of ionized calcium content and up to 33% ($P < 0.05$) of variations in the ratio of ionized to total calcium in blood serum of cows can be caused by the tone of the autonomic nervous system of animals.

Unlike the tone of the autonomic nervous system, the season has a significant effect on calcium content in whole blood ($F = 8.94 > FU = 7.41$; $P < 0.01$), while the content of this element in the serum and blood cells of cows had no relationship with the season. Also, should be noted the significant influence of the vegetative status of animals on the content of ionized calcium in blood serum ($F = 6.19 > FU = 3.55$; $P<0.01$). Thus, both the season and the tone of the autonomic nervous system did not affect the ratio of ionized to total calcium in blood serum ($F = 3.19–3.37 < FU = 3.55–4.41$; $P > 0.05$). Analyzing calcium content in various fractions of blood of cows, a significant relationship between the vegetative status of cows and the season was not established ($F = 0.25–1.94 < FU = 3.55$, $P > 0.05$).

Therefore, the tone of the autonomic nervous system and the time of year affect the content of calcium and phosphorus in blood of cows.

3. Regression analysis of the dependence of calcium content in blood of cows on the tone of the autonomic nervous system, conditional units (n = 16)

Substrate	Indicator			
	summer		winter	
	coefficient	R-squared	coefficient	R-squared
Whole blood	0.01	0.09	0.01	0.10
Blood serum	0.01	0.13	0.01	0.06
Blood cells	0.0001	0.02	0.01	0.26
Ionized Ca^{++}	0.02***	0.75***	0.01	0.16
Ionized Ca/total Ca	0.01*	0.33*	0.15	0.214

Note: differences are significant in comparison with normotonics: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Conclusions and future perspectives

The tone of the autonomic nervous system in cows in summer is significantly inversely related to the content of phosphorus in whole blood ($r = -0.73$; $P < 0.05$), its cells ($r = -0.87$; $P < 0.001$) and directly correlates with the content of ionized calcium in serum ($r = 0.87$; $P < 0.001$). The regression analysis shown a significant effect of the autonomic nervous system tone on the content of ionized calcium in blood serum ($F = 6.19 > FU = 3.55$; $P < 0.01$).

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Анотація. *Провідну роль у мобілізації адаптаційних можливостей організму відіграють нейро-гуморальні механізми, насамперед – діяльність центральної нервової системи. Повноцінність мінерального живлення великої рогатої худоби залежить від забезпеченості тварин макроелементами, які входять до структури багатьох ензимів або є їх активаторами, приймаючи провідну роль в окисно-відновних реакціях. Досліди проводили на коровах української чорно-рябої молочної породи 2–3 лактації. За результатами дослідження тонусу автономної нервової системи було сформовано 3 дослідні групи: I – нормотоніки, II – ваготоніки, III – симпатикотоніки. Відбір крові проводили двічі на рік, влітку та взимку. Цільну кров стабілізували за допомогою гепарину, сироватку крові отримували методом відстоювання, а клітини крові – за допомогою центрифугування гепаринузованої крові, відбирання плазми та триразового промивання клітин у холодному ізотонічному розчині з наступним центрифугуванням. Тонус автономної нервової системи в корів влітку достовірно обернено пов'язаний із вмістом Фосфору в цільній крові (r = -0,73; P < 0,05) та її клітинах (r = -0,87; P < 0,001). Тоді, як у сироватці крові дані взаємозв'язки недостовірні, як влітку так і взимку (r = -0,02–0,24). Взимку тонус автономної нервової системи в корів обернено достовірно пов'язаний лише з вмістом Фосфору в цільній крові (r = -0,81; P < 0,01). На відміну від тонусу автономної нервової системи пора року має достовірний вплив на вміст Кальцію в цільній крові (F = 8,94 > FU = 0,41; P < 0,01), тоді, як вміст цього елементу в сироватці та клітинах крові корів не залежав від пори року.*

Отже, тонус автономної нервової системи та пора року впливають на вміст Кальцію і Фосфору в крові корів.

Ключові слова: *корови, кров, нервові процеси, тонус автономної нервової системи, Кальцій, Фосфор*

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TILMICOSIN INTAKE AND DISTRIBUTION IN THE BODY OF BROILER CHICKENS WITH ORNITHOBACTERIOSIS

A. M. TYSHKIVSKA, *Graduate Student, Department of Pharmacology, Parasitology and Tropical Veterinary Medicine*
http://orcid.org/0000-0003-4419-2174
V. B. DUKHNYTSKYI, *Doctor of Veterinary Sciences, Professor, Department of Pharmacology, Parasitology and Tropical Veterinary Medicine*
http://orcid.org/0000-0002-9670-1244
National University of Life and Environmental Sciences of Ukraine,
15 Heroiv Oborony st., Kyiv 03041, Ukraine
E-mail: annatyshkivska@gmail.com, dukhnytskyjvb@ukr.net

Abstract. *One of the main indicators that determine the effectiveness of the antibiotic in the body is its ability to penetrate and accumulate in high concentrations at the sites of the pathological process. The article presents the results of studies of the intake, distribution, and excretion of tilmicosin phosphate – the active substance of the antibiotic Tilmox 25% from the body of broiler chickens (Cobb-500 cross) with ornithobacteriosis. It was found that in 24 hours after the start of the feeding Tilmox 25% solution to broiler chickens with ornithobacteriosis, most tilmicosin phosphate was contained in the lungs, while in the liver less than 1.6 times, kidneys – 3.0 times, heart muscle – 3.4 times, pectoral muscles – 3.5 times than in the lungs. After 48 and 72 hours, the tilmicosin content increased in all studied organs but the pattern of its distribution was the same as after 24 hours. Tilmicosin phosphate levels in the lungs exceeded the values in the liver, kidneys, heart, and pectoral muscles by 1.8 times, 2.7, 2.9, and 3.9 times, respectively, at 72 hours of the experiment. At 96 hours, tilmicosin levels were highest in the pectoral muscles, kidneys, liver, and lungs, and only slightly less in the heart than in previous research periods. The obtained results testify to the organ affiliation of tilmicosin phosphate to the lung tissues in broiler chickens with ornithobacteriosis. In a day (120 hours of the experiment) after discontinuation of Tilmox 25%, the content of tilmicosin phosphate in the lungs, liver, kidneys, heart, and pectoral muscles of broiler chickens was 53%, 50, 57, 68, and 34%, respectively, in comparison with values after 96 hours. The Ornithobacterium rhinotracheale sensitivity to tilmicosin and its distribution in maximum amounts in the lungs of broiler chickens with ornithobacteriosis provided a therapeutic effect, which was confirmed by microscopic studies.*

Studies on the pharmacokinetic properties of tilmicosin have been performed mainly in healthy birds. Therefore, the optimization of treatment regimens of already known antibiotics, which will be based on the study of pharmacokinetic and pharmacodynamic properties not only on clinically healthy but also on diseased organisms is a relevant and important issue in the field of veterinary pharmacology.

Keywords: *Tilmox 25%, tilmicosin phosphate, broiler chickens, ornithobacteriosis, Ornithobacterium rhinotracheale, pharmacokinetics, distribution, accumulation, excretion*

Introduction

Antibiotics are a key component of modern veterinary medicine used in the treatment of animals and poultry in more than half of all diseases. They are used as a primary therapy – to destroy the pathogen and to treat patients with secondary infections, which often occur as complications of viral and parasitic diseases, mycoses, and immunodeficiency. The pharmacokinetics of the animal's interaction with the drug is studied. It describes the absorption of the drug into the blood – bioavailability, its distribution, biotransformation, accumulation, and excretion. Pharmacokinetic indicators allow the physician to select an effective drug and calculate the most optimal treatment regimen that will provide a therapeutic effect and prevent the development of bacterial resistance (Evans et al., 2002; Lalonde et al., 2007; Ronald et al., 2014).

One of the reasons for the development of bacterial resistance is the insufficient bioavailability of the antibiotic at the site of pathogen localization. This leads to the fact that the concentration required to kill bacteria is not reached in the tissues, or the drug is excreted too quickly (McClary et al., 2011).

In recent years, in European countries, including Ukraine, the semi-synthetic macrolide antibiotic tilmicosin began to actively use for the treatment of birds with respiratory diseases caused by *Mycoplasma gallisepticum, Mycoplasma synoviae, Ornithobacterium rhinotracheale, Pasteurella multocida*.

Desirable pharmacokinetic properties of tilmicosin include rapid absorption, good airway penetration, and slow excretion (Li et al., 2017).

Analysis of recent researches and publications

Tilmicosin is a semi-synthetic macrolide antibiotic synthesized from tylosin, with a broad spectrum of action, which has a pronounced activity against the causative agents of avian respiratory diseases – respiratory mycoplasmosis, ornithobacteriosis, pasteurellosis. Positive properties that characterize the tilmicosin pharmacokinetics are its rapid absorption after oral administration, good penetration into the tissues of the respiratory tract, in particular into the lungs and air sacs, slow excretion, which provides a pronounced prolonged effect (McClary et al., 2011; El-Mahmoudy et al., 2016; Li et al., 2017; Li et al., 2017; Timsit et al., 2017).

The tilmicosin's pharmacokinetic properties have been studied by many scientists in different dosage forms and animals of different species. In particular, in 2007, Jordanian scientists conducted studies on broiler chickens with tilmicosin phosphate in the composition of the drugs Provityl – a ready-made aqueous solution for oral administration and Pulmotyl AS – a powder for solution. The drugs were used in broiler chickens 30 days of age for 5 days. Studies have shown the tilmicosin phosphate's bioequivalence, rapid absorption, and slow excretion (Moore et al., 1996; Shen et al., 2005; Abu-Basha et al., 2007).

A pharmacokinetic study of tilmicosin was also performed in healthy pigs and infected with *Haemophilus parasuis*. The drug was administered orally once at a dose of 40 mg/kg body weight. Tilmicosin concentrations were determined in blood plasma at regular intervals after administration for 96 hours. The maximum serum concentration of tilmicosin in healthy animals was 1.77 ± 0.33 compared to 1.67 ± 0.128 μg/L in patients, indicating no significant differences between pharmacoki-

netic profiles in clinically healthy pigs and infected with *H. parasuis* (Zhang et al., 2017; Zhang et al., 2019).

Comparative characterization of the pharmacokinetic parameters of tylosin and tilmicosin in 12 lactating Holstein cows after intravenous administration showed that the maximum serum concentrations are 1.30 ± 0.12 for tylosin and 4.55 ± 0.23 μg/mL for tilmicosin. The time required to reach the peak concentration for tylosin was 2 hours, while for tilmicosin – 4 hours. The half-lives were 20.46 ± 2.08 hours for tylosin and 26.36 ± 5.55 hours for tilmicosin, indicating long-term excretion of the drug and its prolonged action in animals (Ziv et al., 1995; Dimitrova et al., 2012; Avci et al., 2014).

The research results of the pharmacokinetics of tilmicosin in healthy broiler chickens, when administered orally, indicate that the drug is well absorbed from the digestive tract, and the maximum concentration in serum is reached 2 hours after ingestion. Similar results were obtained in other studies performed on laying hens and broiler chickens. In particular, the maximum tilmicosin concentration in the serum of laying hens was found after 2 h and was 1.28 μg/mL, in broiler chickens – 1.297 μg/mL (Keles et al., 2001; Kowalski et al., 2002; Li, 2003). Other studies have shown that with a single internal application of tilmicosin to birds, the time to reach the maximum concentration in blood plasma is 2.5 hours. The antibiotic rapidly entered organs and tissues, accumulated in macrophages and lung tissues, where its concentrations were higher than in blood plasma (El-Ela et al., 2015).

The research results of the pharmacokinetics of tilmicosin presented in the scientific literature apply mainly to healthy animals and poultry. There is no information on the intake, distribution, and excretion of tilmicosin from broiler chickens with ornithobacteriosis.

The purpose of the research is to investigate tilmicosin phosphate intake, distribution, and excretion when using it in the form of the drug Tilmox 25% in the body of broiler chickens with ornithobacteriosis.

Materials and methods of research

The research was conducted on the basis of one of the poultry farms in the Ivano-Frankivsk region. For research, 20 broiler chickens of Cobb-500 cross aged 25 days, with an average body weight of 1200 g were used. Bacteriological examination in birds diagnosed ornithobacteriosis, which in some individuals was complicated by *Escherichia coli*.

Before performing the experiment, 5 sick broiler chickens in a state of light ether anesthesia were slaughtered and the lungs and trachea were taken for microscopic examinations and the tracheal washes – for bacteriological examinations.

The effectiveness of the treatment was monitored by clinical indicators and microscopic changes in the studied organs of broiler chickens on the second and fourth days after administration of Tilmox 25%. To do this, 3 chickens under light ether anesthesia were slaughtered and the lungs and trachea were removed again.

To detect the causative agent of pneumonia and aerosaculitis, from a bird slaughtered in a state of light ether anesthesia, washes were removed from the tracheal mucosa using a sterile applicator SWAB (Biomerieux). Selected material from the tracheal mucosa was sieved by direct seeding on blood agar. Cultivation was performed under microaerophilic conditions at a tempera-

ture of 37 ºC for 48 hours. After cultivation, the growth of small matte gray colonies was found.

The two-day culture was identified by the method of MALDI TOF mass spectrometry by direct deposition.

Antibiotic sensitivity of the isolated culture of microorganisms was determined using the disc-diffusion method. From pure daily culture, a suspension was prepared in a sterile isotonic sodium chloride solution on a turbidity scale of 0.5 (according to McFarland). Mueller-Hinton blood agar (Biomerieux) was inoculated with the suspension and antibiotic discs were spread on the surface. The culture of the isolated microorganisms was cultured in Petri dishes at a temperature of 37 °C for 24 hours. After cultivation, growth retardation zones were measured according to CLSI: M31-A3.

For studies, a solution of the drug Tilmox 25% of the AVICO trademark (active substance – tilmicosin phosphate) was used, which in an amount of 0.3 ml was mixed with 1 liter of drinking water.

The tilmox solution was fed to broiler chickens for 96 hours instead of drinking water, according to the recommended scheme used in industrial poultry farming. For birds feeding, the complete feed was used taking into account the technological scheme of growing.

Three chickens in a state of light ether anesthesia were slaughtered in 24, 48, 72, and 96 hours after the start of feeding a solution of Tilmox 25%, and the lungs, liver, kidneys, heart, pectoral muscles were taken to control tilmicosin phosphate entry and distribution in the body of broiler chickens with ornithobacteriosis, and 24 hours after discontinuation of a tilmox solution (i.e. 120 hours after the start of the experiment) to control its content in the internal organs and excretion. Selected samples were frozen.

Tilmicosin phosphate detection by liquid chromatography spectrometry was performed after pre-extraction with acetonitrile and subsequent purification of the samples. Extraction, concentration, and purification of test samples were performed on solid-phase extraction columns. Tilmicosin phosphate was extracted from the column with eluent. Identification was carried out by retention time and quantification – by the method of external standards, by the area of peaks. Detection was performed using Waters LC-MS-MS liquid chromatograph with a Premier XE tandem quadrupole detector. The results of the study were calculated by the methods of variation statistics.

Results of the research and their discussion

Clinical examination of sick broiler chickens showed general depression, plumage, apathy, and refusal to feed. Nasal discharge and swelling of the nasal mucosa were observed in some individuals. Histological examinations of organs in broiler chickens with ornithobacteriosis revealed the presence of distinct microscopic changes. In the lungs, the lumens of most parabronchi were markedly reduced due to the swelling of their walls and infiltration, mainly by lymphocytes, among which a small number of monocytes and pseudo-eosinophils were found (Fig. 1, 2). The epithelium on the surface of the parabronchi was almost completely absent. Only a few flattened epithelial cells or their small groups were found.

Most of the air capillaries in all parabronchial complexes, without exception, were narrowed due to the marked expansion and overflow of blood capillaries. Instead, a small part of the air capillaries was clearly dilated

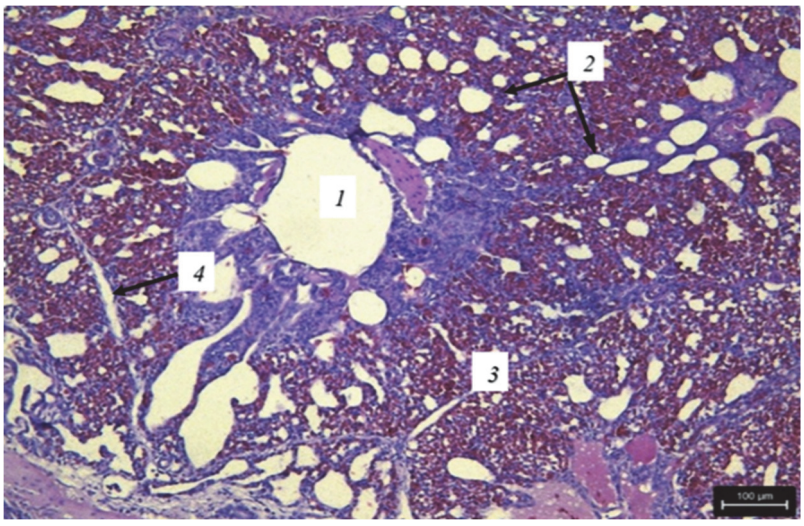

Fig. 1. The lungs of a broiler chicken with ornithobacteriosis: parabronchial lumen (1), dilated air capillaries (2), narrowed air capillaries (3), unevenly swollen connective tissue (4). Hematoxylin Jill 2 and eosin

(Fig. 1–2), which, in our opinion, indicated a compensatory response of the lungs to the loss of a significant part of the gas exchange surface. On the surface of almost all air capillaries, the epithelium was also absent (Fig. 3).

All blood vessels of the lungs were clearly dilated, full of blood cells. In all cases, pronounced edema was found around the blood vessels, and hemorrhages were also found around some of them (Fig. 4). Hemorrhages were also

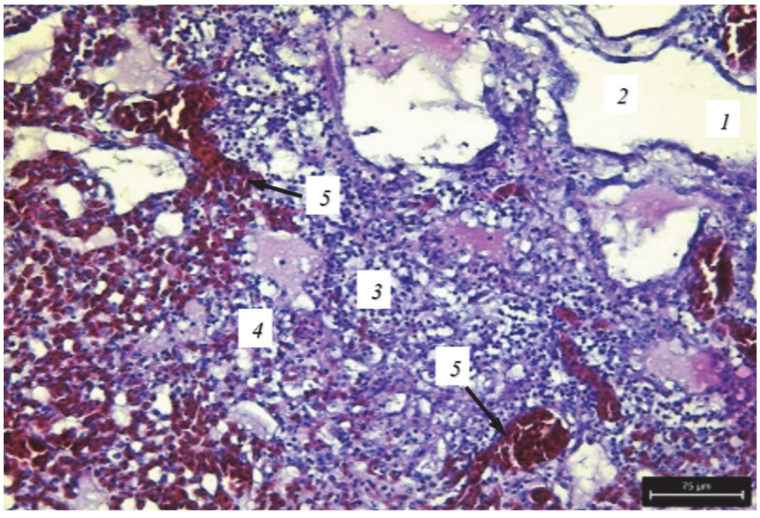

Fig. 2. The lungs of a broiler chicken with ornithobacteriosis: parabronchial lumen (1), absence of respiratory epithelium (2), lymphocytic infiltration, dilated blood-filled capillaries (3), hemorrhage (4). Hematoxylin Jill 2 and eosin

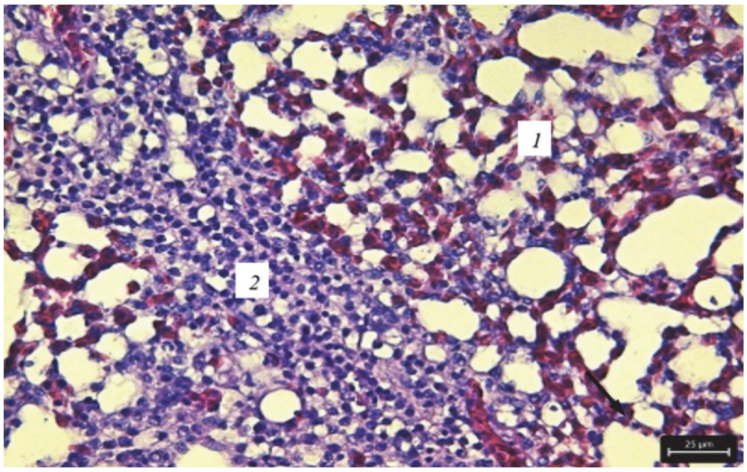

Fig. 3. The lungs of a broiler chicken with ornithobacteriosis:
absence of respiratory epithelium in the air capillary (1), edema and lymphocytic infiltration of connective tissue between two adjacent parabronchial complexes (2). Hematoxylin Jill 2 and eosi

found in the parenchyma of parabronchial complexes (Fig. 2).

The trachea was noticeably less affected than the lungs. The entire surface of the tracheal mucosa was covered with a fairly thick layer of thick mucus (Fig. 5). Part of the epithelial cells in the tracheal mucosa was destroyed and part became clearly flattened. All blood vessels of the mucous membrane were significantly dilated and overflowed with blood cells. The submucosal base was clearly swollen. Instead,

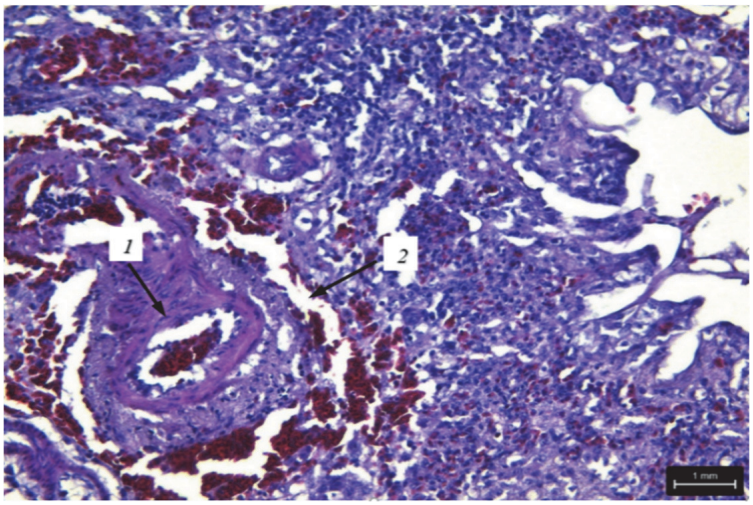

Fig. 4. The lungs of a broiler chicken with ornithobacteriosis:
dilated blood-filled artery (1), edema and hemorrhage around the artery (2). Hematoxylin Jill 2 and eosin

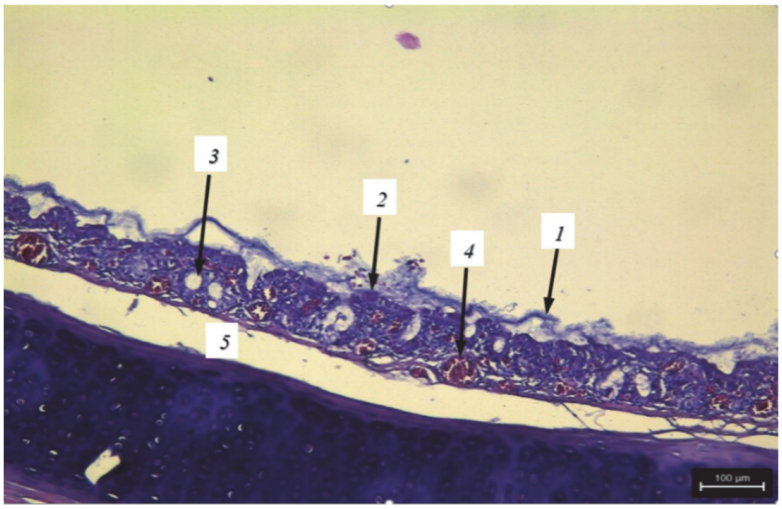

Fig. 5. The trachea of a broiler chicken with ornithobacteriosis:
mucus on the surface of the mucosa (1), destruction of the epithelium (2), foci of edema (3), dilated blood-filled vein (4), edema of the submucosal base (5). Hematoxylin Jill 2 and eosin

microscopic changes were not detected in the cartilage tissue.

Bacteriological examination with the determination by MALDI TOF mass spectrometry identified a culture of microorganisms isolated from the trachea as *Ornithobacterium rhinotracheale*.

According to the results of the antibiotic susceptibility reaction, it was found that the culture of *Ornithobacteria* isolated from the trachea is sensitive to doxycycline, tilmicosin, rifampicin, cefazolin, amoxiclav, and benzylpenicillin, moderately sensitive to enrofloxacin, and resistant to gentamicin (Table 1).

In 24 hours after the start of feeding Tilmox 25% solution to broiler chickens with ornithobacteriosis, the highest content of its active substance (tilmicosin phosphate) was found in the lungs, much less in the liver, and least in the kidneys, heart, and pectoral muscles (Table 2). During this research period, the tilmicosin phosphate content in the lungs was 1.6 times, 3.0, 3.4, and 3.5 times higher than in the liver, kidneys, heart, and pectoral muscles, respectively (Table 2).

Feeding Tilmox 25% solution to broiler chickens the next day was accompanied by a slight increase in tilmicosin phosphate content in all studied organs. In particular, 48 hours after the start of drinking tilmox solution, the tilmicosin phosphate content was higher in the lungs by 14%, liver – by 6%, kidneys – by 17%, heart – by 19%, pectoral muscles – by 3%.

As in previous study periods, 72 hours after the start of feeding tilmox solution to broiler chickens, tilmicosin phosphate was highest in the lungs, less in the liver, and least in the kidneys, heart, and pectoral muscles. Tilmicosin phosphate levels in the lungs during this study period were 1.8 times, 2.7, 2.9, and 3.9 times higher than in the liver, kidneys, heart, and pectoral muscles, respectively.

In 96 hours after drinking tilmox solution, the tilmicosin phosphate content in the lungs, liver, kidneys, and pectoral muscles reached maximum values for the entire study period and exceeded the values observed at 24 hours in the lungs by

1. Antibiotic sensitivity of *Ornithobacterium rhinotracheale* culture isolated from the trachea in broiler chickens

Antibiotic	Code (antibiotic content in the disk, μg/g)	The zones' sizes interpretation of growth retardation			Growth retardation zone, mm	Research result
		R Resistant	I Moderately sensitive	S Sensitive		
Erythromycin	E10	≤ 20	21–22	≥ 23	0	Resistant
Enrofloxacin	EX10	≤ 18	19–22	≥ 23	20	Moderately sensitive
Oxytetracycline	O30	≤ 24 (22)	–	≥ 24	22	Resistant
Benzylpenicillin (penicillin-G)	P1	≤ 17	–	≥ 17	19	Sensitive
Amoxiclav	AMC30	≤ 15	–	≥ 15	28	Sensitive
Gentamicin	GEN30	≤ 23	–	≤ 23	8	Resistant
Cefazolin	CZ30	≤ 14	15–17	≥ 18	22	Sensitive
Rifampicin	RIF15	≤ 16	17–19	≥ 20	21	Sensitive
Doxycycline	DO30	≤ 20	21–24	≥ 25	20	Sensitive
Tilmicosin	TL15	≤ 13	14–20	≥ 21	21	Sensitive

17%, liver – by 8%, kidneys – by 32%, pectoral muscles – by 4%. The tilmicosin phosphate content in the heart muscle in this study period decreased compared to 72 hours by 29% and was even lower than after 24 hours by 4% (Table 2).

The obtained research results convincingly prove that tilmicosin phosphate in the body of broiler chickens with ornithobacteriosis during the period of feeding Tilmox 25% solution in maximum quantities is distributed in the lung tissue.

Although tilmicosin phosphate was distributed in maximum amounts in lung tissue, there is no reason to assert material accumulation in this organ, as its rate increased only by 17% during the period from 24 to 96 hours of drinking tilmox solution (Table 2). We believe that the tilmicosin phosphate distribution in the largest quantities in the lungs indicates its organ affiliation, which is of great practical importance in bacterial diseases of birds with respiratory lesions, in particular in ornithobacteriosis.

Higher levels of tilmicosin phosphate in the liver and kidneys than in the heart and pectoral muscles are ex-

2. Tilmicosin phosphate content in organs and tissues of broiler chickens with ornithobacteriosis while feeding a tilmox solution, μg/g (M ± m, n = 3)

Organ/tissue	Research time, hours				
	24	48	72	96	120
Pectoral muscles	3.18 ± 0.02	3.27 ± 0.02	3.29 ± 0.03	3.31 ± 0.01	1.14 ± 0.01
Kidneys	3.66 ± 0.28	4.27 ± 0.13	4.78 ± 0.12	4.84 ± 0.05	2.75 ± 0.15
Liver	6.73 ± 0.27	7.11 ± 0.07	7.22 ± 0.05	7.25 ± 0.07	3.65 ± 0.05
Lungs	11.05 ± 0.04	12.57 ± 0.34	12.72 ± 0.28	12.91 ± 0.06	6.88 ± 0.37
Heart	3.25 ± 0.03	3.86 ± 0.15	4.37 ± 0.55	3.11 ± 0.03	2.13 ± 0.03

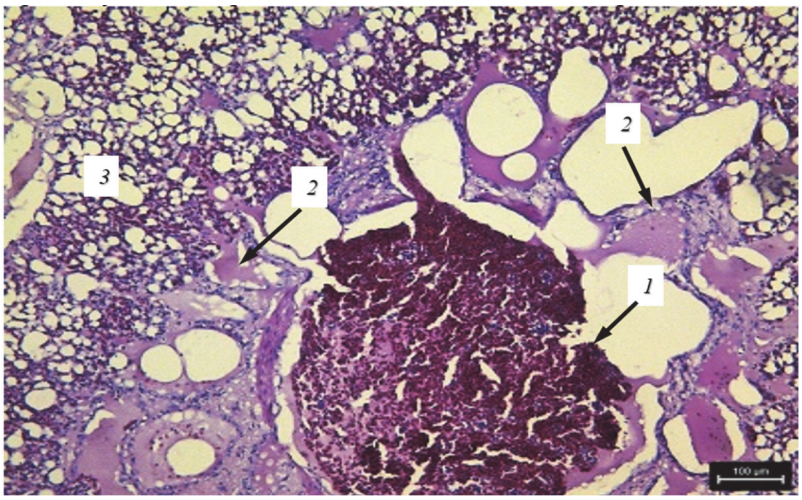

Fig. 6. The lungs of a broiler chicken with ornithobacteriosis on the 2nd day of the treatment with tilmicosin: cellular detritus and red blood cells in the lumen of the parabronchia (1), edema of the parabronchial wall (2), air capillaries (3). Hematoxylin Jill 2 and eosin

plained by better blood supply to these organs, as well as participation in the processes of biotransformation and excretion from the body.

After the application of tilmicosin to broiler chickens, cellular detritus and erythrocytes were detected in the lungs and lumen of the parabronchi for the second day, which, in our opinion, testified to the processes of organ cleansing (Fig. 6).

No marked inflammatory reaction was observed, although the walls of the

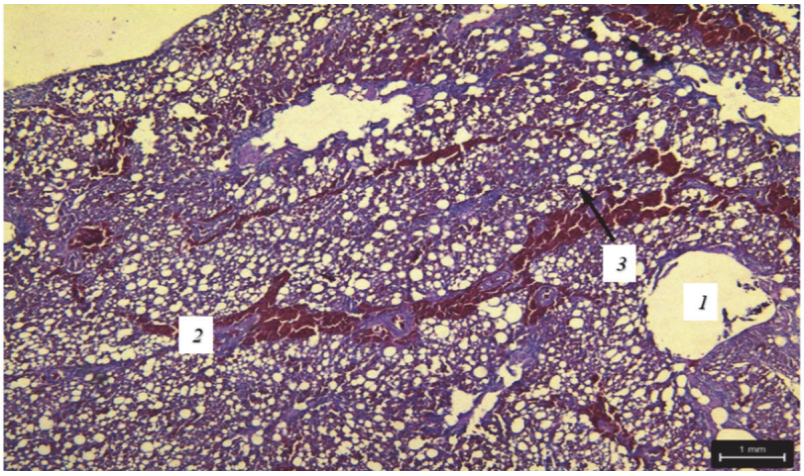

Fig. 7. The lungs of a broiler chicken with ornithobacteriosis on the 4th day of the treatment with tilmicosin: lumen of the parabronchia (1), dilation and overflow of blood vessels (2), air capillaries (3). Hematoxylin Jill 2 and eosin

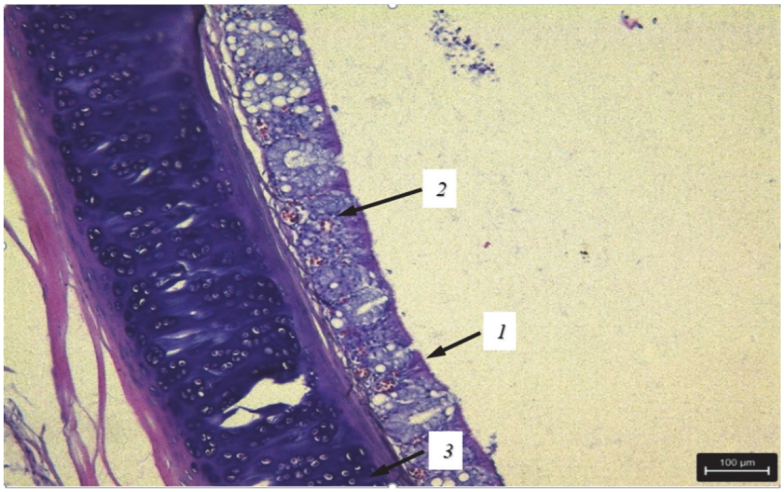

Fig. 8. The trachea of a broiler chicken with ornithobacteriosis on the 4th day of the treatment with tilmicosin: epithelium of the mucous membrane (1), mucous membrane (2), cartilage (3). Hematoxylin Jill 2 and eosin

parabronchi were swollen. The air capillaries were dilated. Small groups of narrowed air capillaries were found only in some places (Fig. 6). Such changes testified to more intensive functioning of the lungs.

Only slight dilation and overflow of mucosal blood vessels were observed in the trachea.

On the 4th day after the tilmicosin application, there was the only hyperemia in the lungs (Fig. 7). In the trachea, no microscopic changes were detected (Fig. 8).

After cessation of feeding broiler chickens with a tilmox solution, the content of its active substance (tilmicosin phosphate) in the internal organs has decreased (Table 1). Thus, in a day after discontinuation of Tilmox 25% (120 hours of the experiment), the tilmicosin phosphate content in the lungs, liver, kidneys, heart, and pectoral muscles was lower than in 96 hours by 47%, 50, 43, 32, and 66%, respectively, which, in our opinion, provides a long-lasting antimicrobial effect.

Conclusions and future perspectives

In the lungs of broiler chickens with ornithobacteriosis, microscopic changes were characterized by a decrease in the lumen of the parabronchi due to swelling of their walls and infiltration, mainly by lymphocytes, lack of epithelium on the surface of the parabronchi; narrowing of air capillaries in parabronchial complexes; hemorrhages in the parenchyma of parabronchial complexes; dilation of the vessels in the lungs, their overflow with blood cells and edema around the vessels.

Microscopic changes in the trachea were characterized by the destruction of the part of epithelial cells in the mucosa and the rest of them became clearly flattened; swelling of the submucosal base; dilation and overflow of mucosal blood vessels with blood cells.

When feeding Tilmox 25% solution to broiler chickens with ornithobacteriosis according to the recommended scheme, the highest content of its active substance (tilmicosin phosphate) was

found in the lungs in all research periods, much less in the liver and least in the kidneys, heart, and pectoral muscles.

The tilmicosin phosphate distribution in the maximum amounts in the lungs indicates its organ affiliation and minor fluctuations in the drug concentration during different research periods – the lack of cumulative properties.

In a day (120 hours of the experiment) after discontinuation of Tilmox 25% solution in broiler chickens with ornithobacteriosis, the tilmicosin phosphate content in the lungs, liver, kidneys, heart, and pectoral muscles was 53%, 50, 57, 68, and 34%, respectively, up to 96 hours. Residual amounts of tilmicosin phosphate in the avian internal organs – 50% or more in a day after discontinuation of feeding tilmox solution indicate a long half-life, and hence a long antimicrobial effect.

The high sensitivity of *Ornithobacterium rhinotracheale* culture to tilmicosin and its maximum distribution in the lung tissue in broiler chickens with ornithobacteriosis, gives reason to recommend Tilmox 25% for practical use.

In four days after Tilmox 25% application to broiler chickens with ornithobacteriosis, only hyperemia in the lungs was observed, and no changes in the trachea, which are signs of the microscopic structure restoration.

To study the pharmacokinetic characteristics of antibiotics of other groups in broiler chickens with ornithobacteriosis are the prospects for our further research.

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Анотація. *Одним з основних показників, що визначають ефективність антибіотика в організмі, є його здатність проникати та накопичуватись у високих концентраціях у місцях патологічного процесу. У статті наведено результати досліджень надходження, розподілу та виведення тилмікозину фосфату – діючої речовини антибіотика тилмокс 25% з організму курчат-бройлерів кросу КОББ-500, хворих на орнітобактеріоз. Встановлено, що через 24 год від початку випоювання розчину препарату тилмокс 25% курчатам-бройлерам, хворим на орнітобактеріоз, найбільше тилмікозину фосфату містилося в легенях, тоді як у печінці менше в 1,6 раза, нирках – у 3,0 раза, серцевому м'язі – у 3,4 раза, грудних м'язах – у 3,5 раза,*

ніж у легенях. Через 48 та 72 год вміст тилмікозину зростав в усіх досліджуваних органах, але закономірність його розподілу була такою ж, як і через 24 год. Вміст тилмікозину фосфату в легенях на 72 год досліду переважав вміст в печінці, нирках, серцевому та грудних м'язах відповідно в 1,8 раза, 2,7, 2,9 та 3,9 раза. На 96 год вміст тилмікозину був максимальним у грудних м'язах, нирках, печінці, легенях, лише в серці його містилося дещо менше, ніж у попередні періоди досліджень. Отримані результати засвідчують органну приналежність тилмікозину фосфату до тканин легень у курчат-бройлерів, хворих на орнітобактеріоз. Через добу (на 120 год досліду) після припинення застосування тилмоксу 25%, вміст тилмікозину фосфату в легенях, печінці, нирках, серцевому та грудних м'язах курчат-бройлерів становив відповідно 53%, 50, 57, 68 та 34% до показника на 96 год. Чутливість Ornithobacterium rhinotracheale до тилмікозину та його розподіл у максимальних кількостях у легенях курчат-бройлерів, хворих на орнітобактеріоз, забезпечувало лікувальний ефект, що підтверджено результатами мікроскопічних досліджень.

Дослідження щодо фармакокінетичних властивостей тилмікозину виконані, в основному, на здоровій птиці. Тому, оптимізація схем лікування вже відомих антибіотиків, яка буде ґрунтуватися на дослідженні фармакокінетичних і фармакодинамічних властивостей не тільки на клінічно здорових, але й на хворих організмах є актуальним та важливим питанням у галузі ветеринарної фармакології.

Ключові слова: *тилмокс 25%, тилмікозину фосфат, курчата-бройлери, орнітобактеріоз, Ornithobacterium rhinotracheale, фармакокінетика, розподіл, накопичення, виведення*

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IMMUNOLOGICAL INDICATORS IN ANIMAL ORGANISMS UNDER THE INFLUENCE OF ALLOGENEIC ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS

***L. V. KLADNYTSKA*[1],** *Doctor of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0002-9360-0587
***A. Y. MAZURKEVYCH*[1],** *Doctor of Veterinary Sciences, Professor*
http://orcid.org/0000-0002-2409-7703
***L. V. GARMANCHUK*[2],** *Doctor of Biological Sciences, Professor*
http://orcid.org/0000-0002-1527-2346
***M. O. MALIUK*[1],** *Doctor of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0003-3019-6035
***S. V. VELYCHKO*[3],** *Candidate of Biological Sciences*
http://orcid.org/0000-0003-1842-5114
***T. A. MAZURKEVYCH*[1],** *Doctor of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0002-1294-5939
***V. V. KOVPAK*[1],** *Doctor of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0003-2419-1246
***V. B. DANILOV*[1],** *Candidate of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0002-2897-5235
***Yu. O. KHARKEVYCH*[1],** *Candidate of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0002-7877-8272
***R. R. BOKOTKO*[1],** *Candidate of Veterinary Sciences, Assistant*
http://orcid.org/0000-0002-6217-5266
***T. L. SAVCHUK*[1],** *Candidate of Veterinary Sciences, Senior Lecturer*
http://orcid.org/0000-0002-7351-5684
[1]National University of Life and Environmental Sciences of Ukraine, 15 Heroiv oborony st., Kyiv 03041, Ukraine
[2]Taras Shevchenko National University of Kyiv, 60 Volodymyrska st., Kyiv 01033, Ukraine
[3] Veterinary hospital, Holosiivskyi Avenue 105 B, Kyiv 01041, Ukraine,
E-mail: Kladlarisa@ukr.net

Abstract. *The studies were conducted on 2–3 months old males of C57BL/6 mice, weighing 20–24 g. Our work aimed at studying the functional state of the organs of the immune system in mice after administration of allogeneic mesenchymal stem cells of adipose tissue origin. Obtaining and cultivating mesenchymal stem cells were carried out in a sterile laminar box with compliance of asepsis and antiseptic conditions. The abdominal adipose tissue from*

C57BL/6 mice was cultured in a CO_2 incubator at 37 °C and 5% CO_2 in DMEM with 10–15% of fetal bovine serum, 1% of antibiotic/antimycotic solution (Sigma-Aldrich, USA). The following groups of animals were formed: 1 group – intact (control); 2 group – animals, to whom 0.5 cm^3 of 0.9% NaCl solution (placebo) were injected into the caudal vein; 3 group – animals, to whom 10^4 of allogeneic mesenchymal stem cells from adipose tissue in 0.5 cm^3 of phosphate buffer solution were injected into the caudal vein. The weight index, the content of lymphoid cells in the thymus and spleen in C57BL/6 mice were examined on days 7, 18, and 25 after the administration of mesenchymal stem cells. To assess the lymphocyte content in lymphoid organs, they were weighed (whole thymus, 50 mg of spleen), triturated, and filtered through the nylon cloth. After that, the tissue homogenate was applied to Ficoll-Urografin density gradient (d = 1.077) in a ratio of 3:2. The test tubes were centrifuged at 1500 rpm for 30–40 minutes. After centrifugation, plasma and layer of lymphocytes were above the density gradient; lymphocytes were collected by a Pasteur pipette and washed twice with an arbitrary amount of Hank's solution by centrifugation at 1500 rpm for 10 minutes. After washing, 1 cm^3 of Hank's solution was added to lymphocytes and they were counted in the Goryaev chamber. Calculation of the cellularity of lymphoid organs was performed in 1 mg of tissue.

The transplantation of allogeneic adipose-derived mesenchymal stem cells affects the central and peripheral organs of the immune system. Administration of allogeneic adipose-derived mesenchymal stem cells causes a significant increase in the content of lymphoid cells in the thymus on days 7, 18, and 25 by 71%, 57, and 53% ($P < 0.05$), respectively, compared to the control. At the 7 and 18 days of the immune response, lymphoid cell content in the spleen significantly increases by 33% ($P < 0.01$) and 19% ($P < 0.05$), respectively, compared to the control under the administration of allogeneic adipose-derived mesenchymal stem cells. On day 25, values of lymphoid cell content and spleen index return to normal. The thymus and spleen weight indices directly correlate with their lymphoid cell content.

Keywords: *mice, weight index, lymphoid cells, thymus, spleen, allogeneic mesenchymal stem cells, adipose tissue*

Introduction

Important biological features of mesenchymal stem cells (MSCs), in particular, the ability to migrate to the inflammation site, low immunogenicity, immunomodulatory activity, and the ability to stimulate hemopoiesis, make them potentially active regulators of reparative processes (Kladnytska et al., 2014).

At the present stage of the development of biological sciences, different approaches are developed for the use of MSCs in the treatment of various diseases, and several preclinical and clinical trials have already been conducted, the results of which have shown the effectiveness of their application (Haghighat et al., 2011; Reich et al., 2012; Arnhold & Wenisch, 2015; Jakobsen et al., 2017).

An alternative source of MSCs is adipose tissue, which contains stem cells in a higher percentage than bone marrow. Obtaining adipose tissue is a less traumatic procedure for the donor than the obtaining of bone marrow both during the process of primary material obtaining and postoperative period (Marx et al., 2014; Kladnytska et al., 2017).

Regardless of MSCs origin, they have pronounced immunosuppressive activity: they block *in vitro* differentiation of naive CD4+ T cells in Th17 and

suppress the synthesis of many cytokines such as IL-17, IL-22, interferon-gamma, and TNFα (Bartholomew et al., 2002; Gryschenko & Tomchuk, 2013).

Despite the large number of publications confirming the immunosuppressive properties of MSCs, some works deny such effects on immune responses (Di Nicola et al., 2002; Aggarwal & Pittenger, 2005; Djouad et al., 2005).

Thus, it was found that transplantation of MSCs stimulates antibody production and increases the cellularity of the bone marrow in recipients. With increasing the number of administered cells, a significant increase in the thymus cellularity and decrease in spleen cellularity were recorded. The authors suggest that a significant dose of MSCs creates a suppressive microenvironment for lymphoid cells that is accompanied by the inhibition of the immune response (Le Blanc et al., 2003; Batten et al., 2006; Lu et al., 2009).

Opposite, a small number of transplanted cells due to homing is collected in the bone marrow niches, contributing to hematopoiesis, where the myeloid sprout can act as an impressive factor in natural immunity (Nikolskaya et al., 2012).

So, it is not well known about the effect of MSCs on the response of the immune organs, in particular, on the functional state of the thymus and spleen. Taking into account such controversial data on the influence of MSCs on the organs of the immune system, these issues require further research.

The purpose of our work was to study the functional state of the organs of the immune system in C57BL/6 mice after the administration of allogeneic adipose MSCs.

Materials and methods of researches

The studies were carried out on 2–3 months old males of C57BL/6 mice, weighing 20–24 g. All experiments were conducted in accordance with the Good Laboratory Practices regulations in research studies using animals, the Law of Ukraine On the Protection of Animals from Brutal Treatment, and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

MSCs obtaining from adipose tissue. Obtaining and cultivating adipose MSCs (aMSCs) were carried out in a sterile laminar box with compliance of asepsis and antiseptic conditions. The mice were euthanized, samples of abdominal adipose tissue were washed three times with sterile phosphate buffer solution with the addition of 1% antibiotic/antimycotic solution (Sigma-Aldrich, USA). Then samples of adipose tissue were chopped into pieces of 1–3 mm^3 and placed to culture dishes filled with DMEM, 10–15% of fetal bovine serum, 1% antibiotic/antimycotic solution (Sigma-Aldrich, USA) and cultured in a CO_2 incubator at 37 ^{0}C and 5% CO_2. The culture medium was partially or completely changed by fresh medium every 3 days during cultivation. After formation of cell monolayer by 80–90%, cells were removed with trypsin/ethylenediaminetetraacetic acid solution (EDTA), washed with phosphate buffer, and placed into Petri dishes for further cultivation. Cell passaging provided a reduction in the heterogeneity of cell culture and the development of biological material for transplantation (Kladnytska et al., 2016). MSCs at passage 4 were used for transplantation.

MSC administration to mice. The following groups of animals were formed: 1 – intact (control); 2 – animals, to whom 0.5 mL of 0.9% NaCl solution (placebo) were injected into the caudal vein; 3 – animals, to whom 10^4 of allogeneic aMSCs in 0.5 mL of phosphate buffer solution were injected into the caudal vein.

Estimation of the thymic and splenic weight indices in mice after aMSC admin-

istration. Indicators of the weight of peripheral lymphoid organs relative to the body weight (weight index) of animals were evaluated on days 7, 18, and 25 after aMSC administration. The mice were pre-weighed for weight control. At each experimental period, 3 animals were euthanized in each group and the weight index of lymphoid organs and their cellularity were studied. Euthanasia of animals was carried out using carbon dioxide; lymphoid organs (thymus and spleen) were removed and their mass was determined. Indices of lymphoid organs in relation to the weight of the animal were calculated according to the formula:

Evaluation of the thymic and splenic cellularity after aMSC administration. To assess the content of lymphocytes in lymphoid organs, the latter were weighed. The whole thymus and 50 mg of spleen were triturated and filtered through the nylon cloth. After that, the cell homogenate was applied to Ficoll-Urografin density gradient (d = 1.077) in a ratio of 3:2. The test tubes were centrifuged at a rate of 1500 rpm for 30–40 minutes. After centrifugation, the layer of lymphocytes, which was located above the density gradient, was collected by a Pasteur pipette and washed twice with an arbitrary amount of Hank's solution by centrifugation at a rate of 1500 rpm for 10 minutes. After washing, 1 ml of Hank's solution was added to lymphocytes. Lymphocytes were counted in the Goryaev chamber. Calculation of the lymphoid organ cells was performed on 1 mg of tissue.

Results of the research and their discussion

The functional state of the organs of immunogenesis largely depends on the ratio of processes of immune cell proliferation and apoptosis, that almost are not studied after MSC administration.

After allogeneic aMSC administration, the content of lymphoid cells in the thymus on days 7, 18, and 25 significantly increased by 71%, 57, and 53%, respectively, compared with control animals (Table 1).

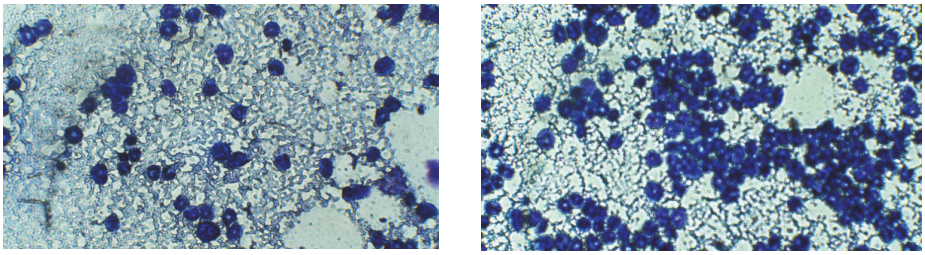
Compared with a placebo group, the content of lymphoid cells in the thymus was significantly increased by 42%, 69, and 86%, respectively. The increase in thymic cellularity is associated with the activation of residential thymocyte proliferation due to antigenic stimulation by MSCs that is consistent with the studies of Huang et al. (2009). The thymus contains T lymphoblasts, immature and mature lymphocytes, supporting and secretory cells of the thymus stromal component (Fig. 2).

A positive correlation between the content of lymphoid cells and thymic weight index was found 7 days after aMSCs administration. Thymic weight index directly correlates with the content of lymphoid cells and its value was

1. The content of lymphoid cells and the weight index of the thymus in C57BL/6 mice after allogeneic aMSC administration (M ± m, n = 9)

Day	The content of lymphoid cells, x 10^6/mg			thymic weight index after administration of aMSC, %
	intact (n = 6)	administration of 0.89% NaCl, placebo (n = 9)	administration of aMSCs (n = 9)	
7	1.4 ± 0.1	1.9 ± 0.2	2.7 ± 0.1* v	0.19 ± 0.03* v
18	1.4 ± 0.1	1.3 ± 0.1	2.2 ± 0.1* v	0.16 ± 0.01* v
25	1.7 ± 0.1	1.4 ± 0.1	2.6 ± 0.3* vv	0.17 ± 0.01* v

Note: * P < 0.05, ** P < 0.01 compared to intact animals; v P < 0.05, vv P < 0.01 compared to placebo group.



A B

Fig. 2. Thymic cellularity on day 7 of the experiment:
A – intact group, B – after aMSC administration (smear-imprint, x 400)

r = 0.57 (P < 0.01) on day 7 and r = 0.50 P < 0.05 on day 18 of the experiment.

Under the influence of MSCs from adipose tissue, the indicator of splenic weight index was significantly increased until day 18 of the experiment (Table 2). On day 25, the splenic weight index did not significantly differ from that in the experimental group and placebo animals, but only observed a tendency to increase it.

The spleen, as the peripheral organ of the immune system, is also involved in the process of forming an immune response to the antigen. After MSC administration, the content of lymphoid cells in the spleen significantly exceeded the parameters of spleen cellularity in intact animals (Table 2). Smear-imprint contains erythroid cells, neutrophil granulocytes, monocytes. and lymphoid cells.

The number of lymphoid cells significantly increased by 33 and 24% compared to intact animals and the placebo group on day 7 of the experiment. On day 18 of the experiment, the spleen cellularity under the influence of MSCs from adipose tissue was significantly higher by 19 and 14%, respectively. On day 25 of the experiment, the lymphoid cell count was higher by 7 and 15% within the tendency.

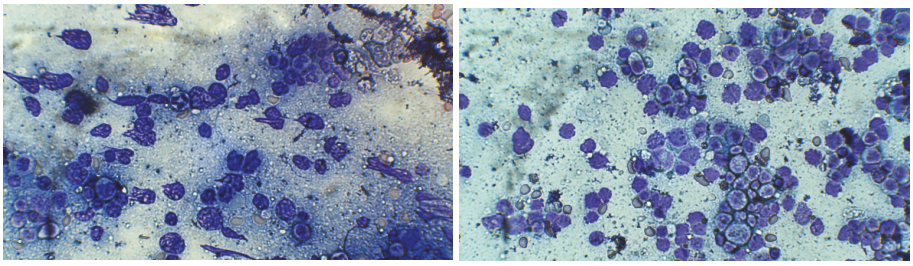
The splenic weight index directly correlates with the content of lymphoid cells in them r = 0.91 (P < 0.001), r = 0.94 (P < 0.001), r = 0.92 (P < 0.001) on day 7, 18 and 25 of the experiment, respectively. Such changes indicate a direct reaction of the spleen to the administration of allogeneic MSC from adipose tissue.

Thus, the administration of allogeneic MSCs isolated from adipose tissue from C57Bl/6 mice causes systemic effects on the thymus and spleen. As a result of antigenic stimulation by allogeneic stem cells, there is an increase in mitotic activity of thy-

2. The content of lymphoid cells and splenic weight index in C57BL/6 mice after allogeneic aMSC administration (M ± m)

Day	The content of lymphoid cells, x 10^6/mg			Splenic weight index after administration of aMSC, %
	intact (n = 6)	administration of 0.89% NaCl, placebo (n = 9)	administration of aMSCs (n = 9)	
7	2.7 ± 0.1	2.9 ± 0.1	3.6 ± 1.1**vv	0.79 ± 0.04* v
18	2.7 ± 0.1	2.8 ± 0.4	3.2 ± 0.1*v	0.79 ± 0.04* v
25	2.7 ± 0.1	2.5 ± 0.1	2.9 ± 0.1	0.46 ± 0.03

Note: * P < 0.05, ** P < 0.01 compared to intact animals; v P < 0.5, vv P < 0.01 compared to placebo group.



A B

Fig. 2. The spleen cellularity on day 7 of the experiment:
A – intact group, B – after aMSC administration, (smear-imprint, x 400)

mocytes and splenocytes. Despite numerous publications that reveal the immunological properties of cells and confirm the presence of immunosuppressive effects, the results of individual scientific studies show that MSCs under certain conditions can be eliminated by cells of the immune system in recipient animals since they have signs of foreignness (Huang et al., 2009).

The increase in the content of lymphoid cells in the thymus and spleen after aMSC administration in our mind may be due to the heterogeneity of introduced cell cultures, an insufficient number of introduced cells for the implementation of immunosuppressive effect, as well as low concentration of immunosuppressive factors synthesized by MSCs.

Conclusion

1. The administration of allogeneic adipose-derived mesenchymal stem cells affects the central and peripheral organs of the immune system.
2. Administration of allogeneic adipose-derived mesenchymal stem cells causes a significant increase in the content of lymphoid cells in the thymus by 71, 57, and 53% ($P < 0.05$) on days 7, 18, and 25, respectively, compared to the control.
3. Thymic weight index directly correlates with the content of lymphoid cells and its value was $r = 0.57$ ($P < 0.01$) on day 7 and $r = 0.50$ ($P < 0.05$) on day 18.
4. Lymphoid cell count in the spleen significantly increase on days 7 and 18 of the immune response by 33 ($P < 0.01$) and 19 % ($P < 0.05$), respectively, compared to the control under the administration of allogeneic adipose-derived mesenchymal stem cells.
5. On the 25th day, the content of lymphoid cells in the spleen and spleen index values return to normal.
6. Splenic weight index directly correlates with the content of lymphoid cells in it – $r = 0.91–0.94$ ($P < 0.001$).

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Анотація. *Дослідження проводили на самцях мишей C57BL/6 вагою 20–24 г, віком 2–3 місяці. Метою нашої роботи було вивчення функціонального стану органів імунної системи у мишей після введення алогенних мезенхімальних стовбурових клітин із жирової тканини. Маніпуляції з отримання первинного матеріалу та культивування мезенхімальних стовбурових клітин здійснювали в стерильному боксі з дотриманням усіх правил асептики й антисептики. Абдомінальну жирову тканину мишей C57BL/6 культивували у CO_2 інкубаторі за температури 37 °C і 5% CO_2 в середовищі DMEM, з додаванням 10–15% фетальної сироватки бичків, 1% антибіотика-антимікотика (Sigma-Aldrich, USA). Для проведення досліджень було сформовано такі групи тварин: 1 група – інтактні (контрольна група); 2 група – тварини, яким у хвостову вену вводили 0,5 см^3 0,9% розчину NaCl (плацебо); 3 група – тварини, яким у хвостову вену вводили 10^4 алогенних мезенхімальних стовбурових клітин із жирової тканини в 0,5 см^3 фосфатно-буферного розчину. Досліджували ваговий індекс, вміст лімфоїдних клітин тимусу та селезінки мишей C57BL/6 за введення мезенхімальних стовбурових клітин із жирової тканини. Для оцінювання вмісту лімфоцитів у лімфоїдних органах, останні зважували (тимус повністю), а селезінку – по 50 мг, потім розтирали та фільтрували через капронову тканину. Після цього гомогенат тканини наносили на градієнт фікол-верографіну (щільність 1,077) у співвідношенні 3:2. Пробірки з вмістом центрифугували зі швидкістю 1500 об/хв., упродовж 30–40 хв. Після центрифугування над шаром градієнта залишається плазма й лімфоцити (не менше 90%), які збирали пастерівською піпеткою і двічі відмивали довільною кількістю розчину Хенкса за допомогою центрифугування за швидкості обертання 1500–1800 об/хв. упродовж 10 хв. Після відмивання до лімфоцитів додавали 1 см^3 розчину Хенкса й підраховували їхню кількість у камері Горяєва. Розрахунок клітинності лімфоїдних органів проводили на 1 мг тканини.*

Трансплантація алогенних мезенхімальних стовбурових клітин із жирової тканини чинить вплив на центральні й периферичні органи імунної системи. За впливу алогенних мезенхімальних стовбурових клітин із жирової тканини відбувається достовірне підвищення вмісту лімфоїдних клітин тимусу на ранніх і пізніх етапах імунної відповіді на 7, 18 та 25 добу відповідно на 71%, 57 і 53% ($P < 0,05$) порівнюючи з контролем. Кількість лімфоїдних клітин у селезінці достовірно зростала на 7 та 18 добу імунної відповіді відповідно на 33% та 19%, ($P <0,01$, $P <0,05$) порівнюючи з контролем за введення алогенних мезенхімальних стовбурових клітин, одержаних із жирової тканини. На 25 добу показники вмісту лімфоїдних клітин та індексу селезінки повертаються до норми. Індекси ваги тимуса й селезінки прямо корелюють з вмістом лімфоїдних клітин у цих органах.

Ключові слова: *миші, ваговий індекс, лімфоїдні клітини, тимус, селезінка, алогенні мезенхімальні стовбурові клітини, жирова тканина*

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IMMUNOLOGICAL INDICATORS IN ANIMAL ORGANISMS UNDER THE INFLUENCE OF ALLOGENEIC ADIPOSE TISSUE-DERIVED MESENCHYMAL STEM CELLS

***L. V. KLADNYTSKA*[1],** *Doctor of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0002-9360-0587
***A. Y. MAZURKEVYCH*[1],** *Doctor of Veterinary Sciences, Professor*
http://orcid.org/0000-0002-2409-7703
***L. V. GARMANCHUK*[2],** *Doctor of Biological Sciences, Professor*
http://orcid.org/0000-0002-1527-2346
***M. O. MALIUK*[1],** *Doctor of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0003-3019-6035
***S. V. VELYCHKO*[3],** *Candidate of Biological Sciences*
http://orcid.org/0000-0003-1842-5114
***T. A. MAZURKEVYCH*[1],** *Doctor of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0002-1294-5939
***V. V. KOVPAK*[1],** *Doctor of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0003-2419-1246
***V. B. DANILOV*[1],** *Candidate of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0002-2897-5235
***Yu. O. KHARKEVYCH*[1],** *Candidate of Veterinary Sciences, Associate Professor*
http://orcid.org/0000-0002-7877-8272
***R. R. BOKOTKO*[1],** *Candidate of Veterinary Sciences, Assistant*
http://orcid.org/0000-0002-6217-5266
***T. L. SAVCHUK*[1],** *Candidate of Veterinary Sciences, Senior Lecturer*
http://orcid.org/0000-0002-7351-5684
[1]National University of Life and Environmental Sciences of Ukraine, 15 Heroiv oborony st., Kyiv 03041, Ukraine
[2]Taras Shevchenko National University of Kyiv, 60 Volodymyrska st., Kyiv 01033, Ukraine
[3] Veterinary hospital, Holosiivskyi Avenue 105 B, Kyiv 01041, Ukraine,
E-mail: Kladlarisa@ukr.net

Abstract. *The studies were conducted on 2–3 months old males of C57BL/6 mice, weighing 20–24 g. Our work aimed at studying the functional state of the organs of the immune system in mice after administration of allogeneic mesenchymal stem cells of adipose tissue origin. Obtaining and cultivating mesenchymal stem cells were carried out in a sterile laminar box with compliance of asepsis and antiseptic conditions. The abdominal adipose tissue from*

C57BL/6 mice was cultured in a CO_2 incubator at 37 °C and 5% CO_2 in DMEM with 10–15% of fetal bovine serum, 1% of antibiotic/antimycotic solution (Sigma-Aldrich, USA). The following groups of animals were formed: 1 group – intact (control); 2 group – animals, to whom 0.5 cm^3 of 0.9% NaCl solution (placebo) were injected into the caudal vein; 3 group – animals, to whom 10^4 of allogeneic mesenchymal stem cells from adipose tissue in 0.5 cm^3 of phosphate buffer solution were injected into the caudal vein. The weight index, the content of lymphoid cells in the thymus and spleen in C57BL/6 mice were examined on days 7, 18, and 25 after the administration of mesenchymal stem cells. To assess the lymphocyte content in lymphoid organs, they were weighed (whole thymus, 50 mg of spleen), triturated, and filtered through the nylon cloth. After that, the tissue homogenate was applied to Ficoll-Urografin density gradient (d = 1.077) in a ratio of 3:2. The test tubes were centrifuged at 1500 rpm for 30–40 minutes. After centrifugation, plasma and layer of lymphocytes were above the density gradient; lymphocytes were collected by a Pasteur pipette and washed twice with an arbitrary amount of Hank's solution by centrifugation at 1500 rpm for 10 minutes. After washing, 1 cm^3 of Hank's solution was added to lymphocytes and they were counted in the Goryaev chamber. Calculation of the cellularity of lymphoid organs was performed in 1 mg of tissue.

The transplantation of allogeneic adipose-derived mesenchymal stem cells affects the central and peripheral organs of the immune system. Administration of allogeneic adipose-derived mesenchymal stem cells causes a significant increase in the content of lymphoid cells in the thymus on days 7, 18, and 25 by 71%, 57, and 53% ($P < 0.05$), respectively, compared to the control. At the 7 and 18 days of the immune response, lymphoid cell content in the spleen significantly increases by 33% ($P < 0.01$) and 19% ($P < 0.05$), respectively, compared to the control under the administration of allogeneic adipose-derived mesenchymal stem cells. On day 25, values of lymphoid cell content and spleen index return to normal. The thymus and spleen weight indices directly correlate with their lymphoid cell content.

Keywords: *mice, weight index, lymphoid cells, thymus, spleen, allogeneic mesenchymal stem cells, adipose tissue*

Introduction

Important biological features of mesenchymal stem cells (MSCs), in particular, the ability to migrate to the inflammation site, low immunogenicity, immunomodulatory activity, and the ability to stimulate hemopoiesis, make them potentially active regulators of reparative processes (Kladnytska et al., 2014).

At the present stage of the development of biological sciences, different approaches are developed for the use of MSCs in the treatment of various diseases, and several preclinical and clinical trials have already been conducted, the results of which have shown the effectiveness of their application (Haghighat et al., 2011; Reich et al., 2012; Arnhold & Wenisch, 2015; Jakobsen et al., 2017).

An alternative source of MSCs is adipose tissue, which contains stem cells in a higher percentage than bone marrow. Obtaining adipose tissue is a less traumatic procedure for the donor than the obtaining of bone marrow both during the process of primary material obtaining and postoperative period (Marx et al., 2014; Kladnytska et al., 2017).

Regardless of MSCs origin, they have pronounced immunosuppressive activity: they block *in vitro* differentiation of naive CD4+ T cells in Th17 and

suppress the synthesis of many cytokines such as IL-17, IL-22, interferon-gamma, and TNFα (Bartholomew et al., 2002; Gryschenko & Tomchuk, 2013).

Despite the large number of publications confirming the immunosuppressive properties of MSCs, some works deny such effects on immune responses (Di Nicola et al., 2002; Aggarwal & Pittenger, 2005; Djouad et al., 2005).

Thus, it was found that transplantation of MSCs stimulates antibody production and increases the cellularity of the bone marrow in recipients. With increasing the number of administered cells, a significant increase in the thymus cellularity and decrease in spleen cellularity were recorded. The authors suggest that a significant dose of MSCs creates a suppressive microenvironment for lymphoid cells that is accompanied by the inhibition of the immune response (Le Blanc et al., 2003; Batten et al., 2006; Lu et al., 2009).

Opposite, a small number of transplanted cells due to homing is collected in the bone marrow niches, contributing to hematopoiesis, where the myeloid sprout can act as an impressive factor in natural immunity (Nikolskaya et al., 2012).

So, it is not well known about the effect of MSCs on the response of the immune organs, in particular, on the functional state of the thymus and spleen. Taking into account such controversial data on the influence of MSCs on the organs of the immune system, these issues require further research.

The purpose of our work was to study the functional state of the organs of the immune system in C57BL/6 mice after the administration of allogeneic adipose MSCs.

Materials and methods of researches

The studies were carried out on 2–3 months old males of C57BL/6 mice, weighing 20–24 g. All experiments were conducted in accordance with the Good Laboratory Practices regulations in research studies using animals, the Law of Ukraine On the Protection of Animals from Brutal Treatment, and the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes.

MSCs obtaining from adipose tissue. Obtaining and cultivating adipose MSCs (aMSCs) were carried out in a sterile laminar box with compliance of asepsis and antiseptic conditions. The mice were euthanized, samples of abdominal adipose tissue were washed three times with sterile phosphate buffer solution with the addition of 1% antibiotic/antimycotic solution (Sigma-Aldrich, USA). Then samples of adipose tissue were chopped into pieces of 1–3 mm^3 and placed to culture dishes filled with DMEM, 10–15% of fetal bovine serum, 1% antibiotic/antimycotic solution (Sigma-Aldrich, USA) and cultured in a CO_2 incubator at 37 ^{0}C and 5% CO_2. The culture medium was partially or completely changed by fresh medium every 3 days during cultivation. After formation of cell monolayer by 80–90%, cells were removed with trypsin/ethylenediaminetetraacetic acid solution (EDTA), washed with phosphate buffer, and placed into Petri dishes for further cultivation. Cell passaging provided a reduction in the heterogeneity of cell culture and the development of biological material for transplantation (Kladnytska et al., 2016). MSCs at passage 4 were used for transplantation.

MSC administration to mice. The following groups of animals were formed: 1 – intact (control); 2 – animals, to whom 0.5 mL of 0.9% NaCl solution (placebo) were injected into the caudal vein; 3 – animals, to whom 10^4 of allogeneic aMSCs in 0.5 mL of phosphate buffer solution were injected into the caudal vein.

Estimation of the thymic and splenic weight indices in mice after aMSC admin-

istration. Indicators of the weight of peripheral lymphoid organs relative to the body weight (weight index) of animals were evaluated on days 7, 18, and 25 after aMSC administration. The mice were pre-weighed for weight control. At each experimental period, 3 animals were euthanized in each group and the weight index of lymphoid organs and their cellularity were studied. Euthanasia of animals was carried out using carbon dioxide; lymphoid organs (thymus and spleen) were removed and their mass was determined. Indices of lymphoid organs in relation to the weight of the animal were calculated according to the formula:

Evaluation of the thymic and splenic cellularity after aMSC administration. To assess the content of lymphocytes in lymphoid organs, the latter were weighed. The whole thymus and 50 mg of spleen were triturated and filtered through the nylon cloth. After that, the cell homogenate was applied to Ficoll-Urografin density gradient (d = 1.077) in a ratio of 3:2. The test tubes were centrifuged at a rate of 1500 rpm for 30–40 minutes. After centrifugation, the layer of lymphocytes, which was located above the density gradient, was collected by a Pasteur pipette and washed twice with an arbitrary amount of Hank's solution by centrifugation at a rate of 1500 rpm for 10 minutes. After washing, 1 ml of Hank's solution was added to lymphocytes. Lymphocytes were counted in the Goryaev chamber. Calculation of the lymphoid organ cells was performed on 1 mg of tissue.

Results of the research and their discussion

The functional state of the organs of immunogenesis largely depends on the ratio of processes of immune cell proliferation and apoptosis, that almost are not studied after MSC administration.

After allogeneic aMSC administration, the content of lymphoid cells in the thymus on days 7, 18, and 25 significantly increased by 71%, 57, and 53%, respectively, compared with control animals (Table 1).

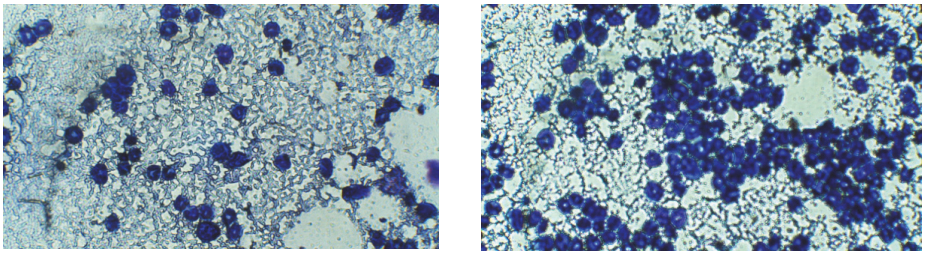
Compared with a placebo group, the content of lymphoid cells in the thymus was significantly increased by 42%, 69, and 86%, respectively. The increase in thymic cellularity is associated with the activation of residential thymocyte proliferation due to antigenic stimulation by MSCs that is consistent with the studies of Huang et al. (2009). The thymus contains T lymphoblasts, immature and mature lymphocytes, supporting and secretory cells of the thymus stromal component (Fig. 2).

A positive correlation between the content of lymphoid cells and thymic weight index was found 7 days after aMSCs administration. Thymic weight index directly correlates with the content of lymphoid cells and its value was

1. The content of lymphoid cells and the weight index of the thymus in C57BL/6 mice after allogeneic aMSC administration (M ± m, n = 9)

Day	The content of lymphoid cells, x 10^6/mg			thymic weight index after administration of aMSC, %
	intact (n = 6)	administration of 0.89% NaCl, placebo (n = 9)	administration of aMSCs (n = 9)	
7	1.4 ± 0.1	1.9 ± 0.2	2.7 ± 0.1* v	0.19 ± 0.03* v
18	1.4 ± 0.1	1.3 ± 0.1	2.2 ± 0.1* v	0.16 ± 0.01* v
25	1.7 ± 0.1	1.4 ± 0.1	2.6 ± 0.3* vv	0.17 ± 0.01* v

Note: * P < 0.05, ** P < 0.01 compared to intact animals; v P < 0.05, vv P < 0.01 compared to placebo group.



A B

Fig. 2. Thymic cellularity on day 7 of the experiment:
A – intact group, B – after aMSC administration (smear-imprint, x 400)

r = 0.57 (P < 0.01) on day 7 and r = 0.50 P < 0.05 on day 18 of the experiment.

Under the influence of MSCs from adipose tissue, the indicator of splenic weight index was significantly increased until day 18 of the experiment (Table 2). On day 25, the splenic weight index did not significantly differ from that in the experimental group and placebo animals, but only observed a tendency to increase it.

The spleen, as the peripheral organ of the immune system, is also involved in the process of forming an immune response to the antigen. After MSC administration, the content of lymphoid cells in the spleen significantly exceeded the parameters of spleen cellularity in intact animals (Table 2). Smear-imprint contains erythroid cells, neutrophil granulocytes, monocytes. and lymphoid cells.

The number of lymphoid cells significantly increased by 33 and 24% compared to intact animals and the placebo group on day 7 of the experiment. On day 18 of the experiment, the spleen cellularity under the influence of MSCs from adipose tissue was significantly higher by 19 and 14%, respectively. On day 25 of the experiment, the lymphoid cell count was higher by 7 and 15% within the tendency.

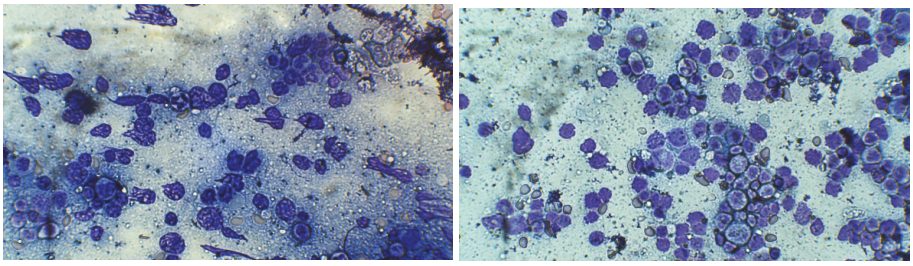
The splenic weight index directly correlates with the content of lymphoid cells in them r = 0.91 (P < 0.001), r = 0.94 (P < 0.001), r = 0.92 (P < 0.001) on day 7, 18 and 25 of the experiment, respectively. Such changes indicate a direct reaction of the spleen to the administration of allogeneic MSC from adipose tissue.

Thus, the administration of allogeneic MSCs isolated from adipose tissue from C57Bl/6 mice causes systemic effects on the thymus and spleen. As a result of antigenic stimulation by allogeneic stem cells, there is an increase in mitotic activity of thy-

2. The content of lymphoid cells and splenic weight index in C57BL/6 mice after allogeneic aMSC administration (M ± m)

Day	The content of lymphoid cells, x 10^6/mg			Splenic weight index after administration of aMSC, %
	intact (n = 6)	administration of 0.89% NaCl, placebo (n = 9)	administration of aMSCs (n = 9)	
7	2.7 ± 0.1	2.9 ± 0.1	3.6 ± 1.1**vv	0.79 ± 0.04* v
18	2.7 ± 0.1	2.8 ± 0.4	3.2 ± 0.1*v	0.79 ± 0.04* v
25	2.7 ± 0.1	2.5 ± 0.1	2.9 ± 0.1	0.46 ± 0.03

Note: * P < 0.05, ** P < 0.01 compared to intact animals; v P < 0.5, vv P < 0.01 compared to placebo group.



A B

Fig. 2. The spleen cellularity on day 7 of the experiment:
A – intact group, B – after aMSC administration, (smear-imprint, x 400)

mocytes and splenocytes. Despite numerous publications that reveal the immunological properties of cells and confirm the presence of immunosuppressive effects, the results of individual scientific studies show that MSCs under certain conditions can be eliminated by cells of the immune system in recipient animals since they have signs of foreignness (Huang et al., 2009).

The increase in the content of lymphoid cells in the thymus and spleen after aMSC administration in our mind may be due to the heterogeneity of introduced cell cultures, an insufficient number of introduced cells for the implementation of immunosuppressive effect, as well as low concentration of immunosuppressive factors synthesized by MSCs.

Conclusion

1. The administration of allogeneic adipose-derived mesenchymal stem cells affects the central and peripheral organs of the immune system.
2. Administration of allogeneic adipose-derived mesenchymal stem cells causes a significant increase in the content of lymphoid cells in the thymus by 71, 57, and 53% ($P < 0.05$) on days 7, 18, and 25, respectively, compared to the control.
3. Thymic weight index directly correlates with the content of lymphoid cells and its value was $r = 0.57$ ($P < 0.01$) on day 7 and $r = 0.50$ ($P < 0.05$) on day 18.
4. Lymphoid cell count in the spleen significantly increase on days 7 and 18 of the immune response by 33 ($P < 0.01$) and 19 % ($P < 0.05$), respectively, compared to the control under the administration of allogeneic adipose-derived mesenchymal stem cells.
5. On the 25th day, the content of lymphoid cells in the spleen and spleen index values return to normal.
6. Splenic weight index directly correlates with the content of lymphoid cells in it – $r = 0.91–0.94$ ($P < 0.001$).

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Анотація. *Дослідження проводили на самцях мишей C57BL/6 вагою 20–24 г, віком 2–3 місяці. Метою нашої роботи було вивчення функціонального стану органів імунної системи у мишей після введення алогенних мезенхімальних стовбурових клітин із жирової тканини. Маніпуляції з отримання первинного матеріалу та культивування мезенхімальних стовбурових клітин здійснювали в стерильному боксі з дотриманням усіх правил асептики й антисептики. Абдомінальну жирову тканину мишей C57BL/6 культивували у CO_2 інкубаторі за температури 37 °C і 5% CO_2 в середовищі DMEM, з додаванням 10–15% фетальної сироватки бичків, 1% антибіотика-антимікотика (Sigma-Aldrich, USA). Для проведення досліджень було сформовано такі групи тварин: 1 група – інтактні (контрольна група); 2 група – тварини, яким у хвостову вену вводили 0,5 см^3 0,9% розчину NaCl (плацебо); 3 група – тварини, яким у хвостову вену вводили 10^4 алогенних мезенхімальних стовбурових клітин із жирової тканини в 0,5 см^3 фосфатно-буферного розчину. Досліджували ваговий індекс, вміст лімфоїдних клітин тимусу та селезінки мишей C57BL/6 за введення мезенхімальних стовбурових клітин із жирової тканини. Для оцінювання вмісту лімфоцитів у лімфоїдних органах, останні зважували (тимус повністю), а селезінку – по 50 мг, потім розтирали та фільтрували через капронову тканину. Після цього гомогенат тканини наносили на градієнт фікол-верографіну (щільність 1,077) у співвідношенні 3:2. Пробірки з вмістом центрифугували зі швидкістю 1500 об/хв., упродовж 30–40 хв. Після центрифугування над шаром градієнта залишається плазма й лімфоцити (не менше 90%), які збирали пастерівською піпеткою і двічі відмивали довільною кількістю розчину Хенкса за допомогою центрифугування за швидкості обертання 1500–1800 об/хв. упродовж 10 хв. Після відмивання до лімфоцитів додавали 1 см^3 розчину Хенкса й підраховували їхню кількість у камері Горяєва. Розрахунок клітинності лімфоїдних органів проводили на 1 мг тканини.*

Трансплантація алогенних мезенхімальних стовбурових клітин із жирової тканини чинить вплив на центральні й периферичні органи імунної системи. За впливу алогенних мезенхімальних стовбурових клітин із жирової тканини відбувається достовірне підвищення вмісту лімфоїдних клітин тимусу на ранніх і пізніх етапах імунної відповіді на 7, 18 та 25 добу відповідно на 71%, 57 і 53% ($P < 0,05$) порівнюючи з контролем. Кількість лімфоїдних клітин у селезінці достовірно зростала на 7 та 18 добу імунної відповіді відповідно на 33% та 19%, ($P <0,01$, $P <0,05$) порівнюючи з контролем за введення алогенних мезенхімальних стовбурових клітин, одержаних із жирової тканини. На 25 добу показники вмісту лімфоїдних клітин та індексу селезінки повертаються до норми. Індекси ваги тимуса й селезінки прямо корелюють з вмістом лімфоїдних клітин у цих органах.

Ключові слова: *миші, ваговий індекс, лімфоїдні клітини, тимус, селезінка, алогенні мезенхімальні стовбурові клітини, жирова тканина*

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STERILITY MONITORING OF CANINE PACKED RED BLOOD CELLS

O. V. YEHOROV, *PhD student, Department of Surgery and Pathophysiology named after Academician I. O. Povazhenko*
https://orcid.org/0000-0002-6095-2244
M. O. MALYUK, *Doctor of Veterinary Sciences, Professor, Department of Surgery and Pathophysiology named after Academician I. O. Povazhenko*
https://orcid.org/0000-0003-3019-6035
H. V. KOZLOVSKA, *Candidate of Veterinary Sciences, Associate Professor, Department of Epizootology, Microbiology and Virology*
https://orcid.org/0000-0003-1149-9970
National University of Life and Environmental Sciences of Ukraine,
15 Heroiv Oborony st., Kyiv 03041, Ukraine
E-mail: o.yehorov@it.nubip.edu.ua; nikolai_malyuk@ukr.net; kozlovska@nubip.edu.ua

Abstract. *A constant risk factor of transfusion is microbial contamination of whole blood and its components. Because using contaminated blood products can lead to sepsis and high risks to the health of recipients. Blood can be a good nutrient medium for microorganisms, so the risk of bacteria growth in any blood component after it has been donated is significant. Violation in the rules of asepsis during blood donation, processing of blood products, damage to blood collection systems or their tightness, etc. can cause microbial contamination.*

We examined 5 samples of preserved canine packed red blood cells. The donor animals were 5 clinically healthy dogs. Blood was collected in closed systems with a CPDA anticoagulant. After following centrifuging, plasma was separated from red blood cells in different containers. The remaining packed red blood cells were stored at a temperature of +2–6°C for 30 days.

The bacteria cultivation method is considered the "gold standard" for assessing the presence of microbial contamination in most blood transfusion centers. The tested canine packed red blood cells samples were inoculated into thioglycolate and Sabouraud media and incubated in a thermostat at +20–25 °C. The incubation period was 14 days. According to the results of the bacteriological examination of these samples of canine packed red blood cells after their storage, a non-sterile sample was no found.

Thereby, the method of blood collection using closed systems with CPDA anticoagulant is reliable and allows obtaining donor blood and its components without loss of sterility during long-term storage.

Keywords: *canine packed red blood cells, microbial contamination, donor blood sterility*

Introduction

This work is a continuation of a series of our studies on animal transfusion, in which we study the effect of blood sampling techniques and type of using systems on the sterility of animals' canned whole blood and blood components during long-term storage under hypothermic conditions (Yehorov et al., 2020).

Bacterial contamination of animals' donor blood and blood components always are high risk and a problem of clinical veterinary transfusion. The probability of contamination exists at all stages of blood sampling and processing of its components. For assessing the quality and safety of such preparations, it is important to control sterility by microbiological tests (Ischenkova et al., 2015).

Sterility monitoring of canned animals' blood and blood components needs to identify possible contamination by aerobic and anaerobic microorganisms. The reason for this is the permanent risk of iatrogenic factors even all rules of asepsis during blood collection and storage are complied, and microbial contamination can further endanger the health and life of animal recipients (Lyubich, 2014).

Frequent reasons for microbial contamination of blood components during their processing may be leaks in any part of containers or improper techniques during plasma extraction or division of the volume of the transfusion preparation into several doses (Lasta et al., 2020).

Contamination is also possible during blood sampling from donors. Thus, there have been reported cases of microbial contamination of collected blood with *S. Marcescens*, when using inappropriate concentrations of disinfectant solutions for treating the animal skin at the site of blood sampling (Miglio et al., 2016).

Analysis of recent researches and publications

Before using, blood components usually are visually inspected and bacterial infection should be suspected when there is a discoloration of the component, hemolysis of the upper layers of red blood cells, or presence of visible clots. If such signs are detected, a bacteriological examination of this unit should be provided to determine if contamination occurred (Rodrigues et al., 2020).

The bacteria cultivation method is considered the "gold standard" for assessing blood contamination in most blood transfusion centers.

The process of bacterial culture growth is slow because microorganisms need time to develop and reach a significant number of cells. For this reason, it is possible to use an alternative PCR method because its analytical sensitivity is higher and the time required to obtain the result is much shorter (Wardrop et al., 2005).

However, the virulence of contaminating microorganisms determines their ability to grow under storage conditions. Only the presence of bacteria in the blood is less important than their ability to replicate, which causes serious septic complications in patients (Miglio et al., 2016).

During testing sterility of blood transfusion preparations, the possibility of microorganisms detecting is directly proportional to their number in the test sample and depends on the ability of these microorganisms to grow on the nutrient media with visible signs. At a low degree of contamination, the probability of detecting microorganisms is very low, even in the case of uniform microbial contamination.

Therefore, the purpose of the sterility test is to prove the absence of viable microorganisms in the blood component sample with maximum reliability. The

main factors that determine the effectiveness of sterility monitoring are the testing sample volume, seeding technique, nutrient composition, time, and temperature of culture incubation (Lyubich, 2014).

Purpose. The purpose of the study is to test the sterility of canine packed red blood cells during quality control of these blood components after long-term hypothermic storage.

Materials and methods of research

The material for the study was samples of packed red blood cells that were collected from 5 blood donor dogs. Donor animals were clinically healthy.

Blood was collected from the jugular vein of dogs by closed systems (Fig. 1).

Before donation, a puncture site was shaved and treated with 70% alcohol. Donor blood from dogs was collected in double polymer containers with CPDA anticoagulant (sodium citrate, sodium phosphate, dextrose, adenine) (Kisielewicz & Self, 2014).

The volume of blood sampling was calculated at 12 cm^3 per 1 kg of animal weight. This amount minimizes unwanted donor cat risks during blood collection (blood pressure and heart rate) and after donation (Helm & Knottenbelt, 2010).

All blood samples were typed by a test "RapidVet-H Canine DEA 1". All animals in this study had a DEA 1.1 negative blood group (Proverbio et al., 2019). For morphological analysis of canine donor whole blood, we tested samples by automatic Mindray BC2800 hematology analyzer, the main results are shown in Table 1.

After blood collection, the double containers were centrifuged at 2500 rpm for 20 minutes on Hettich ROTANTA 460R centrifuge. After that, blood plasma was separated from the red blood cells in different containers by a plasma extractor with keeping the system closed (Guide, 2013) (Fig. 2).

The erythrocyte mass of canine blood, or canine packed red blood cells (pRBC), was stored at a temperature of + 2–6 °C for 30 days in VESTFROST AKG 317 refrigerator in the

Fig. 1. Blood sampling using a closed system in a dog

1. The results of hematology analysis in donor dogs (M ± m, n = 5)

Red blood cells, 10^{12}/L	Hemoglobin, g/L	White blood cells, 10^{9}/L	Hematocrit, %
7.3 ± 1.1	175.4 ± 7.3	9.8 ± 2.1	52.2 ± 4.9

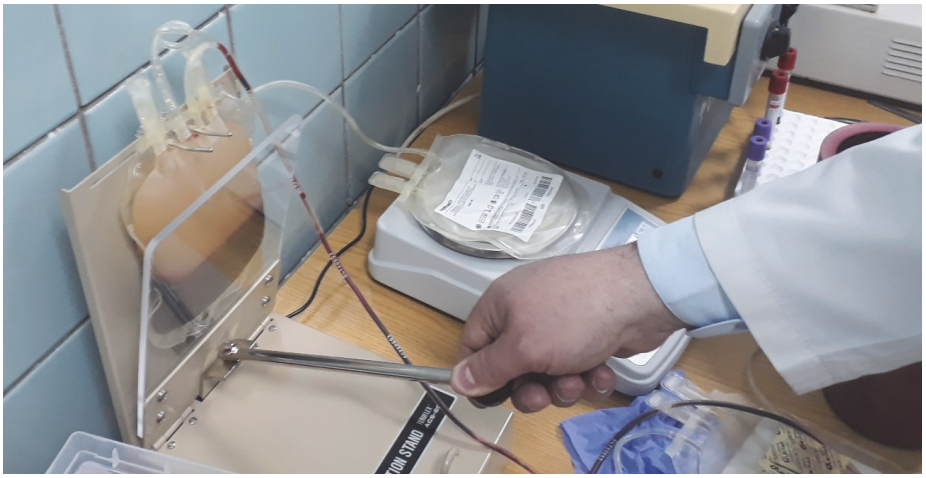

Fig. 2. Separation of blood plasma from red blood cells

educational and scientific laboratory (ESL) "Animals Blood Bank" at the National University of Life and Environmental Sciences of Ukraine (Fig. 3).

Bacteriological examination for sterility of canned pRBC samples was performed according to the approved Instruction of the Ministry of Health of Ukraine (Instruction, 1999) in the laboratory of the Department of Epizootology, Microbiology and Virology at NULES of Ukraine.

For testing, we used sterile pipettes, closed with cotton plugs, rubber bulbs, sterilized tools, which were in a container with 96 % ethanol during manipulation.

Polymer containers with pRBC were checked for leaks in the lab pre-box, then wiped with 70% ethanol. Before inoculation, we clamped the container tube above the node and cut the tube between the node and clamp. The cut end of the tube was quickly passed through the flame, and a pipette was introduced into it. Reducing the pressure to the clamp, we pressed the container and piped at least 2 cm^3 of sample.

Fig. 3. The refrigerator for pRBC storage

Inoculation of each sample was made in a thickness of culture medium without blowing a separate pipette but with a rubber bulb and without previous flame processing. Used pipettes laid in a disinfectant solution.

The tested pRBC sample was inoculated with 1 ml into two tubes containing 20 cm^3 of thioglycollate broth and one tube with 20 cm^3 of Sabouraud medium. In parallel, two tubes with thioglycolate broth and one with Sabouraud medium were left untreated for controlling the sterility of the nutrient medium during the entire period of samples incubation. Cultures in thioglycolate broth and control tubes were incubated in a thermostat at 20–25 °C and 30–35 °C, with Sabouraud medium – at 20–25 °C (Fig. 4). The incubation period was 14 days for both culture media (Instruction, 1999).

Accounting and interpretation of sterility test results were viewed daily. The presence of microorganisms in nutrient media was assessed visually macroscopically (we looked for turbidity, film, sediment, inclusions) and microscopically.

Fig. 4. The thermostat in the Microbiological laboratory

Results of the research and their discussion

Currently, in Ukraine, there is no legal regulation of animal blood donation, including the quality control of canned blood and its components. There are few publications on the results of experimental studies of bacterial contamination monitoring during storage of animals' donor blood with CPDA. However, there are similar norms and research in human medicine.

According to the Sterility Control Instruction (Instruction, 1999), sterility monitoring of canned donor blood and its components is one of the main parameters for assessing their quality. This rule applies in the field of human health. Given this, the purpose of our work was aimed to assess the suitability of closed systems for blood collection in dogs and following preparation of pRBC as a risk factor for bacterial contamination of these preparations during prolonged hypothermic storage.

In total, we examined 5 samples of pRBC from dogs aged 2 to 5 years, which were used as donors in ESL "Animals Blood Bank" of the Department of Surgery and Pathophysiology named after academic I. O. Povazhenko at NULES of Ukraine. The mentioned samples of canine pRBC were tested after 30 days of refrigeration storage at +2–6 °C.

During hypothermic storage, an external inspection of polymer containers with test samples of pRBC showed no signs of bacterial growth: color changes from dark purple to light red or green, signs of red blood cell hemolysis, presence of visible clots.

2. The results of pRBC samples examination for sterility

The animal	Contamination	The degree of contamination
1	–	–
2	–	–
3	–	–
4	–	–
5	–	–

Note: "–" – no contamination, "+" – presence of contamination, "*" – low degree, "**" – mild degree, "***" – significant contamination.

The results of the bacteriological examination of pRBC samples using thioglycolate and Sabouraud media showed that all 5 test samples with a shelf life of 30 days were sterile. The research results were entered into the journal in the form of a table (Table 2).

Conclusions and future perspectives

The results of our research indicate that closed blood collection systems in dogs with aseptic manipulations are reliable and allow to obtain sterile donor blood and its erythrocyte mass for 30 days.

However, there is a need for further research on the quality of canned canine packed red blood cells to assess the level of hemolysis, red blood cell count, and other indicators to obtain a safe animal transfusion product for a recipient.

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Анотація. *Під час проведення трансфузії цільною кров'ю та препаратами крові постійним ризиком є фактор їхнього мікробного забруднення. Адже застосування контамінованих препаратів крові може спричиняти сепсис та високі ризики для здоров'я реципієнтів. Кров може бути живильним середовищем для мікроорганізмів, тому досить значним є ризик росту кількості бактерій у будь-якому компоненті крові після її відбору. До мікробного забруднення може призвести порушення правил асептики під час забору донорської крові, виготовлення препаратів крові, пошкодження систем забору крові, їхньої герметичності тощо.*

Було досліджено 5 проб консервованої еритроцитарної маси собак. Тваринами-донорами були 5 клінічно здорових собак. Кров відбирали з використанням закритих систем із антикоагулянтом ЦФДА. Після наступного центрифугування плазму відокремлювали від еритроцитів у інший контейнер. Еритроцитарну масу, що залишалася, зберігали за температури + 2–6 °С упродовж 30 діб.

Метод посіву бактерій розглядається як «золотий стандарт» для оцінювання наявності мікробного забруднення в більшості центрів переливання крові. Досліджувані зразки еритроцитарної маси собак інокулювали в тіогліколеве середовище та середовище Сабуро й інкубували в термостаті за 20–25 °С. Інкубаційний період становив 14 діб. За результатами бактеріологічного дослідження проб еритроцитарної маси собак після їхнього зберігання, жодної нестерильної проби не було виявлено.

Отже, метод забору крові з використанням закритих систем, що містять антикоагулянт ЦФДА, є надійним і дає змогу одержувати донорську кров та компоненти крові без втрати стерильності за тривалого терміну зберігання.

Ключові слова: *еритроцитарна маса крові собак, мікробна контамінація, стерильність донорської крові*

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THE USE OF GADOLINIUM ORTHOVANADATE NANOPARTICLES FOR THE CORRECTION OF REPRODUCTIVE ABILITY IN BOARS UNDER OXIDATIVE STRESS

V. I. KOSHEVOY*[1]*, *PhD Student , Department of Veterinary Reproductology*
https://orcid.org/0000-0003-2938-2762
S. V. NAUMENKO*[1]*, *PhD, Associate Professor,*
Department of Veterinary Reproductology
https://orcid.org/0000-0002-7340-5186
V. K. KLOCHKOV*[2]*, *PhD, Senior Research Fellow,*
Department of Nanostructured Materials
https://orcid.org/0000-0002-8080-1195
S. L. YEFIMOVA*[2]*, *Doctor of Physical and Mathematical Sciences, Professor,*
Department of Nanostructured Materials
https://orcid.org/0000-0003-2092-1950
[1]Kharkiv State Zooveterinary Academy, 1 Academic st.,
Mala Danylivka 62341 Kharkiv Oblast, Ukraine
[2]Institute of Scintillation Materials of the National Academy of Sciences of Ukraine, 60 Nauky ave., Kharkiv 61072, Ukraine
E-mail: vsevolod_koshevoy@yahoo.com

Abstract. *The search for effective and safe means for correcting male hypofertility is an urgent problem of modern reproductology. Researchers have proved the possibility of the use of nanoparticles based on oxides of rare-earth elements for the treatment of disorders of male reproductive function. We investigated the effectiveness of the use of gadolinium orthovanadate nanoparticles activated by europium, the size of which was 25×8 nm in the dose of 0.0125 mg per kg of live weight to correct reproductive ability decrease in boars under oxidative stress. After 14 days of hydrosol nanoparticles administration, we experimentally established the dynamics of the content of oxidative stress markers and stable metabolites in the nitrogen oxide cycle, and also determined the changes in sperm quality indicators. Thus, conjugated dienes concentration in the blood serum of boars on the 15th day tended to decrease, and on the 30th day of the study was lower by 9.4% compared with the group of animals before administration. At the same time, the amount of thiobarbituric acid reactive substances decreased on the 15th day of the study by 24.7%, and the 30th day – by 48.2%, which indicates the normalization of oxidative processes in males. We noted positive changes in the system*

[1] scientific supervisor – PhD, Associate Professor S. V. Naumenko

of the nitrogen oxide cycle, the content of stable metabolites on the 15th day was lower by 25.2% than in animals before administration and on the 30th day – by 42.6%. At the same time, we observed an improvement in the boar sperm quality, especially motility and the number of motile sperm in the ejaculate increased by 42.9 and 57.1% on the 60th day of the study and by 95.2% and 1.48 times on the 90th day, respectively. In this case, the volume of ejaculate, sperm concentration, and sperm content with morphological anomalies with the introduction of nanoparticles normalized and almost reached the values of the control group. The researchers are interested in further elucidating the effect of correction of reduced reproductive ability in boars under oxidative stress with gadolinium orthovanadate nanoparticles on the hormonal background and the state of enzymatic and non-enzymatic systems of antioxidant protection.

Keywords: *sperm quality, prooxidant-antioxidant system, peroxidation, oxidative stress markers, nitrogen oxide cycle, nanomaterials*

Introduction

The main cause of reduced reproductive ability in males (hypofertility) is a negative impact of oxidative stress accompanied by an intensification of the synthesis of reactive oxygen species (ROS), and at the same time, nitrogen (RNS) and sulfur, an accumulation of toxic peroxidation products and reduced antioxidant activity of antioxidant defense system (ADS) (Bisht et al., 2017; Barik et al., 2019; Koshevoy & Naumenko, 2020; Otasevic et al., 2020).

The problem of research is a significant spread of factors that cause oxidative stress in males and the lack of harmless means for the correction, which would also have pronounced effectiveness and contribute to the rapid improvement of sperm quality (Mayorga-Torres et al., 2016; Agarwal et al., 2018).

Analysis of recent researches and publications

The main means for the correction of oxidative stress include groups of drugs that directly or indirectly have an antioxidant effect or reduce the intensity of peroxidation processes by eliminating toxic products. The mechanism of action of these drugs depends on the peculiarities of their pharmaceutical composition. Vitamin and mineral drugs make an effect by improving the activity of the non-enzymatic part of ADS. For instance, carotenoids in the body contribute to vitamin A synthesis in the liver and activation of ADS processes, as well as the introduction of tocopherols and ascorbic acid (Domoslawska et al., 2018; Skliarov et al., 2020).

The enzymatic ADS is affected by the introduction of microelements such as zinc and copper. They are a part of one of the main enzymes in the first link of ADS – superoxide dismutase, which acts as a substrate for another powerful enzyme – catalase (Palani, 2018; Scarlata & O'Flaherty, 2020). Drugs based on succinic acid, which is the main component of Roberts' shunt and has its effect in the nervous system (Zarubina et al., 2012), are characterized by a powerful antioxidant effect. In this case, the significant role of the nitrogen oxide cycle and its metabolites in the functioning of the male reproductive system and the relationship with sperm activation and sperm quality (Liman & Alan, 2016) were proved.

A promising direction in the creation of oxidative stress correction is the development of nanomaterials that exhibit redox properties, such as cerium dioxide and vanadates based on rare-earth elements, in particular gadolinium (Koreneva et al., 2016). A positive effect of such nanoparticles on the reproductive function of male rats in the case of reproductive diseases and experimental prostatitis has been proved (Belkina et al., 2017; Karpenko et al., 2020). A peculiar feature of vanadium compound action is the effect on the ADS by increasing the activity of enzymes such as superoxide dismutase, catalase, and, especially, glutathione link – glutathione peroxidase, as indicated in human hepatocytes in vitro (Kim et al., 2012).

The aim of the study is to investigate the effectiveness of the use of gadolinium orthovanadate nanoparticles activated by europium to correct reduced reproductive ability in boars under oxidative stress.

Materials and methods of research

The research was conducted at the Department of Veterinary Reproductology, Faculty of Veterinary Medicine at Kharkiv State Zooveterinary Academy. The studies were performed on boars kept on a standard diet with free access to water.

Animals in terms of sperm quality and the content of oxidative stress markers – conjugated dienes (CD), as primary of lipid peroxidation products, the final product – thiobarbituric acid reactive substance (TBARS), as well as stable metabolites of the nitrogen oxide (NO_x) cycle were divided into two groups. The sperm quality in males of the control group (n = 5) met the standards. It was reduced in the experimental group (n = 5), especially in terms of sperm motility and the number of motile sperm in the ejaculate, while there was an intensification of peroxidation processes by the content of oxidative stress markers in blood serum.

To correct oxidative stress, nanoparticles (NPs) of oxides of rare-earth elements synthesized at the Department of Nanostructured Materials of the Institute of Scintillation Materials at the National Academy of Sciences of Ukraine under the agreement on scientific and practical cooperation (№ 48, 22/07/2020) were used. Males of the experimental group were orally administered hydrosol of gadolinium orthovanadate NPs activated by europium, size 25×8 nm granular form at a dose of 0.0125 mg per kg of live weight for 14 days. It has been investigated that gadolinium orthovanadate NPs belong to the toxicity class IV – low-toxic compounds, which allows use in biomedical studies (Koreneva et al., 2016).

The effectiveness of the developed method for the correction was evaluated by the changes in sperm quality and the content of oxidative stress markers in blood serum of males. Blood serum samples were taken on the 1st, 15th, and 30th days. Sperm quality was assessed on the 1st, 60th, and 90th day of the study. Macroscopic (volume) and microscopic (sperm motility and number of motile sperm in the ejaculate, concentration, percentage of sperm content with morphological abnormalities) sperm quality indicators were evaluated according to common methods (Yablonskyi, 2005). The content of oxidative stress markers in blood serum was estimated using spectrophotometric methods in order to determine the concentrations of CD and TBARS (Vlizlo et al., 2012) and NO_x (Golikov, 2004).

All manipulations with animals were carried out in accordance with the Europe-

an Convention for the protection and vertebrate animals used for experimental and other scientific purposes (2006) and General ethical principles of animal experiments adopted by the First National Congress on Bioethics (Kyiv, Ukraine, 2001).

All digital data obtained during the study were processed statistically using Microsoft EXCEL. The Student's criterion was used to determine the probability of differences between mean values.

Results of the research and their discussion

Determination of the content of oxidative stress markers in biological fluids – blood, serum or plasma, sperm or sperm plasma allows obtaining objective information concerning the intensity of biological oxidation processes in males. This becomes important taking into account the seasonality of these processes and possible lack of adequate response from antioxidant defense system, especially in case of combined action of negative factors – inducers of peroxidation. Analyzing the results of biochemical studies, we determined the state of oxidative stress in males of the experimental group (Fig. 1) – the content of primary LPO products – CD was higher than the control rate by 19.3% ($P < 0.01$). There was also an increase in the concentration of TBARS in blood serum by 1.07 times ($P < 0.001$), which is the final product of LPO.

The number of NO_x was also significantly increased (Fig. 2) – by 83.6% ($P < 0.001$) compared with the control group. The increase of these indicators shows an increase in the intensity of peroxidation processes and the presence of nitrosative damage in boars, which is the main cause of reduced reproductive ability and is consistent with previously obtained data (Koshevoy & Naumenko, 2020).

We conducted a chronic experiment in order to establish the effectiveness of the use of NPs oxides of rare-earth elements as a safe pharmacological agent to correct reduced reproductive ability in boars under oxidative stress with pronounced redox and sperm-modulating activities.

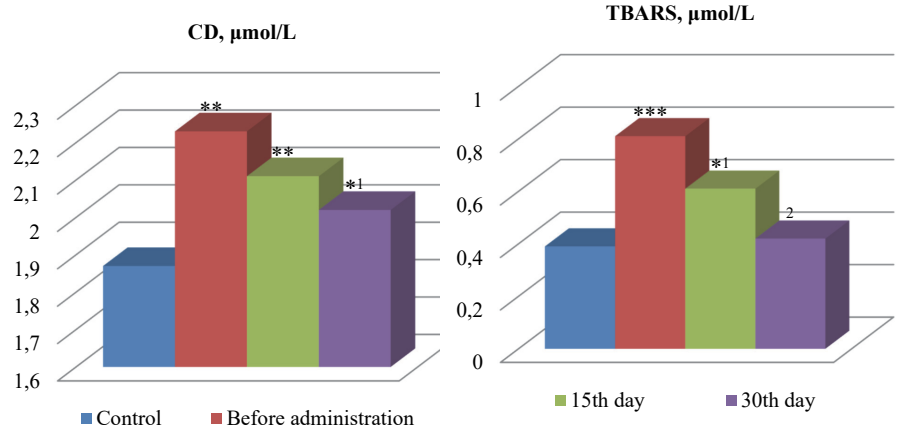

Fig. 1. The intensity of peroxidation processes in blood serum of boars during the correction by gadolinium orthovanadate nanoparticles (M ± m, n = 5)

Notes. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ – statistically significant changes in relation to the control group; $^1P < 0,05$; $^2P < 0,001$ – statistically significant changes in relation to the group of animals before administration.

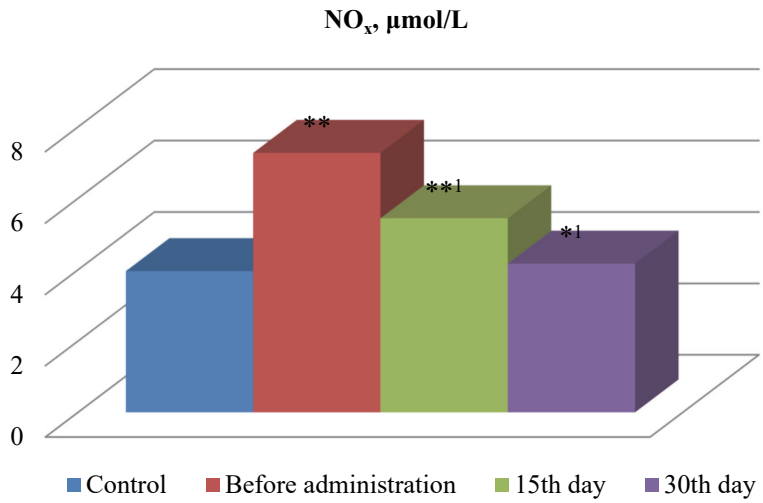

Fig. 2. The content of stable metabolites of the nitrogen oxide cycle in blood serum of boars after the correction by gadolinium orthovanadate nanoparticles (M ± m, n = 5)

Notes. * $P < 0.05$; ** $P < 0.001$ – statistically significant changes in relation to the control group; [1]$P < 0.001$ – statistically significant changes in relation to the group of animals before administration.

Thus, after the correction by gadolinium orthovanadate NPs activated by europium, a decrease in the intensity of peroxidation processes was observed in the blood serum of boars. At the same time, the studied indicators had a positive dynamics of changes on the 15th day of the study: the amount of CD tended to decrease and was 5.4% less than before administration, and the concentration of TBARS was lower by 24.7% ($P < 0.05$). The amount of NO_x also decreased by 25.2% ($P < 0.001$). This indicates that the administration of NPs has a positive effect on the dynamics of peroxidation.

Restoration of prooxidant balance, which almost reached the indicators of the control group was observed in the blood serum on the 30th day of the study. The CD content was reduced by 9.4% ($P < 0.05$), the TBARS concentration was lower than before administration by 48.2% ($P < 0.001$). This indicates a decrease in the intensity of LPO processes and the presence of a prolonged effect of the introduction of NPs hydrosol. Similar changes were observed in the nitrogen oxide cycle – its amount was lower by 42.6% ($P < 0.001$). The obtained data confirm the effectiveness of the use of gadolinium orthovanadate NPs for the correction of oxidative stress in boars.

Reduced reproductive ability in boars under oxidative stress is determined by the deterioration of sperm quality. It was found that males of the experimental group had significantly reduced sperm motility (by 51.2%, $P < 0.001$) and the number of motile sperm in the ejaculate (by 63.2%, $P < 0.001$). There was also a decrease in ejaculate volume by 17.2% ($P < 0.001$) and sperm concentration by 10.5% ($P < 0.01$), while the content of sperm with morphological abnormalities was higher by 24% ($P < 0.001$) compared with the indicators of the control group.

The presence of a correlation between LPO intensity and indicators of male sperm quality has been proved by many authors. In particular, our studies have confirmed a significant decrease in motility and the number of motile sperm in boar ejaculate. Analyzing the obtained data, we can conclude that the influence of the accumulation of ROS and RNS in boars, i.e. oxidative stress leads to deterioration of spermogram indicators and requires correction. This coincides with the opinion of most authors of the cited literature. The correction of oxidative stress in boars in case of reduced reproductive ability by gadolinium orthovanadate NPs has a positive effect on the dynamics of spermiogenesis, which was established by assessing the sperm quality in animals of the experimental group (Table 1).

Thus, on the 60th day of the study, the sperm motility indicator was higher than in animals before the administration of hydrosol NPs by 42.9% ($P < 0.01$), while the number of motile sperm in the ejaculate was higher by 57.1% ($P < 0.01$). In general, the quality of the obtained sperm was higher than before the administration of NPs – ejaculate volume by 5.8% ($P < 0.05$), sperm concentration – by 5.9% ($P < 0.05$), and sperm content with morphological abnormalities tended to decrease. This is likely to ensure the cost-effectiveness of this method of correction.

Evaluating the effectiveness of the oxidative stress correction by gadolinium orthovanadate NPs it was noted that on the 90th day of the study the quality indicators of ejaculate almost reached the values of the control in males. Sperm motility indicators are particularly sensitive to the action of oxidative stress and the number of motile sperm in the ejaculate increased by 95.2% ($P < 0.001$) and 1.48 times ($P < 0.001$) in relation to the group of animals before administration. A positive effect of nanoparticles was observed on the dynamics of ejaculate volume, which on the 90th day of the study was higher by 15.1% ($P < 0.001$), sperm concentration, which was higher by 11.8% ($P < 0.01$). The sperm content with morphological anomalies decreased by 18.3% ($P < 0.01$).

The effectiveness of the introduction of gadolinium orthovanadate NPs can

1. Indicators of boar sperm quality after the correction by gadolinium orthovanadate nanoparticles (M ± m, n = 5)

№	Indicator	Animal group			
		control	experiment		
			before administration	day 60	day 90
1	Ejaculate volume, mL	209.5 ± 3.2	173.5 ± 3.0***	183.6 ± 2.9***[1]	199.7 ± 2.5*3
2	Sperm concentration, billion/mL	0.19 ± 0.003	0.17 ± 0.003**	0.18 ± 0.002*[1]	0.19 ± 0.0032
3	Mobility, scores	8.6 ± 0.3	4.2 ± 0.4***	6.0 ± 0.3***[2]	8.2 ± 0.4
4	The number of motile sperm in the ejaculate, billion	34.2 ± 1.02	12.6 ± 1.32***	19.8 ± 1.19***[2]	31.3 ± 1.623
5	The content of sperm with morphological abnormalities, %	15.4 ± 0.6	19.1 ± 0.4***	18.2 ± 0.4**	15.6 ± 0.62

Notes. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ – statistically significant changes in relation to the control group; [1] $P < 0.05$; [2] $P < 0.01$; [3] $P < 0.001$ – statistically significant changes in relation to the group of animals before administration.

be explained by the increased activity of these compounds in the transition to the nanoform, as they do not manifest such properties in the form of industrial pharmaceutical compositions, which are known today. The results of the study of boar spermogram after oxidative stress correction prove the presence of sperm-modeling action of NPs based on oxides of rare-earth elements. It can be recommended for practical implementation and development of methods for correction of hypofertility of different genesis in males of different species.

Conclusions and future perspectives

The efficiency of the developed correction method for the reduced reproductive ability in boars under oxidative stress by gadolinium orthovanadate NPs is proved:

1. Positive dynamics of changes in the intensity of peroxidation processes with the introduction of hydrosol NPs of gadolinium orthovanadate were observed, namely: the concentration of conjugated dienes in blood serum of boars, which on the 15th day tended to decrease, and on the 30th day of the study was less by 9.4% compared with the indicators before administration.
2. Normalization of oxidative processes in males was confirmed by a decrease in the amount of the final product of LPO – thiobarbituric acid reactive substance on the 15th day of the study – by 24.7%, and on the 30th day – by 48.2%.
3. A decrease in the activity of the Nitrogen oxide cycle was determined. The content of stable metabolites in the blood serum of boars on the 15th day was lower than in animals before administration by 25.2%, and on the 30th day – by 42.6%. Such changes indicate the action of nanoparticles as scavengers of oxygen radicals.
4. The method of correction had a significant effect on sperm quality indicators, especially on sperm motility and the number of motile sperm in the ejaculate, which increased by 42.9 and 57.1% on the 60th day and by 95.2% and 1.48 times on the 90th day of the study, respectively.
5. Ejaculate volume, sperm concentration, and sperm content with morphological anomalies after the administration of nanoparticles normalized and almost reached the values of the control group.

Perspectives for further research are to elucidate the effect of correction of reduced reproductive ability in boars under oxidative stress by gadolinium orthovanadate NP on the hormonal background indicators and the state of enzymatic and non-enzymatic systems of antioxidant defense, as well as to determine additional spermogram parameters.

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В. І. Кошевой, С. В. Науменко, В. К. Клочков, С. Л. Єфімова (2021). ВИКОРИСТАННЯ НАНОЧАСТИНОК ГАДОЛІНІЮ ОРТОВАНАДАТУ ДЛЯ КОРЕКЦІЇ РЕПРОДУКТИВНОЇ ЗДАТНОСТІ КНУРІВ ЗА ОКСИДАТИВНОГО СТРЕСУ. *Ukrainian Journal of Veterinary Sciences, 12(2): 74–82, https://doi.org/10.31548/ujvs2021.02.008*

Анотація. *Пошук ефективних і безпечних засобів корекції гіпофертильності самців є актуальною проблемою сучасної репродуктології. Дослідниками доведена можливість застосування наночастинок на основі оксидів рідкісноземельних елементів для лікування розладів чоловічої репродуктивної функції. Нами досліджено ефективність використання наночастинок гадолінію ортованадату активованих європієм розміром 25×8 нм зерноподібної форми в дозі 0,0125 мг на кг живої маси для корекції зниження репродуктивної здатності кнурів за оксидативного стресу. Експериментально, після 14-добового введення гідрозолю наночастинок нами встановлена динаміка вмісту маркерів оксидативного стресу і стабільних метаболітів циклу Нітрогену оксиду, а також визначено зміни показників якості сперми. Так, концентрація дієнових кон'югатів у сироватці крові кнурів на 15-ту добу мала тенденцію до зниження, а на 30-ту добу дослідження була вірогідно меншою на 9,4% показників групи тварин до введення. Водночас, кількість кінцевого продукту ліпопероксидації – малонового діальдегіду вірогідно зменшувалася на 15-ту добу дослідження на 24,7%, а на 30 добу – на 48,2%, що свідчить про нормалізацію окисних процесів в організмі самців. Позитивні зміни відзначено в системі циклу Нітрогену оксиду, вміст стабільних метаболітів якого на 15 добу був вірогідно меншим показників тварин до введення на 25,2%, а на 30-ту добу – на 42,6%. Одночасно відмічали покращення показників якості сперми кнурів, особливо рухливості й кількості рухливих спермiїв у еякуляті, які підвищилися відповідно на 42,9 і 57,1% на 30-ту добу та на 95,2% і в 1,48 раза на 60-ту добу дослідження. Водночас, об'єм еякуляту, концентрація спермiїв і вміст спермiїв із морфологічними аномаліями за введення наночастинок нормалізувалися й майже досягли значень групи контролю. Інтерес дослідників полягає у подальшому з'ясуванні впливу корекції зниження репродуктивної здатності кнурів за оксидативного стресу наночастинками гадолінію ортованадату на показники гормонального фону та стан ензиматичної й неензиматичної систем антиоксидантного захисту.*

Ключові слова: *якість сперми, прооксидантно-антиоксидантна система, пероксидація, маркери оксидативного стресу, цикл нітрогену оксиду, наноматеріали*