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The National Scientific Center 'Institute of the Experimental and Clinical Veterinary Medicine' is the leading specialized research institution in Ukraine created for solving scientific and practical tasks of veterinary animal. NSC IECVM's basic research are focused on: immunogenesis and disease pathogenesis, indications, authentications, isolations and studies of biological features of their causative agents, developments of facilities and systems of monitoring, diagnostics, prophylaxis and prognostication of infectious diseases of animals, monitoring of quality and unconcern of agricultural produce and development of the normative basis for animal diseases control and biosafety. NSC IECVM coordinates implementation of scientific researches on questions veterinary medicine, that conduct scientific establishments of NAAS, State Service of Ukraine for Food Safety and Consumer Protection, and Higher educational establishments of Ukraine of agrarian profile.

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ETIOLOGICAL STRUCTURE OF LEPTOSPIROSIS AMONG THE WILD BOARS AND DOMESTIC PIGS IN THE TERRITORY OF UKRAINE, ITS ANALYSIS AND CHARACTERISTICS

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Summary. Wild boars (*Sus scrofa*) are a reservoir of leptospirosis in nature and a source of infection for domestic pigs and the human, especially those at risk. Wild boars (n = 516) and domestic pigs (n = 1042) were tested for *Leptospira* spp. using microscopic agglutination test (MAT), which was conducted with 21 *Leptospira*'s serological groups. The circulation of common pathogenic leptospires among wild boars and domestic pigs in the territory of Ukraine was established. Registered positive-responding animals in the MAT among wild boars were 86.8%, among domestic pigs — 38.1%. Analysis of the etiological structure of leptospirosis among wild boars and domestic pigs showed that the dominant serological groups were *Icterohaemorrhagiae*, *Australis* (serovar *bratislava*), *Pomona*, *Canicola*, *Grippotyphosa*, *Sejroe*, *Hebdomadis*, *Tarassovi*.

Keywords: leptospirosis, etiological structure, wild boars, domestic pigs, microscopic agglutination test, Ukraine

Introduction. Leptospirosis is the natural foci-associated disease, the other name of which is 'water fever'. This zoonosis is very closely related to water, since the main route of transmission of infection is water (according to the Ukrainian Center for Monitoring and Control of Infectious Diseases of The Ministry of Healthcare of Ukraine, 164 cases of human infections in leptospirosis through the waterway of transmission throughout Ukraine, which was 50.8% of the total number of registered cases) (Adler, 2015; Mandyhra et al., 2014).

In natural foci, the source and reservoirs of pathogenic leptospires are small mammals from the genus of rodents, insectivores, predators and marsupials. The farm animals and synanthropic rodents could be the reservoirs as well in anthropological centers (Vinohrad et al., 2005; Uhovskyi, Kucheryavenko and Stepna, 2014).

In addition, recent studies have shown a significant role in the epizootology of leptospirosis in wildlife infections, in particular wild boars, which are an integral part of the fauna in many countries around the world. These animals play an important role for most viral and bacterial pathogens, including leptospirosis. It is necessary to take into account factors such as: their migration ability; ecological peculiarities of existence in the wild; omnivorous and consumption of corpses of rodents; they share pastures and rates for watering with other wildlife and livestock. Thus, infected wild boars become reservoirs and carriers of pathogenic leptospires, resulting in the creation of ideal conditions for the formation of natural foci of leptospirosis by the transfer of pathogenic leptospires from wild boars to domestic pigs and to human (Bolotskiy, 1998; Levett, 2001).

According to the literature data, wild boars (*Sus scrofa*) could be the potential reservoir for a variety of pathogenic leptospires.

For the first time, the problem of studying infectious diseases in wild boars began to be engaged in the last century. In 1986, in the USA (Texas) group of researchers examined 10 populations of this species of animals and found that they all are vectors of pathogenic leptospires (Corn et al., 1986).

Similar studies were conducted in Australia in 1998. This discovered antibodies to leptospiras in wild boars serum samples using MAT (Mason et al., 1998).

In Europe, such studies began to be conducted only in the 21st century. His first results were presented in Spain in 2002 (Vicente et al., 2002).

A year later, Italian scientists discovered specific antibodies to leptospiras and brucellas in wild boars' serum (Ebani et al., 2003). In Zagreb (Croatia), veterinarians were tested blood serums and kidney samples from wild boars and rodents and isolated three major serogroups from isolates — *Pomona*, *Australis*, *Icterohaemorrhagiae* (Cvetnic et al., 2003).

In 2006–2007, conducted a study of blood serum from wild boars caught in the suburban of Berlin (Germany), which resulted in the carriage of serogroups *Pomona* and *Australis* (serovar *bratislava*) (Jansen and Schneider, 2011).

Leptospirosis in wild boars and deer was established in Japan in 2009 (Koizumi et al., 2009). In the same year, American scientists have found that wild boars constitute a direct threat to farm animals and humans (Meng, Lindsay and Sriranganathan, 2009).

Scientists from different region in the World discovered specific leptospira antibodies in wild boars and other wildlife species during 2010–2015 (Chatfield et al., 2013; Durfee and Presidente, 1979; Espí, Prieto and Alzaga, 2010; Fornazari et al., 2009; Pappas et al., 2008; Pedersen et al., 2015; Vale-Gonçalves et al., 2015).

Multiple researchers and scholars in Russia carried out the study of wild fauna (Ananyina, 2002; Bolotskiy, 1998; Malakhov, Panin and Soboleva, 2001; and others).

In Ukraine, the study of the etiological structure of leptospirosis in wild boars was not undertaken.

Many researchers and scientists have been involved in the research of farm animals. Study of the etiological structure of leptospirosis is a very labor-intensive process, since the main hosts of the leptospirosis of one serovar can be different animals (Dovgan, Atamas and Fuchidgi, 1998).

The leading position in the etiological structure of leptospirosis among domestic pigs in Europe is occupied by serogroups *Pomona* and *Icterohaemorrhagiae* (Nardone et al., 2004; Schönberg, Staak and Arbeitsgruppe, 1987).

At the pig farms in the countries of Western Europe, North America and Asia, great attention is paid to the increasing number of positive reactions to leptospirosis with serovar *Bratislava* (serogroup *Australis*) (Meites et al., 2004; Mendoza and Prescott, 1992). For the first time in Ukraine the circulation of this serovar among the pig population was reported in 1999 (Ntahonshikira, 1999). This pathogen was registered in 80.7% of the total number of positively responsive pigs in 2004 (Ukhovskyi, 2005).

According to the results of the analysis of the data of the veterinary report of the Central State Veterinary Medicine Laboratory, the etiological structure of the pigs' leptospirosis was as follows: *Icterohaemorrhagiae* — 41.6%, *Pomona* — 14.9%, *Tarassovi* — 10.6%, *Grippotyphosa* — 2.2%, *Canicola* — 2.0%, *Hebdomadis* — 0.6%, *Sejroe* — 0.5% (Nedosekov, Ukhovskyi and Kucheryavenko, 2011).

According to the results of recent studies, the etiological structure of pigs' leptospirosis in farms of Ukraine as of the beginning of 2017 is as follows: *Icterohaemorrhagiae* — 40.2%, *Pomona* — 14.8%, *Australis* (serovar *bratislava*) — 13.4%, *Canicola* — 8.5%, *Sejroe* — 7.3%, *Hebdomadis* — 7.2%, *Tarassovi* — 4.7%, *Grippotyphosa* — 3.9% (Kulykova et al., 2016).

An analysis of literary sources about the role of wild boars in the spread of leptospirosis among wild and farm animals and human infection was the basis for research on the spread of leptospirosis among this species of animals on the territory of Ukraine.

The aim of the study was to investigate the large number of blood sera samples from wild boars and domestic pigs, to determine the etiological structure of leptospirosis, and to analyze it and to characterize the connections of leptospirosis infection in these species, taking into account the genetic affinity between wild boars and domestic pigs.

Materials and methods. All researches were performed during 2014–2016 on the basis of the Leptospirosis Laboratory of Farm Animals with the Museum of Microorganisms, on the basis of which the Scientific Research Reference Center for the study and prevention of leptospirosis in the territory of Ukraine.

Leptospira strains: Twenty-one pathogenic *Leptospira* spp. strains were genotyped. These strains were part of the bacterial collection of the Leptospirosis Laboratory of Farm Animals with the Museum of Microorganisms of the Institute of Veterinary Medicine of the National Academy of Agrarian Sciences of Ukraine.

Study sites: Blood sera samples from 516 wild boars were obtained by shooting hunters on the territory in hunting grounds from 375 administrative districts of all oblasts of Ukraine and the Autonomous Republic of Crimea from the State Program 'On the control of the number of wild boars in the territory of Ukraine', and kindly provided by Dr. M. P. Sytiuk.

Blood sera samples from 1,042 domestic pigs came to the laboratory from dysfunctional leptospirosis farms of Ukraine.

Serological test (MAT): The research was carried out by microscopic agglutination test (MAT) using antigens of 21 *Leptospira* serogroups recommended for research in state laboratories of veterinary medicine of Ukraine in dilutions 1:50, 1:100, 1;500, and 1:2,500. The study of blood serum from wild boars was carried out by MAT using antigens of 21 *Leptospira* serogroups (large diagnostic series), and domestic pigs — 8 *Leptospira* serogroups (small diagnostic series), which listed in Table 1.

Table 1 — List of strains used for research

No.	Serogroup	Serovar	Strain
1	*Javanica* [L]	*javanica*	Veldrat Bataviae 46
2	*Bataviae* [L]	*djatzi*	HS 26
3	*Mini* [L]	*szwajizak*	Szwajizak
4	*Sejroe* [L,S]	*polonica*	493 Poland
5	*Hebdomadis* [L,S]	*kabura*	Kabura
6	*Tarassovi* [L,S]	*tarassovi*	Perepelicyni
7	*Pomona* [L,S]	*pomona*	Pomona
8	*Grippo-typhosa* [L,S]	*grippotyphosa*	Moskva V
9	*Canicola* [L,S]	*canicola*	Hond Utrecht IV
10	*Icterohaemor-rhagiae* [L,S]	*copenhageni*	M 20

Table 1 — continuation

No.	Serogroup	Serovar	Strain
11	*Louisiana*[L]	*louisiana*	LSU
12	*Shermani*[L]	*shermani*	LT 821
13	*Panama*[L]	*panama*	CZ 214 K
14	*Semaranga*[L]	*patoc*	Patoc 1
15	*Celledoni*[L]	*whitcombi*	Whitcomb
16	*Australis*[L]	*erinacei-europaei*	Jez 1
17	*Autumnalis*[L]	*autumnalis*	Akiyami A
18	*Cynopteri*[L]	*cynopteri*	Vleermuis 3868
19	*Pyrogenes*[L]	*pyrogenes*	Saline
20	*Ballum*[L]	*ballum*	Mus 127
21	*Australis*[S]	*bratislava*	Jez-bratislava

Notes: [L] — large diagnostic series, [S] — small diagnostic series.

Results. We tested 1,558 samples of blood serum in the MAT, namely: 516 — from wild boars, 1,042 — from domestic pigs, and analyzed the results.

Research blood sera samples on leptospirosis of wild boars. In order to study the etiological structure of leptospirosis among population wild boars, we conducted a study of blood sera from all regions of Ukraine that arrived at the Laboratory of leptospirosis of IVM NAAS. The results of the research are shown in Table 2.

Table 2 — Results of studies of blood sera of wild boars on leptospirosis in MAT

Indexes	Years			Total
	2014	2015	2016	
Tested blood sera samples	109	170	237	**516**
Positive results	95	158	195	**448**
Percentage of positively blood sera samples	87.2	92.9	82.3	**86.8**
Positive reactions	288	536	553	**1,377**

In order to study leptospirosis and detect the spectrum of the etiological structure of the disease among wild boars population in Ukraine, we conducted a study of serum blood in the MAT. The results of studies in the area of regions are shown in Table 3.

Analysis of the results of Table 2 shows that in the vast majority of Ukraine (13 regions), the percentage of positive reactions ranges from 80–89%, which indicates a significant infection of wild pigs with pathogenic serogroups leptospires. In eight regions, this percentage is between 90% and 100%. Only in three regions the percentage of infection remained at the level of 70–79%.

According to numerous publications of scientists from different countries of the world, the seroprevalence of leptospirosis infection among wild boars population varies from 3% to 95% (Fig. 1). Taking into account the results of its own research, Ukraine occupies the second position in this list and is unfriendly in relation to the leptospirosis of wild boars.

Table 3 — Results of serological examination of blood serum wild boars in the territory of Ukraine

No.	Region	Studied samples of blood sera	Positive results	
			Total	%
1	AR Crimea	23	21	91.3
2	Vinnytsia	13	13	100.0
3	Volyn	15	13	86.7
4	Dnipropetrovsk	18	15	83.3
5	Donetsk	18	15	83.3
6	Zhytomyr	18	16	88.9
7	Zakarpattia	17	14	82.4
8	Zaporizhia	16	13	81.3
9	Ivano-Frankivsk	18	17	94.4
10	Kyiv	19	16	84.2
11	Kirovohrad	19	15	78.9
12	Luhansk	22	19	86.4
13	Lviv	17	17	100.0
14	Mykolaiv	20	17	85.0
15	Odesa	22	20	90.9
16	Poltava	34	31	91.2
17	Rivne	20	15	75.0
18	Sumy	25	20	80.0
19	Ternopil	22	19	86.4
20	Kharkiv	22	20	90.9
21	Kherson	19	16	84.2
22	Khmelnitskyi	22	21	95.5
23	Cherkasy	32	29	90.6
24	Chernivtsi	22	18	81.8
25	Chernihiv	23	18	78.3
	Total:	**516**	**448**	**86.8**

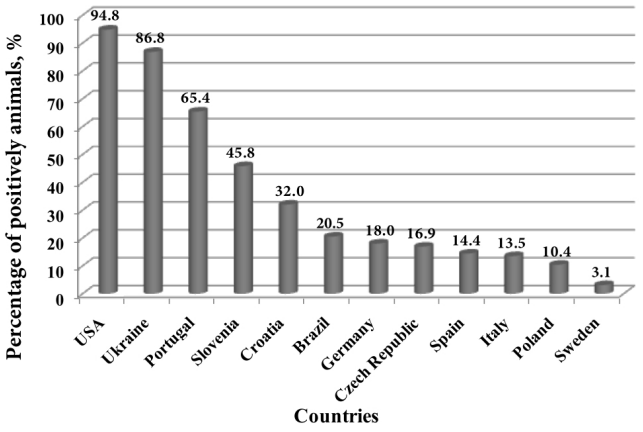

Figure 1. Seroprevalence level of leptospirosis among wild boars population in countries (2014–2016)

As a result of the serological study, 448 animals reacted positively, representing 86.8% of the total number of

investigated ones. As shown in Table 3, the highest level of infection was in 2016, and the lowest — in 2014.

The general etiological structure of the leptospirosis of wild pigs is presented in Fig. 2.

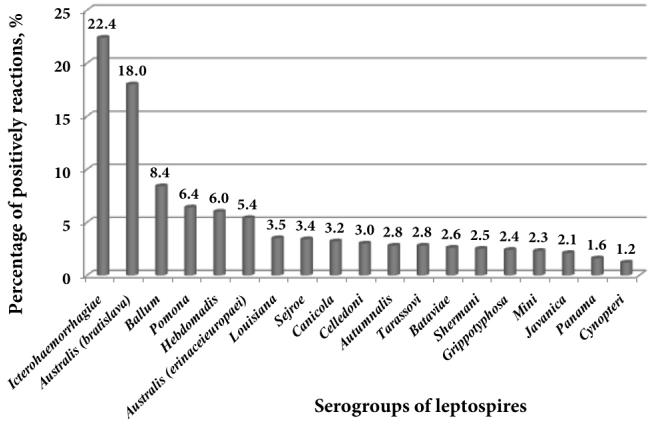

Figure 2. General etiological structure of leptospirosis of wild boars (n = 516)

Analyzing the overall etiological structure of the leptospirosis of wild pigs, shown in Fig. 2, among all positively reactive animals, the antibodies to the serotypes *Icterohaemorrhagiae* (22.4%), *Australis* (serovar *bratislava*) (18%) and *Ballum* (8.4%) were most frequently detected. *Pomona* (6.4%) and *Hebdomadis* (6%) were slightly less registered. Other serological groups ranged from 2.1% to 5.4%. The smallest etiological role was played by serogroups *Cynopteri* (1.2%) and *Panama* (1.6%).

Testing of blood sera samples on leptospirosis of domestic pigs. In total, we examined and analyzed 1,042 blood sera samples domestic pigs in MAT from 26 farms in different regions of Ukraine. The results of the research are shown in Table 4.

Table 4 — Results of studies of blood sera samples domestic pigs on leptospirosis in MAT

Indexes	Years			Total
	2014	2015	2016	
Tested blood sera samples	565	149	328	**1,042**
Positive results	237	60	100	**397**
Percentage of positively blood sera samples	41.9	40.3	30.5	**38.1**
Positive reactions	333	81	143	**557**

Positive reactions were diagnosed in 397 samples, representing 38.1% of the total number of examined animals. As shown in Table 4, with during 2015–2016 leptospirosis of domestic pigs was recorded in a relatively equal number of animals, respectively, 41.9% and 40.3%; in 2016 there was a decrease in the level to 30.5%.

The leading role in etiology of leptospirosis infection of domestic pigs was played by the serological group *Icterohaemorrhagiae*. Antibodies to it were diagnosed in 262 animals, which is 47.0%. Serogroup *Australis* was found in sick animals less frequently (16.2%). *Pomona* and *Canicola* were recorded at almost the same level (respectively 10.8% and 10.4%). The smallest etiological role was played by the serological group *Grippotyphosa*, which was noted only in 2.3% of cases (Fig. 3).

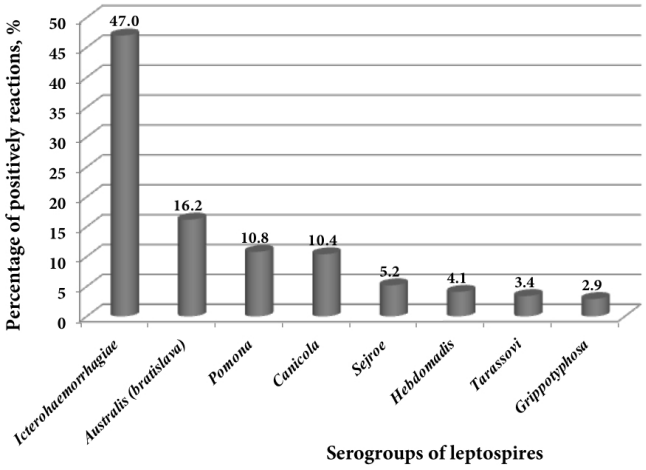

Figure 3. General etiological structure of leptospirosis of domestic pigs (n = 1,042)

Having analyzed the obtained data, common serogroups were established between positive reactions in wild boars and domestic pigs whose blood serum was investigated in the Laboratory of Leptospirosis with the Museum of Microorganisms of IVM NAAS during 2014–2016. The systematized results are shown in Table 5.

Table 5 — Etiological structure of leptospirosis of wild boars and domestic pigs on the territory of Ukraine (2014–2016)

Serogroup	Wild boars, %	Domestic pigs, %
Icterohaemorrhagiae	22.4	47.0
Australis (serovar *bratislava*)	18.0	16.2
Pomona	6.4	10.8
Canicola	3.2	10.4
Sejroe	3.4	5.2
Hebdomadis	6.0	4.1
Tarassovi	2.8	3.4
Grippotyphosa	2.4	2.9

As can be seen from Table 5, the main etiological role of wild boars and domestic pigs is played by the serological groups *Icterohaemorrhagiae* and *Australis* (serovar *bratislava*). Antibodies to the following serogroups: *Pomona*, *Hebdomadis*, *Canicola*, and others were registered less rarely. The difference in the percentage of positive reactions to serogroups due to the variability of the etiological structure, which is characteristic of leptospirosis.

Discussion and conclusions. An analysis of the publications of scientists from other countries regarding leptospirosis among wild boars populations indicates their significant level of infectiousness around the world. In particular, they can be infected by eating rodents. In order to study leptospirosis among populations of this species of animals on the territory of Ukraine, we conducted serological monitoring. Blood sera were obtained as a result of shooting at the territory of hunting grounds from 375 administrative districts of all regions and the Autonomous Republic of Crimea within the framework of the State Program 'On the control of the number of wild pigs in the territory of Ukraine'.

According to the results of our research, it has been established that the entire territory of Ukraine is unsuccessful in relation to the leptospirosis of the specified species of animals. In particular, it was investigated in MAT and analyzed 516 samples of blood sera. Of these, 448 responded positively to leptospirosis, which is 86.8%.

Based on the analysis of the results obtained, we found that in serotypes positive for wild boars, antibodies to serogroups were *Icterohaemorrhagiae* (22.4%), *Australis* (serovar *bratislava*) (18.0%), *Ballum* (8.4%), *Pomona* (6.4%), and *Hebdomadis* (6.0%) were slightly less registered. Other serological groups ranged from 2.1% to 5.4%. The smallest etiological role was played by serogroups *Cynopteri* (1.2%) and *Panama* (1.6%) (Stepna, Ukhovskyi and Sytiuk, 2015).

According to German researchers, among wild pigs, the serogroups *Pomona* and *Australis* (serovar *bratislava*) are predominant (Jansen et al., 2007).

As a result of our studies, the leptospirosis of these serogroups was also detected: *Pomona* in 6.4%, *Australis* (serovar *bratislava*) — 18.0%. Having analyzed the obtained data, there were established common serogroups between positive reactions in wild boars and domestic pigs whose blood serum was investigated in the Laboratory of Leptospirosis with the Museum of Microorganisms of IVM NAAS during 2014–2016.

The main etiological role of wild boars and domestic pigs is played by the serological groups *Icterohaemorrhagiae* and *Australis* (serovar *bratislava*). Antibodies to the following serogroups: *Pomona*, *Hebdomadis*, *Canicola*, and others were registered less rarely. The difference in the percentage of positive reactions to serogroups is due to the variability of the etiological structure, which is characteristic of leptospirosis (Ukhovskyi, 2005).

Among the domestic pig's populations, during the research period, the highest percentage of positive reactions was caused by the serological groups *Icterohaemorrhagiae* (47.0%), *Australis* (16.2%) and *Pomona* (10.8%). Together, they recorded 73.6% of the total number of positive reactions to leptospirosis. Antibodies to the serotype *Icterohaemorrhagiae* were diagnosed in 262 animals out of 365 positive-responsive, representing 66.0%. Serogroups *Australis* and *Pomona* were found in sick animals less frequently (respectively, 22.7% and 15.1%).

The obtained results confirm the data of Ukhovskyi (2005), Zon et al. (2001), Atamas, Maslennikova and Dovgan (2003), Ivanchenko and Gontar' (2010), etc., who reported the leading role of the serogroups *Icterohaemorrhagiae* and *Pomona* in the etiological structure of leptospirosis infection among domestic pigs in Ukraine. Ntahonshikira (1999), and later Ukhovskyi (2005), the significant role of the serologic group *Australis* (serovar *bratislava*) in the disease of these animals has been proven. Particular attention to the causative agent is on the part of foreign researchers. As of 2008, antibodies to it were found in 7.98% of the positively responding to the leptospirosis of the pig population in Poland, in the Netherlands — 28.67%, in the Czech Republic — 13.37%, in Denmark — about 70.0% (Ukhovskyi, 2005).

A lower percentage of positive reactions were recorded with serogroups *Canicola* (9.7%), *Sejroe* (6.2%), *Hebdomadis* (6%), and *Tarassovi* (3.4%). Antibodies to the serogroup *Grippotyphosa* have been diagnosed in only six reactions out of 535, representing 1.1% (Kulykova et al., 2016). The obtained results logically agree with the data of official reporting and foreign scientists with minor fluctuations in the percentage of certain serogroups (Atamas, Maslennikova and Dovgan, 2003; Zon et al., 2001; Ivanchenko and Gontar', 2010; Ukhovskyi, 2005; Nardone et al., 2004; Schönberg, Staak and Arbeitsgruppe, 1987).

Having systematized and analyzed the results of the research, it can be argued that the etiological structure of leptospirosis among wild boars and domestic pigs is common and has the same serological group's leptospires.

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ANTI-EXUDATIVE ACTIVITY OF 7-DISUBSTITUTED 8-METHYLPIPERAZINE-1,3-DIMETHYLXANTHINES

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Summary. It has been established that the greatest anti-exudative action was shown by 7-(3-chlorobutane-2-diol-1)-8-N-ethylpiperazinetheophylline (compound 4). Derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines are a promising group of heterocyclic compounds for more effective anti-exudative substances search.

Keywords: derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines, anti-exudative activity

Introduction. Inflammation in animals and humans is the most frequent symptom of a variety of diseases and represents an important clinical problem. In the development of inflammatory reaction, the central role belongs to the leukocytes migration from the microvasculature into the tissues. The interaction of leukocytes, thrombocytes, and endothelium in the focus of inflammation is mediated by adhesion molecules. In addition, inflammation involves interleukin-1, tumor necrosis factor, thrombocytes activation factor, cytokines, leukotriene B4, as well as prostaglandins (PG). The last participate in the inflammatory reaction development, as well as in pain and fever occurrence. Violation of PG synthesis by not narcotic analgesics leads to the implementation of the anti-inflammatory effect (Hinz, Dormann and Brune, 2006; Kato et al., 2001).

At present, the interest in the treatment of the inflammatory process has increased, which has contributed to the expansion of pathogenesis studies and the search for more effective and safe pharmacological substances for the drug regulation of inflammation at various diseases that are not accompanied by ulcerogenic effects that is typical for modern NSAIDs that cause erosion and gastrointestinal bleeding.

Because of the peculiarities of inflammation development mechanisms, drugs of different pharmacological groups are used for treatment, among which a special place is occupied by drugs of symptomatic therapy — nonsteroidal anti-inflammatory drugs (NSAIDs) (Nasonov, 2002).

The anti-inflammatory effect of NSAIDs is mediated by two independent mechanisms. Low concentrations of NSAIDs, interacting with the arachidonate-COX complex, prevent the stable prostaglandins (PG) formation. High NSAIDs concentrations block the association of arachidonate with G protein and suppress the cellular activation of stable PG formation. It leads to the violation of the class E prostaglandin synthesis in the mucous membrane of the stomach and the development of erosive and ulcerative lesions, and the inhibition of cyclooxygenase-2 (COX-2) in the site of inflammation, a key enzyme in the prostaglandins synthesis of anti-inflammatory activity (Sorotskaya and Karateev, 2005; Goldstein et al., 2000) and selective blockers COX-2 are a threat to the development of thrombotic events, myocardial infarction (Crofford et al., 2000).

The moderate anti-inflammatory effect has theophylline, which inhibits the formation of free oxygen radicals, the synthesis and release of cytokines. When searching for new anti-exudative substances, we used 7-substituted 8-N-methylpiperazin-1,3-dimethylxanthine derivatives (Kornienko, Tarasevičius and Samura, 2013).

The aim of the study was to study the dependence of anti-exudative activity on the chemical structure of the newly synthesized 7-substituted 8-N-methylpiperazine theophylline.

Materials and methods. New derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines (compounds 1–12) were taken as a research object. Synthesis of substances was carried out at the Department of Biological Chemistry of the Zaporizhia State Medical University under the direction of Dr. Pharm. Sci., Prof. N. I. Romanenko (Romanenko et al., 2013).

The structure of the synthesized substances was confirmed by the modern physicochemical methods of elemental analysis, UV-, IR-, PMR-, and mass spectrometry, counter synthesis, and the purity of the synthesized substances was monitored by thin-layer chromatography. Non-linear rats weighing 170–195 g were used for the study. The anti-exudative activity of 7-substituted 8-N-methylpiperazine theophylline was studied on the acute inflammatory edema model caused by the subplantar administration of a 1% carrageenan solution. The investigated substances in the form of a finely dispersed aqueous suspension, stabilized by Tween-80, at the volume of 0.5 ml were administered intraperitoneally at doses of 0.05 LD_{50}. The control group of animals received in the same way an isotonic 0.9% solution of sodium chloride and Tween-80 in the appropriate volume and appropriate doses (Mokhort, Yakovleva and Shapoval, 2001).

After 30 min, 0.1 ml of a 1% aqueous suspension of carrageenan was injected under the aponeurosis of the hind paw of the rat. Using the oncometer, the paw volumes

were measured in rats prior to the beginning of the experiment and hourly for 4 hours.

The anti-exudative activity was determined by the degree of reduction of the experimental edema in the experimental rats in comparison with the control animals. It was expressed as a percentage of the control. The drug of comparison was Diclofenac sodium at a dose of 8 mg/kg. The degree of edema oppression was calculated by the formula (1):

$$\% \text{ oppression} = \frac{Yc-Ye}{Yc} \times 100, \quad (1)$$

where Yc and Ye, respectively, the volume of the paw in the control and in the experiment (Mokhort et al, 2001; Sernov and Gatsura, 2000).

Experimental studies were conducted according to the regulations on the use of animals in biomedical research (Strasbourg, 1986) and the 'General Ethical Principles of Experiments in Animals' (Kiev, 2001), and agreed with the requirements of the 'European Convention for the Protection of Vertebrate Animals, used for experimental and scientific goals'.

The statistical verification of the data was carried out using a standard analysis package for the statistical processing of the results of the version of Microsoft Office Excel 2003. The results are presented as a sample mean and a standard error of the mean value. The reliability of the differences between the experimental groups was assessed using the Student's *t*-criterion and the Mann-Whitney U test of the computer program Statistica® for Windows 7.0 (Statsoft Inc. No.AXXR712D833214 Fan5). Differences at a significance level of < 0.05 (Lapach, Chubenko and Babich, 2001) were considered statistically significant for all types of analysis.

Results and discussion. Table 1 shows the results of studying the anti-exudative activity of heterocyclic derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines.

It has been found that the most pronounced anti-exudative effect was shown by compound 4 — 7-(3-chlorobutane-2-diol-1)-8-N-ethylpiperazine theophylline which, at a dose of 19.3 mg/kg in 4 hours after administration, caused a decrease in edema of the paw in rats by 45.2%. Replacement in the 7th position of 7-(3-3-chlorobutane-2-diol-1)-8-N-ethylpiperazine teophylline 3-chlorobutane-2-diol radical (compound 4) molecule with *м*-bromobenzyl (compound 1), β-phenylethyl, (compound 2), α-methylbenzyl (compound 3), α-naphthylmethyl (compound 5), β-phenylethyl (compound 6) led to decreasing in the development of experimental carrageenan paw edema in rats from 45.2% to 28.9%.

Replacement of the ethyl radical in the 8th position of the piperazine fragment of the molecule 1-*n*-fluorobenzyl-8-(4-ethylpiperazinyl-1)-theobromine (compound 9) by methyl (compound 12) led to a decrease in anti-nociceptive activity by 3.5%.

Table 1 — Anti-exudative activity of the derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines

Compound No.	Code	Dose, mg/kg	Anti-exudative activity	
			paw volume after 4 hours, ml	paw volume after 4 hours, %
1	α-2466	23.0	1.48±0.14*	35.1
2	α-2660	25.5	1.59±0.12*	32.5
3	α-4253	27.0	1.62±0.19*	28.9
4	α-4255	19.3	1.25±0.06*	45.2
5	α-4256	15.5	1.45±0.08*	36.4
6	α-4258	34.0	1.64±0.16*	28.1
7	α-4259	27.5	1.59±0.17*	30.3
8	α-4260	23.7	1.68±0.18*	26.3
9	α-8431	12.3	1.28±0.15*	43.9
10	α-8319	37.5	1.86±0.07	18.4
11	α-8314	39.0	1.63±0.13*	28.5
12	α-8430	13.0	1.36±0.15*	40.4
Diclofenac sodium		8.0	1.22±0.14*	46.5
Control		—	2.28±0.11	100.0

Note: * — for $p < 0.05$ compared with the control.

Introduction of 7-β-phenylethyl-8-N-ethylpiperazine theophylline molecule instead of the methyl radical of the hydrogen atom to the first position, and to the 7th position instead of phenylethyl *н*-heptyl (compound 7) or *н*-docyl (compound 8) fragments, and anti-exudative activity was 30.3% and 26.3%, respectively.

Compounds 10 and 11 revealed a tendency to suppress the development of paw edema in rats by an average of 18.4 and 28.5%, respectively.

The anti-exudative activity of the comparative drug — diclofenac sodium at a dose of 8 mg/kg was 46.5%.

It can be assumed that the anti-inflammatory effect of the newly synthesized derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines is realized by reducing the release of inflammatory mediators from mast cells and inhibiting the expression of genes responsible for the synthesis of anti-inflammatory cytokines (Kato et al., 2001).

Thus, among the studied derivatives of 7-disubstituted 8-methylpiperazine-1,3-dimethylxanthines, the anti-exudative activity of compound 4 is comparable to the anti-inflammatory effect of the diclofenac sodium comparator.

Conclusions. 1. Expressed anti-exudative activity was shown by the compound 4 — 7-(3-chlorobutane-2-diol-1)-8-N-ethylpiperazinetheophylline, which caused a decrease in the development of experimental carrageenan edema in rats by 45.2%.

2. Derivatives of 7-substituted 8-methylpiperazin-1,3-dimethylxanthines are the promising group of organic substances for the subsequent purposeful synthesis and pharmacological screening in order to create new agents with anti-inflammatory activity on their basis.

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Part 2. Biosafety

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STUDY OF THE INFLUENCE OF BACTERICIDAL PREPARATION ON CELL CULTURES

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Summary. Pressing problem today is the existence of a large number of microorganisms — causative agents of viral diseases, highly resistant to bactericidal preparations. At the same time, an important direction of research is the study of the toxic effects of new disinfectants not only on the organism of laboratory animals, but also on cell cultures, which is more humane and meets the modern standards of bioethics. That's what determined the purpose of research — the study of the antiviral and toxic effects of the bactericidal preparation 'Barez' on the Aujeszky's disease virus strain 'Clone-B', tested and adapted to the continuous cell cultures SNEV and PTP. When conducting research there were studied methods for assessing toxic effects of antibacterial drug 'Barez' on the cell cultures of swine origin SNEV, PTP, and its effect on the titer of infectious activity of the vaccine strain 'Clone-B' of Aujeszky's disease virus from the collection of strains of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms. When studying the antiviral properties of the bactericidal preparation on the Aujeszky's disease virus 'Clone-B' strain, tested and adapted for continuous cell cultures SNEV and PTP, there was determined that 0.2–0.5% solutions of the drug 'Barez' are not toxic when effecting cell cultures, but exhibit inactivating effect on the Aujeszky's disease virus. Therefore, it is possible to use it for preventive disinfection of objects of veterinary medicine in the presence of animals in recommended concentrations and exposures.

Keywords: bactericidal drug 'Barez', cell cultures, toxicity, Aujeszky's disease virus, silver nanoparticles, benzalkonium chloride, essential oils

Introduction. A serious problem in prevention and control of animal viral diseases is the resistance of pathogens to antiseptics and disinfectants. The species composition of antibiotic resistant microorganisms is highly varied; they are characterized by a higher resistance to disinfectants of various chemical groups and the ability not only to survive, but also to multiply in solutions of disinfectants and on various objects of the environment (Stadnytska et al., 2011; Ababutain, 2011).

Methods of studying the toxic effects of new disinfectants on laboratory animals are not humane, expensive and do not meet the global standards for biotic evaluation of experiments. Therefore, the application of the method for determining the toxic effect of disinfectants on cell cultures, as an alternative to experiments using animals, is very relevant in today's research practice (Ababutain, 2011; Pathmanathan et al., 2010; Kovalenko and Nedosiekov, 2011).

An important condition for the reliable biotesting of new disinfectants is the use of genetically homogeneous laboratory cultures, since they are tested for sensitivity, they are stored in special standard-determined laboratory conditions, which ensures the necessary reliability and reproducibility of the results of studies, as well as the maximum sensitivity to toxic substances.

The study of antiviral activity of disinfectants on viruses that can cause disease in animals and humans, is relevant in the practice of veterinary medicine as well as in many other areas where it is necessary to determine the action of disinfectants against specific infectious agents (Kovalenko et al., 2010; Deyneka, 1999).

The study of toxicity of bactericidal agents on cell cultures can be recommended for: an approximate accelerated assessment of the toxicity of bactericidal drugs; establishment of harmlessness of animal protection means during selective control; in order to obtain an indicative evaluation of the toxicity of active substance, solvent, filler, etc. at the stage of development or changes in the production technology; in cases where the amount of the preparation is too small to determine its toxicity on laboratory animals; ecological testing of bactericidal agents that may pose a threat to the environment (Pathmanathan et al., 2010; Kovalenko and Nedosiekov, 2011).

The aim of the study was to research the antiviral and toxic effects of bactericidal preparation 'Barez' on the

Aujeszky's disease virus strain 'Clone-B', tested and adapted for continuous cell cultures SNEV and PTP.

Materials and methods. In the course of the research, methods for assessing the toxic effects of disinfectants on the cell cultures of swine origin SNEV, PTP, as well as their influence on the titer of the infectious activity of Aujeszky's disease virus vaccine strain 'Clone-B' from the collection of strains of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms.

The objects of the research were the solutions of bactericidal preparation 'Barez' based on silver nanoparticles, benzalkonium chloride and essential oils. In the course of the experiment there were used: a continuous cell culture of swine embryo kidney (SNEV) and a continuous cell culture of piglets testicular (PTP) grown on the surface of the well bottom of a 96-well microplate as a monolayer. To grow continuous cell cultures there were used following media: RPMI 1640, DMEM, lactalbumin hydrolysate medium, cattle blood serum series 07, phosphate-salt buffer. As a test object, a continuous cell culture with known characteristics and stable for at least three consecutive passages SNEV, PTP was used.

To comply with the standard cultivation conditions, cultural 96-well sterile microplates for cell cultures 'Sarstedt' were used in a quantity of two for each culture, and sterile 96-well microplates of the same firm for dilutions of the tested bactericidal agent. The criteria for toxic effects were: indicators of statistically reliable reduction of the cell culture proliferation index in comparison with control (weak toxicity); suppression for some time or complete loss of cell ability to multiply (strong toxicity); the effect of a bactericidal agent on a cell culture, accompanied by instantaneous visible destructive changes in the cells of the culture (extrusion of cells from the carrier surface), and its strong fixation to the surface of the carrier, or coagulation, can be described as super toxicity.

To determine the degree of antiviral action of the disinfectant on the vaccine strain 'Clone-B' of the Aujeszky's disease virus, its various concentrations were prepared in a microplate with U-shaped bottom. Dilutions of the drug were performed on the basis of phosphate-salt buffer.

After that, in the prepared dilution of the disinfectant, a virus suspension of Aujeszky's disease virus strain 'Clone-B' with activity (10^7 TCD_{50}/cm^3 — the dose of the virus, which caused in 24–28 hours without treatment cytopathic effect in the cell cultures SNEV and PTP) was added. The contact of virus with disinfectant was carried out in a titration microplate for 60 minutes. After the contact virus + drug prepared dilutions were placed onto the surface of the cell monolayer for possible adsorption of the virus, which was not inactivated by a disinfectant.

Identical consecutive dilutions of the preparation for each culture were introduced in two microplates with cell cultures SNEV and PTP, one plate for each culture. The inserted dilutions of the preparation were left for contact with 'Clone-B' strain virus suspension for 15 minutes.

At the end of the contact time, the microplates with the cell cultures were washed three times with phosphate-salt solution, the maintenance medium with cattle blood serum was introduced and placed for further incubation. At that, 16 wells with cell culture in each microplate were left as control, in which the preparation was not introduced. Microplates were observed two times a day by microscopy.

Results and discussion. The visual evaluation of the state of monolayer of taken in the experiments cell cultures, that came into direct contact with the consecutive dilutions of the drug, clearly reflected the result of the interaction culture-disinfectant compared with the control microplate wells. In the wells of a microplate with cell culture in which the drug was introduced at a concentration below the toxicity threshold, the monolayer remained intact and visually did not differ from the control wells with culture.

At the same time, during the visual evaluation, there were also found wells with culture of cells with signs of cytopathic action and degenerative changes of the monolayer in comparison with the control. The cause of degenerative changes in cell culture was the effect of the drug 'Barez' in the corresponding concentration, which was found to be toxic to this type of cell culture (Table 1).

Table 1 — Results of determination of the cytopathic effect of Aujeszky's disease virus treated with a bactericidal preparation 'Barez' on a cell cultures monolayer (n = 10)

Concentration, %	Cell culture, exposure 20 min		Manifestation of cytopathic action of the Aujeszky's disease virus treated with disinfectant, exposure 60 min			
	SNEV	PTP	Number of wells			
0.05	–	–	+	+	+	+
0.1	–	–	+	–	–	+
0.2	–	–	–	–	–	–
0.5	–	–	–	–	–	–
1.0	–	–	0	0	–	–
2.0	+	+	0	0	0	0

Notes: (–) — no cytopathic effect; (+) — reflects the cytopathic effect of the drug on the cell.

The results of the study were taken into account until the moment of degenerative changes in the microplate control wells. The 'Barez' drug in 1.0–2.0% concentrations on cell cultures SNEV and PTP showed cytopathic effects, so these concentrations were not evaluated concerning the effect of disinfectant on the Aujeszky's disease virus.

As a result of studies conducted to determine the toxic effect of the preparation after cell culture subcultivation under the influence of 0.2% solution of the drug 'Barez',

there was found that the drug at this concentration did not affect the change of the culture monolayer compared to the control (Table 1).

Subsequent studies have found that the bactericidal agent 'Barez' in concentrations of 0.2–0.5% exhibited inactivating effect on the Aujeszky's disease virus. Therefore, the drug in these concentrations can be recommended for disinfection in order to prevent viral diseases.

At the same time, the degree of cell proliferation was determined after the action of the investigated disinfectant on them. Observing the cell culture was carried out by microscopy from the 1st to the 6th day so far the completion of the experiment, and counting the number of cells in the microplate wells was carried out from the 4th to the 6th day inclusively. The time of formation of a monolayer in experimental carriers with culture or the absence of such a fact in general compared with the control culture is decisive in assessing the degree of toxic effects of a disinfectant.

For the statistical processing of the obtained research results, in addition to the visual evaluation of the cell culture monolayer, a method of counting cells from each carrier with cell culture, taken in the experiment separately, was used. After 6 days, the observations were discontinued due to degenerative changes in the control cell culture, which was due to the aging of the cell monolayer.

After re-seeding (removal of the cell culture monolayer for further reproduction), the following results were obtained. Under the influence of the drug 'Burez' in 0.3% concentration on the 4th day, the number of cells SNEV was 15,650.0 ± 34.1, on the 6th day — 16,320.0 ± 42.5 ($p \leq 0.05$). In the control experiment, 17,730.0 ± 53.4 SNEV cells were observed.

The same results were obtained with the cell culture PTP, where under the influence of the drug 'Barez' in 0.3% concentration on the 4th day the number of PTP cells was 18,320.0 ± 35.2 ($p \leq 0.05$), on the 6th day — 19,700.0 ± 41.3. In the control experiment, 19,630.0 ± 52.6 PTP cells were observed. In these cases, non-toxic effects of disinfectant were observed.

Conclusions. Bactericidal preparation 'Barez' in a concentration of 0.2–0.5% is non-toxic for exposure to cell culture, but exhibits inactivating effect on the Aujeszky's disease virus. Therefore, it is possible to use it for preventive disinfection of objects of veterinary medicine in the presence of animals in recommended concentrations and exposures.

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INFLUENCE OF MODERN VACUUM PACKAGING ON QUALITY AND SAFETY OF SAUSAGE PRODUCTS

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Summary. The necessity to provide the quality and safety of the product during storage, transportation and sale is determined not only strict observance to veterinary and sanitary measures, but has also forced manufacturers to look for modern packaging methods that meet the claimed requirements. The system of vacuum packaging into the food gas is widely used modern type of packaging — 'Modified Atmosphere Packaging' (MAP). The special feature of MPA is to substitute the air in the package with a mixture of gases (oxygen, carbon dioxide and nitrogen), the ratio of which, especially O_2, depends on the type of packaged product. Low oxygen level prevents the development and reproduction of fungus, bacterium and other microorganisms. The use of a modified gas atmosphere allows you to maintain the quality, taste and the product's appearance, increase shelf life.

The aim of this work has been to carry out the influence of modern vacuum packaging on quality and safety of the sausage products and to install the shelf life term of the ready product in the conditions of packaging into modified gas mixture (carbon dioxide, nitrogen and oxygen) and shrink wrap under vacuum.

The automatic line 'Multivak' has been used for packing of sausage products in the modified gas mixture. Experimental studies have been carried out during the use a gas mixture, which has been consisted of carbon dioxide (30%), nitrogen (40%) and oxygen (30%).

The use of vacuum packaging machines 'Cryovak' and 'Supervak' has provided the reliable sealing of sausage products in a shrink-wrap under vacuum (vacuum depth 9 mbar).

In article terms of realization of sausage products in the natural coating, that are packed in a modified gas mixture (carbon dioxide, nitrogen and oxygen) and the shrink wrap under vacuum with the storage conditions at the temperature 4 ± 1 °C and the relative humidity 85 ± 2% have been detected. It has been defined that the use of the modified gas mixture extends the implementation terms of sausage products in the natural coating from 7 to 20 days. The use of the automatic packing line 'Criovak' and 'Supervak' for the packaging of products in the shrink-wrap at vacuum (9 mbar) allows prolonging shelf life of sausage products in the natural coating from 7 to 15 days.

Keywords: sausage products, packaging, vacuuming, modified gas mixture

Introduction. The meat industry has a great economic importance, because it is designed to provide the population with high quality and safe products: meat, sausage, meat-canned food, products for children and dietary foods, semi-finished products, etc. (Agulnik and Teternik, 1971; Karmas, 1981).

One of the main reasons of the extreme decreasing of the quality and food value of sausage products is the violation of optimal temperature regimes during the storage period, transportation and sale (Münch et al., 1985; Marmuzova, 2006). The storage condition violation for meat and meat products tends to quick microbiological spoilage. Even the short-term presence of products in the air, which contains pathogenic or conditionally pathogenic bacteria, is completely enough to contaminate them (Shmarina, Ryaskova and Rodionova, 2016; Rodionova and Paliy, 2017). Microorganisms, that contaminate products, worsen the product's appearance, reduce its taste, because of changes in proteins and fats, but provoke food poisoning, dysbacteriosis, allergic reactions, and metabolic disorders in humans due to the ability to produce various toxins. In addition, many types of mold, even at low storage temperature, form mycotoxins that intrude into the product. In this case, the removal of mold from the product surface does not exclude the presence of dangerous metabolites (Protchenko, 2002; Agul'nik and Korneyev, 1972; Hultman et al., 2015).

The necessity to provide the quality and safety of the product during storage, transportation and sale is determined not only strict observance to veterinary and sanitary measures (Rodionova and Paliy, 2016; Paliy and Rodionova, 2017), but has also forced manufacturers to look for modern packaging methods that meet the claimed requirements. The system of vacuum packaging into the food gas is widely used modern type of packaging (Shuba, 2008; Kostenko, Gutnik, and Iskakov, 2009; Kainash, Ofilenko and Burbak, 2014).

The technology of product preserving with the help of modified gas atmosphere has received the common English name 'Modified Atmosphere Packaging (MAP)'. The special feature of MPA is to substitute the air in the package with a mixture of gases (oxygen, carbon dioxide and nitrogen), the ratio of which, especially O_2, depends on the type of packaged product. Low oxygen level prevents the development and reproduction of fungus, bacterium and other microorganisms. MPA is the natural

and environmentally friendly technology of product preservation. The use of a modified gas atmosphere allows you to maintain the quality, taste and the product's appearance, increase shelf life (Cachaldora et al., 2013; Hur et al., 2013).

Therefore, using of modern vacuum technologies to vacuum sausage and meat is the promising technology in the food industry all over the world (Potekha, Potekha and Kurilo, 2016; Buzoverov and Postnikova, 2013; Semyenova et al., 2013).

The aim of the work has been to carry out the influence of modern vacuum packaging on quality and safety of the sausage products and to install the shelf life term of the ready product in the conditions of packaging into modified gas mixture (carbon dioxide, nitrogen and oxygen) and shrink wrap under vacuum.

Materials and methods. Experimental studies has been carried out in the Laboratory of Veterinary Sanitation and Parasitology of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv, Ukraine), Meat Processing Enterprise 'X' and the Department of Infectology, Quality and Safety of Agricultural Products of the Luhansk National Agrarian University (Kharkiv, Ukraine), respectively to the existing standard documents in accordance to generally accepted methods applied on the state level in Ukraine (DSTU 4436:2005).

The objects of the study have been sausages in the edible coating, which have been packed in a modern shrink-wrap in vacuum and modified gas mixture.

The automatic line 'Multivak' has been used for packing of sausage products in the modified gas mixture. Experimental studies have been carried out during the use a gas mixture, which has been consisted of carbon dioxide (30%), nitrogen (40%) and oxygen (30%).

The use of vacuum packaging machines 'Cryovak' and 'Supervak' has provided the reliable sealing of sausage products in a shrink-wrap under vacuum (vacuum depth 9 mbar).

Results and discussion. In order to study the influence of modern vacuum packaging on the quality and safety of sausage products and to install the shelf life term for the ready products in the conditions of packing into modified gas mixture (carbon dioxide, nitrogen and oxygen) and shrink wrap under vacuum, all experimental sausages have been divided into three groups:

1st group (packed in a modified gas mixture (carbon dioxide, nitrogen and oxygen) — 'Varena z molokom' 1st class, 'Dytiachia' highest class, 'Sosysky bavarsky' highest class, 'Sosysky molochni' 1st class;

2nd group (packed in a shrink wrap under vacuum) — 'Varena s molokom' 1st class, 'Doctorska' highest class, 'Dytiachia' highest class;

3rd group (control) — 'Varena z molokom' 1st class, 'Doctorska' highest class, 'Dytiachia' highest class, 'Sosysky molochni' 1st class, 'Sosysky bavarsky' highest class.

The samples have been saved in the refrigerator at the 4 ± 1 °C temperature and 85 ± 2% relative humidity.

Microbiological, organoleptic, physical and chemical studies of sausages have been carried out for 25 days to establish the optimal terms, storage conditions and temperature regimes.

Before making the experiment, microbiological studies have been carried out to determine the accordance of the experimental samples with the requirements of DSTU 4436:2005.

In accordance with DSTU 4436:2005 according to microbiological parameters, sausage products must meet the requirements that are given in Table 1.

It has been established that the number of mesophilic aerobic and facultative-anaerobic microorganisms (MAFAnM) in 1 g of experimental sausage products at the time of the end of the technological process was:

'Varena z molokom' — 1.15×10^2 CFU/g,
'Doctorska' — 1.1×10^2 CFU/g,
'Dytiachia' — 1.3×10^2 CFU/g,
'Sosysky molochni' — 1.1×10^2 CFU/g,
'Sosysky bavarsky' — 9.0×10^2 CFU/g.

In the microbiological study of the first group of sausages (Fig. 1), the microbial composition of experimental product has remained at the original level for 5 days. The moisture mass concentration of the researched samples decreased in average in 3% on the 5th day, which is in 6 times less compared with the control.

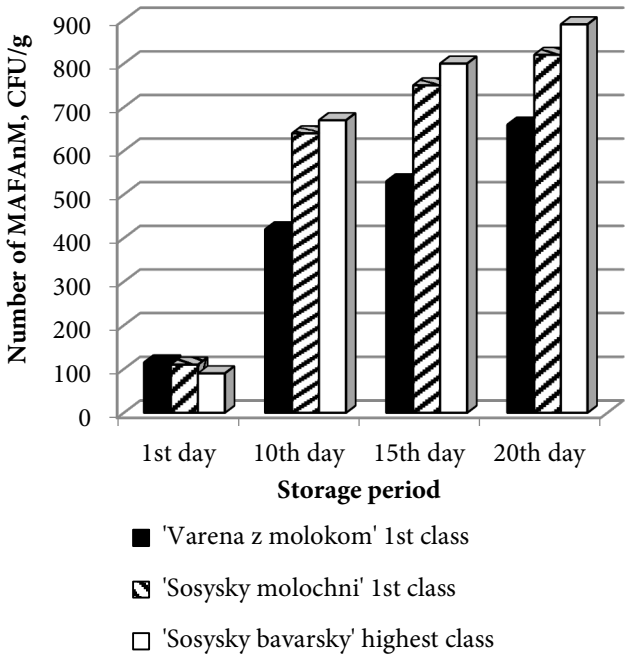

Figure 1. Dynamics of the development of MAFAnM in sausage products packed in modified gas mixture (carbon dioxide, nitrogen and oxygen)

Table 1 —Microbiological indicators of sausage products

Name of the index	Standard			Method of control
	Boiled sausages of the highest, 1st and 2nd classes, frankfurters, sardellas, meat loaves	**Boiled sausages of the 2nd class with the use of cereals, meatmass, meat by-products**	**Boiled sausages of the 3rd class**	
Number of mesophilic aerobic and extra-anaerobic microorganisms, CFU/g of product, not more	1.0×10^3	2.5×10^3	5.0×10^3	According to GOST 9958-81
Pathogenic microorganisms, in particular bacteria of the genus *Salmonella*, in 25 g of product	Not allowed			According to GOST 9958-81 or DSTU 12824:2004
Bacteria of the group of intestinal sticks (BGKP), in 1 g of product				According to GOST 9958-81
Sulphide reductant *Clostridia*: in 0.01 g of product or in 1 g of product for vacuum packed				
Coagulase-positive *Staphylococci* in 1 g of product for children and dietary foods				
Staphylococcus aureus in 1 g of product				According to GOST 10444.2-94 or DSTU 6888-1 or DSTU 6888-2
Listeria monocytogenes, in 25 g of product				According to DSTU 11290-1 or DSTU 11290-2

On the 10th days from the beginning of the study, in accordance with the results of microbiological studies, it has been found that the amount of MAFAnM in the experimental samples is:

'Varena z molokom' — $4.2 \pm 0.46 \times 10^2$ CFU/g,

'Sosysky molochni' — $6.4 \pm 0.26 \times 10^2$ CFU/g,

'Bavarsky sosysky' — $6.7 \pm 0.32 \times 10^2$ CFU/g.

Pathogenic microorganisms have not been detected. According to the results of organoleptic studies, the smell and taste are up to quality of these types of product. The experimental rolls of sausage products have the clean lightly damp surface. Consistency is elastic. The specific smell of the gas mixture disappears within 3–5 seconds. The moisture mass concentration of the researched samples has dropped in general in 3.4%.

According to the results of microbiological research on the 15th day of storage of this experimental group samples, it has been determined that the amount of MAFAnM in the experimental samples is:

'Varena z molokom' — $5.3 \pm 0.25 \times 10^2$ CFU/g,

'Sosysky molochni' — $7.5 \pm 0.31 \times 10^2$ CFU/g,

'Bavarsky sosysky' — $8.0 \pm 0.22 \times 10^2$ CFU/g,

which is in 4.6, 6.8, and 8.9 times more than at the beginning of the experiment. The analysis of the MAFAnM amount, which has been made on the 10th day and 15th day of storage, concluded that the MAFAnM amount in the experimental samples increased in average in 18.2% during the 5-days period. Pathogenic microorganisms have not been detected. As the result of organoleptic studies, all the experimental samples meet the requirements of DSTU 4436:2005. The moisture mass concentration of experimental samples decreased more in 0.3%.

At the 20th day, the number of MAFAnM has been increased in average in 7.5 times.

Pathogenic microorganisms have not been detected. According to the results of organoleptic studies the deviations from standard indicators (DSTU 4436:2005) are not detected. The specific smell of the gas mixture disappears within 5–10 seconds. On the packing surface there it appeared single evaporations droplets. The moisture mass concentration of the experimental samples decreased in 4.1%.

At 22nd day of the research on the packing surface there were droplets of dew, as a result of the interaction of the residual amount of oxygen with a food gas mixture. The surface of sausage products had an extraneous smell.

Therefore, as a result of the carried out research it has been established that during the packaging of sausage products in the modified gas mixture at the automatic

packing line 'Multivak', the realization terms of sausages in the natural coating could be extended up to 20 days underprovided preserve condition at 4 ± 1 °C temperature and 85 ± 2% relative humidity.

The microbial composition also has remained at the original level in the second group of sausages in a vacuum package during 5-days storage (Fig. 2).

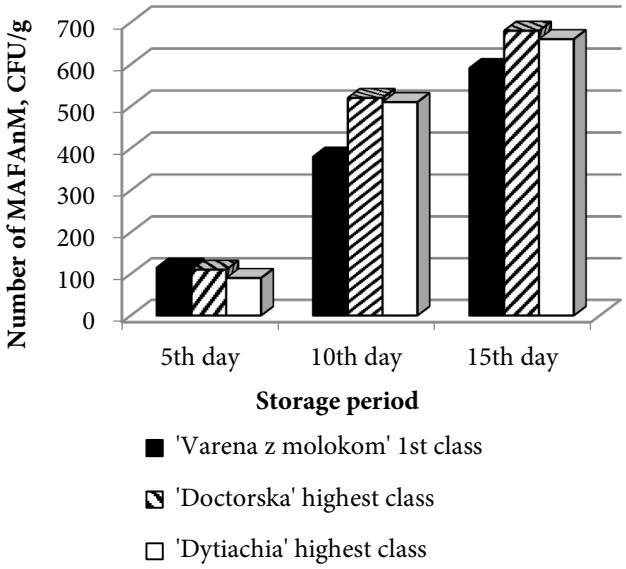

Figure 2. Dynamics of development of MAFAnM in sausages during storage in vacuum packaging

On the 10th days after the beginning of the study, according to the results of microbiological studies, it has been found that the amount of MAFAnM in the experimental samples is:

'Varena z molokom' — $3.8 \pm 0.16 \times 10^2$ CFU/g,

'Doktorska' — $5.2 \pm 0.41 \times 10^2$ CFU/g,

'Dytiacha' — $5.1 \pm 0.15 \times 10^2$ CFU/g.

Pathogenic microorganisms have not been detected. According to the results of organoleptic studies, the smell and taste are up to quality of these types. Shrink wrap sticks tightly to the rolls. The structural integrity of the seal is not damaged. The content of the broth under the wrap is absent. The consistency of sausage rolls is elastic. The specific smell of the gas mixture disappears within 3–5 seconds. The moisture mass concentration of the experimental samples decreased for 3.4%.

At the 15th day, the number of MAFAnM is increased in sausages: 'Varena z molokom' and 'Dytiacha' in 5.1 times, and 'Doktorska' in 6.2 times respectively. Pathogenic microorganisms have not been detected. According to the results of organoleptic studies, it has been found that the color of the sausage rolls 'Doktorska' has changed from pink to pale-pink that indicates about the decomposition of sodium nitrite.

The moisture mass concentration of the experimental samples decreased in 5.4%, which is in 1.8 times more than the first experimental group, but 3.3 times less than the control one.

According to the results of the organoleptic study of sausages of the second group at the 17th day of storage, it has been found that the sausage 'Doctorska' highest class a greenish tinge on the cut surface due to the decomposition of sodium nitrite under the light influence during the storage. In all experimental samples there is the separation of the broth under the shrink-wrap, due to the product losses the vacuum and, consequently, it losses of the commercial appearance of the test specimens.

Making the analysis of the obtained results presented in Fig. 2 it was established that the packaging sausages in a shrink wrap under vacuum, thanks to the automatic packing line 'Criovak' and 'Supervak', allows extend the shelf life of sausage products in natural coating for up to 15 days provided preserved condition at 4 ± 1 °C temperature and 85 ± 2% relative humidity.

As a result of the microbiological study of sausage products in the natural coating (Fig. 3) in storage conditions at 4 ± 1 °C and 85 ± 2% of relative humidity, it has been found that during 3 days in the given temperature, the total amount of bacteria in 1 g of sausages 'Varena z molokom' 1st class, 'Doctorska' highest class, 'Dytiacha' highest class has remained at the original level.

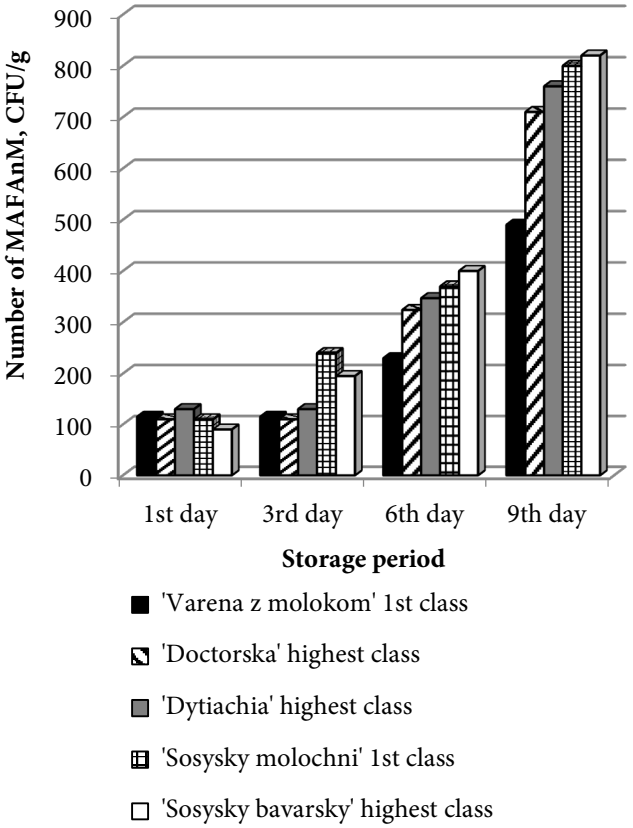

Figure 3. Dynamics of development of MAFAnM in sausage products without packaging

In the sausage products 'Molochni sosysky' and 'Sosysky bavarski' at 3rd day the total amount of MAFAnM has been $2.4 \pm 0.12 \times 10^2$ and $1.95 \pm 0.27 \times 10^2$ CFU/g,

respectively. According to the results of organoleptic studies the deviations from standard parameters (DSTU 4436:2005) were not detected. The moisture mass concentration has in average decreased in 3%.

Making the analysis of the microbiological data which were obtained at 6th day of storage at the given temperature, it was determined that the number of MAFAnM is in average in 3 times higher than at the beginning of the experiment. Pathogenic microorganisms have not been detected. During the organoleptic studies has found that the smoked smell is light, the surface of sausage rolls is slightly wrinkled. The moisture mass concentration of the researched sausages has in average decreased in 18%.

In accordance with the results of bacteriological research the amount of MAFAnM increased in sausages 'Varena z molokom' — in 4.2 times, 'Doktorska' — in 6.4 times, 'Dytiacha' — in 5.8 times, 'Sosysky bavarski' and 'Sosysky molochni' — in 9.0 and 7.2 times respectively at the 9th day. Bacteria of the *Escherichia coli* group (BGKP) have been detected during the analysis on presence of pathogenic microorganisms in the experimental samples of sausages.

During the organoleptic study of control group sausages the extraneous smell has been detected at the 9th day. The sausages surface is sticky. Molds and BGKP has been found during the study of the swabs from the sausages surface.

The moisture mass concentration of the researched sausages has in average decreased in 22%, at the 9th day which is almost in 6 times more than in the first and second control groups.

As a result of the study which were carried out in the control group of sausages, it was found that during the storage of sausages in the natural coating at 4 ± 1 °C temperature and 85 ± 2% of relative humidity, its shelf life period is 7 days.

According to the results of scientific research the Ukrainian patent for utility model No. 119865 'Method of food packaging' (Rodionova, Paliy and Brahinets, 2017) were received.

Conclusions. Nowadays vacuum packaging and modified atmosphere packaging are the most up-to-date ways to maintain the quality and food freshness.

The packaging of sausages in the natural coating in modified atmosphere mixture (carbon dioxide, nitrogen and oxygen) by the automatic packaging 'Multivak' extends its shelf life from 7 to 20 days at storage conditions of 4 ± 1 °C temperature and 85 ± 2% of relative humidity.

The packaging of sausages in vacuum shrink wrap thanks to the automatic packing line 'Criovak' and 'Supervak' allows extend the shelf life of sausage products in the natural coating from 7 to 15 days at 4 ± 1 °C storage temperature and 85 ± 2% of relative humidity.

It has been established that the use of modern vacuum packaging could prevent the losses of the finished product's weight in 6 times compared with the control group.

Using of the modern vacuum packaging, allows to create the high barrier for oxygen, that prevents from microbial contamination, saves the casings protect against breakage, punctures and other mechanical damages, which in reduces the possibility of contaminating products with pathogenic microflora.

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Part 3. Biotechnology and genetics

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EFFECTS OF MTHFR GENE ON REPRODUCTIVE HEALTH AND PRODUCTIVE TRAITS OF DAIRY COWS

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Summary. One-carbon metabolism of mammals is one of the key points of metabolism and the pleiotropic effects of human MTHFR gene determining reproductive health are known. The aim of this study was an evaluation the role of MTHFR gene in lactating dairy cows. Cows were genotyped by sequencing. The plasma homocysteine level, bone mineral density, traditional production and reproduction traits were analyzed. Statistical methods included Pearson's chi-squared and *t* criteria, Pearson's and Spearmen correlation coefficient *r* and ANOVA. Allele frequencies of SNP 8137C/T of MTHFR gene were: 0.943 (C) and 0.057 (T). Distribution of genotypes was 88.6% (CC) : 11.4% (CT) : 0% (TT). Investigated group of animals was in Hardy-Weinberg equilibrium. We had founded that calving interval were shorter in cows with the CC genotype than in CT animals, 378.6 vs 405.9 days, and than in the herd as a whole — 378.6 vs 388.5 days. Lactation period of CC cows shorter by 10 days than in CT cows, 321.7 vs 331.5 days. Analysis of traits of CC cows and animals in the herd had demonstrated that the age of first insemination and the age of first calving are significantly lower in CC cows than in the herd as a whole, 525.8 ± 17.8 vs 642.9 ± 7.5 days, and 808.6 ± 18.5 vs 936.6 ± 8.0 days. Higher bone mineral density values as an indicator of body health are observed in CC cows compared with CT animals, 3,580.3 vs 3,359.0 mg/mm^3. The study of MTHFR gene associated with reproductive traits in cows is relevant as a basis for breeding and biochemical correction of gene effects causing the reproductive disorders of animals.

Keywords: dairy cows, MTHFR gene, homocysteine, bone mineral density, milk production, reproduction of cows

Introduction. One-carbon metabolism of mammals is one of the key points of metabolism, and its research is a perspective direction for the development of pharmacological correction of failures. Methylenetetrahydrofolate reductase (MTHFR) is one of the main regulatory enzymes in the metabolism of homocysteine that catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Single nucleotide polymorphisms (SNPs) in MTHFR gene lead to decreased activity of enzyme, efficiency of the homocysteine-methionine cycle and hyperhomocysteinemia. The C677T transition in the exon 4 in human MTHFR gene leading to a thermolabile protein, with decreased enzymatic activity and it has been investigated for the past 20 years in different countries and ethnic groups. Numerous investigations have been performed on the associations of MTHFR gene SNPs with low fertility of women. Results of these studies have demonstrated a relationship between genotypes of MTHFR gene and pregnancy complications (placental insufficiency, premature detachment of normally situated placenta, late gestosis), fetal malformations (cleft neural tube, anencephaly, deformities of the facial skeleton), children born with a chromosomal abnormality.

So this MTHFR enzymatic activity process is lowered in subjects with MTHFR 677TT and 677CT genotypes and these individuals might require an increased intake of folate or another dietary factors to maintain or control blood levels of plasma folate or homocysteine (Soligo, Barini and Annichino-Bizzacchi, 2017; Hwang et al., 2017; Al-Achkar et al., 2016; Asim et al., 2015; Vanilla et al., 2015; Luo et al., 2015; Poursadegh Zonouzi et al., 2012; Altomare, Adler and Aledort, 2007; Stonek et al., 2007; Ananth et al., 2007; Doolin et al., 2002; Zetterberg et al., 2002).

It was presented by Zimin et al. (2009), that cows and humans have sufficient DNA sequence similarity to enable to map the human genome almost entirely onto cow. Authors were able to demonstrate a large majority, approximately 91%, of the genome has been placed onto the 30 *Bos taurus* chromosomes. Researchers identified 25,710 RefSeq proteins representing 18,019 distinct human genes, and aligned these to the cow genome. Of the 18,019 human genes, 17,253 (95.7%) mapped to cow (Zimin et al., 2009). In this connection, we believe that cows could be a suitable model for studying the effects of genes associated with mammalian reproduction.

The results obtained Yapan et al. (2011) after research of cows reproductive genetics and MTHFR gene were demonstrated that 8137C/T SNP was identified in exon 7 of MTHFR. Association analysis revealed that the 8137C/T was associated with cow abortion. According to the conclusion of researchers MTHFR may be a beneficial candidate gene to control cow abortion.

At the same time it could be noted a number of factors that affect one carbon metabolism such as age, gender, nutrition, genetics, medication, physical activity, climatic conditions and management of animals. That is why it is actual to conduct research on the Ukrainian selection breeds in the technological and fodder conditions of Ukrainian farms.

The aim of the study was the evaluation of role of MTHFR gene in lactating dairy cows.

Material and methods. The study was carried out with the analysis of materials and biological samples of lactating dairy cows from the State Enterprise Research Farm 'Nyva' of Institute of Animal Breeding and Genetics named after M. V. Zubets of the National Academy of Agrarian Sciencies of Ukraine during 2016–2017.

The type of production in SE RF 'Nyva' is organic. In this farm cows had milk production (6,514 L), milk fat (3.65%) and milk protein (3.20%) concentration during 2016–2017. The base total mixed ration (TMR) was alfalfa/haylage based with corn silage, corn and other silage. Part of soybean meal is 3–5% of the grain mixture. The system of keeping cows is traditional. All animals had the same light/dark schedule, humidity and temperature. Part of time animals spent in the walking areas. They do not receive hormonal and other medications, even to stimulate ovulation. The animals are excluded from infectious diseases.

For the study 35 cows at the age of 2^{nd}–7^{th} lactation after calving were selected randomly. All cows have the same pedigree — on average, every animal has 79.5 ± 1.9% of Holstein breed, 16.3 ± 1.92% of Simmental, 2.89 ± 0.82 of Montbeliarde. Traditional production and reproduction parameters were analyzed.

Cows were milked between 6.00 and 8.00, and from 17.00 to 19.00 daily. Samples of milk were individually stored and analyzed by Ecomilk-Standart (Bulteh 2000 Ltd, Bulgaria) for fat, protein. Bone mineral density (BMD) of cows was estimated by ultrasonic densitometry of the middle third of 12 pair rib-bone by Sunlight Omni 7000 (Sunlight Medical Ltd, Israel).

The plasma homocysteine levels were analyzed using commercial ECLIA test kits. The PCR reaction was carried out according Yapan et al. (2011). PCR products were analyzed with ethidium bromide stained 1.0% agarose gels electrophoresis, purified DNA fragments were isolated from gel using GeneJET Gel Extraction Kit (Life Technologies, USA) and then they were sequenced. Analysis of the gel images was carried out by ChemiDoc™ XRS+ System (Bio-Rad, USA). Sequencing was performed on an Applied Biosystems 3130 Genetic Analyzer (Life Technologies, USA). The results were analyzed with Chromas 2.1 Sequencing Software (Technelysium Pty Ltd, Australia) and BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis was performed with the Shapiro-Wilk and Kolmogorov-Smirnov tests for normality and hypotheses — criteria t and χ^2. The deviation of allele frequencies from Hardy-Weinberg equilibrium was tested using Pearson's chi-squared test. The relationship between traits was estimated by the Spearman and Pearson correlation analysis. Means for two groups were compared by ANOVA (Atramentova and Utevskaya, 2008).

Results and discussion. The analysis of the results obtained in the present study indicated cows allele and genotype frequencies of 8137C/T in exon 7 of MTHFR gene (Table 1). Investigated group of animals was in Hardy-Weinberg equilibrium. C allele frequency was 0.943, T allele frequency — 0,057.

Table 1 — Allele and genotype frequencies of MTHFR gene in cows group, $\bar{x} \pm s_x$

Parameter	**SNP 8137C/T of MTHFR gene**		
Allele	**C**	**T**	
Frequency	0,943	0,057	
Genotype	**CC**	**CT**	**TT**
n factual	31	4	0
%	88.6	11.4	0
n theoretical	31	4	0
%	88.9	10.7	0.4
BW, kg	528.7 ± 7.3	524.5 ± 8.0	—
BMD, mg/mm^3	3,580.3 ± 61.4*	3,359.0 ± 89.5*	—
Hcy, μmol/L	5.85 ± 0.82	4.70 ± 0.54	—
CI, days	378.8 ± 5.7**	405.9 ± 10.7**	—
First lactation			
MY, kg/year	5,973.5 ± 92.5	5,868.0 ± 118.8	—
F, %	3.64 ± 0.03	3.67 ± 0.05	—
P, %	3.24 ± 0.02	3.27 ± 0.02	—
LP, days	321.7 ± 5.3	331.5 ± 13.7	—

Notes: $\bar{x} \pm s_x$ — mean ± standard error; BW — body weight, BMD — bone mineral density, Hcy — homocysteine level, CI — calving interval, LP — lactation period, MY — milk yield, F — milk fat, P — milk protein, * — differences are significant at $p < 0.05$, ** — $p < 0.01$.

Distribution of genotypes in cows group is 88.6% : 11.4% : 0%, CC : CT : TT, respectively. Our findings are consistent with the results published before by Yapan et al. (2011) — no homozygote of TT were found. In the work of Chinese authors the genotypic frequencies for CC, CT, and TT were 76.3% : 32,7% : 0%, and the deviation from the Hardy-Weinberg equilibrium was observed. Probably, analysis of a large group of cows (n = 569) allowed to show

the negative effects of the T allele on the fertility of animals: cows with CT genotype had significantly greater relative risk of abortion (OR = 2.05, $p = 0.0002$) than those with CC genotype (Yapan et al., 2011).

The our analysis had demonstrated that calving interval was shorter in cows with the CC genotype — 378.6 vs 405.9 days in CT animals ($p = 0.01$). Evidently, on the one hand, more inseminations were required for CT animals, since it is known that subjects, human and cows, with CT genotype are characterized by lower MTHFR enzymatic activity, DNA and RNA synthesis, protein methylation and a higher homocysteine level in the blood. We have already shown a significant direct correlation between homocysteine level in the blood and the number of inseminations carried out before successful fertilization and development of pregnancy of cows — $r = 0.36$ ($p = 0.05$).

On the other hand, longer days-open in CT cows may be associated with and more active lactation and prolactin's effects include inhibition of ovulatory cycle, follicle-stimulating hormone and gonadotropin-releasing hormone secretion, prolongation of luteal phase and prevents pregnancy during the lactation. In our research — lactation period of CC cows shorter by 10 days than in CT animals, 321.7 vs 331.5 days.

The analysis showed that the homocysteine level in blood of animals was in the range of 2.96 to 27.9 μmol/L, reaching an average of 5.85 ± 0.82 μmol/L in CC cows group and 4.70 ± 0.54 μmol/L in CT group. At the same time, other authors found differences in homocysteine levels in cows with CC and CT genotypes in a large herd (Yapan et al., 2011). According them, the blood homocysteine levels of animals with CT genotype were significantly higher than those with CC genotype during the first 6 months of pregnancy and in non-pregnant cows.

It is well known that a higher level of homocysteine in the blood plasma associated with the CT genotype was also shown in humans (Doolin et al., 2002) and could be associated with various reproductive problems. For example, it had been demonstrated that in women the lower level of homocysteine in the follicular fluid was associated with a better chance of clinical pregnancy (Ocal et al., 2012). A meta-analysis of human studies had documented that high homocysteine level increased the abortion risk by 40% (Ren and Wang, 2006).

Bone mineral density is an indicator of body health and its effect contributed to successful inseminations, good fertility of cows and health pregnancy. Higher BMD values were observed in CC cows compared with CT animals, 3,580.3 vs 3,359.0 ($p = 0.05$). It is important to note that previously we found a negative correlation between bone mineral density and duration of days-open of cows — $r = –0.50$ ($p = 0.05$), and, respectively, the number of inseminations carried out before successful fertilization and development of pregnancy of cows — $r = –0.46$ ($p = 0.08$). More insemination was required for animals with a lower level of bone mineral density.

Analysis of traits of CC cows and all animals in the herd had demonstrated that the age of first insemination and the age of first calving are statistically significantly lower in CC cows than in the herd as a whole, 525.8 ± 17.8 vs 642.9 ± 7.5 days ($p = 0.000007$), and 808.6 ± 18.5 vs 936.6 ± 8.0 days ($p = 0.000003$), respectively. Calving interval were shorter in cows with the CC genotype than in the herd — 378.6 ± 5.7 vs 388.5 ± 4.6 ($F = 5.65$, $p = 0.01$).

Our study found no association between cows with different genotypes on productivity traits, although this position requires further study.

Conclusions. We analyzed association of 8137C/T MTHFR gene with reproductive traits of dairy cows.

The relationship between genotypes and reproduction traits of dairy cows — calving interval, age of first insemination, age of first calving, efficiency of insemination, homocysteine level in the blood plasma and bone mineral density of cows was shown.

Polymorphism 8137C/T of MTHFR gene might play an important role in development and fertility of cows and could be useful in breeding programs.

The study of genes associated with reproductive traits in cows is relevant as a basis for selection and biochemical correction of gene effects causing the reproductive disorders of animals.

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THE EFFECT OF FOOD ADDITIVE 'AQUACAROTIN' ON MAIN PHYSIOLOGICAL BODY PARAMETERS IN MODEL EXPERIMENTS ON ANIMALS

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Summary. The purpose of the work was to carry out experimental studies to determine the effectiveness of the domestic vitamin complex — dietary food additives with biologically active action (DFA BAA) 'Aquacarotin' (LLC 'Bionaftusia', Ukraine), which contains 2% of water-soluble β-carotene (provitamin A) in combination with water soluble vitamin E and vitamin C on physiological body parameters, such as body weight, body weight gain, and peripheral blood parameters. The research was conducted on 60 males of white non-breeding rats that had not reached the age of puberty, and achieved it in the process of research. The rats were grouped into control and main groups of 30 individuals in each group. The main group of animals received DFA BAA 'Aquacarotin' during 3 weeks in addition to main ration, and the control group of animals were kept on a standard rat diet. The influence of DFA BAA 'Aquacarotin' on organism systems was evaluated with body weight, body weight gain, and peripheral blood leukogram indices. The obtained data shows positive influence of DFA BAA 'Aquacarotin' on such physiological parameters as body weight, body weight gain, and hematopoiesis reflected by the peripheral blood indices. Biological activity in DFA BAA 'Aquacarotin' on body weight appears as its significant and accelerated gaining, and body weight increases directly in the process of growth. Active body weight gaining terminated when DFA BAA 'Aquacarotin' was stopped to use. It is valuable that after termination of food additives' introduction to animals' ration, the growth of their body weight slowed down and normalized, thus, it did not continue by itself, which further proves the controllability of the DFA effect, and makes it safe for consumption. DFA BAA 'Aquacarotin' impacts on myelocytic and monocytic leukopoiesis more significantly, which affects increasing of the neutrophil pool, and probably increases the pool of eosinophilic granulocytes and monocytes in peripheral blood stream while increasing of lymphopoiesis. Therefore, DFA BAA 'Aquacarotin' is controllable and therefore safe in regard of its effect on physiological parameters, animals' body weight gaining during growth, as well as peripheral blood parameters. The use of DFA BAA 'Aquacarotin' is beneficial for improving physiological body parameters (rapid increase in body weight), especially in the stage of growth. DFA BAA 'Aquacarotin' activates the myelocytic and monocytic leukopoiesis, which appears in increasing of macro- and microphage pool, as well as enhancement of their functional activity. The obtained data can be interesting and useful in medicine, agriculture, military affairs and nutrition technologies.

Keywords: vitamins A, E and C, dietary food additive 'Aquacarotin', body weight, body weight gain, white blood cells, peripheral blood leukogram

Introduction. The national economy of today is not without food additives all over the world (Raksha-Slusareva, 2014; Shubin, 2010). Dietary food additives (dietary food products) stand out among other dietary additives, which are introduced to food ratio for its enrichment with nutritious or useful (irreplaceable) ingredients (Raksha-Slusareva, 2014; Shubin, 2010). In such areas as agriculture, food medicine, industry, military affairs, and conventional life, dietary food additives with biologically active action (DFA BAA) or functional foods that contain DFA BAA are used. The need to use such DFA BAA like vitamins and vitamin complexes in all periods of organism's existence, especially during its period of growth, is undeniable (Maligina, Raksha-Slusareva and Popova, 2017; Shadrin and Gayduchik, 2016; Anjos et al., 2013).

The most essential vitamins in the period of growth and body formation are fat-soluble vitamins, such as A (β-carotene) and E (tocopherol) and water-soluble vitamin C (Shadrin and Gayduchik, 2016; Anjos et al., 2013; Aslam et al., 2017). Each of the abovementioned vitamins provides the functioning of many organs and systems in the body. Fat-soluble vitamin A, and especially vitamin E, are the most important bioantioxidants of blood lipoproteins and cell membranes and provide the stability of the latter. In this case, the antioxidant action of vitamins is known. Especially the antioxidant effect of vitamin C increases along with the use of tocopherol and retinol, and vitamin A protects vitamin C from oxidation. Vitamin A is a highly effective immune system stimulant, vitamin E is a natural immune regulator, and vitamin C helps to increase the body's resistance to adverse environmental effects. According to recent studies, not only vitamins A and E, but also vitamin C play a significant role, both in non-specific resistance of the organism, and in specific immune response (Alam and

Pawelec, 2012; Mora, Iwata and von Andrian, 2008; Bono et al., 2016; Ströhle and Hahn, 2009; Camarena and Wang, 2016).

Vitamin C refers to vitamins of seasonal, mostly summer, origin, although the development of modern scientific technology makes it possible to get its synthetic analogues into the body at any time of the year. The consumption of synthetic vitamins A and E eliminates almost constant shortage of food intake. Synthetic analogues of fat-soluble vitamins are difficult to metabolize by the body and often cause allergic reactions (Raksha-Slusareva, 2014; Shubin, 2010; Maligina, Raksha-Slusareva and Popova, 2017).

Native scientists of LLC 'Bionaftusia' created a new DFA BAA 'Aquacarotin' on the basis of nanotechnologies. It is a complex vitamin product, which contains 2% of β-carotene (provitamin A) combined with vitamins E and C in form water-soluble form. The micellar form of the product protects the body from side effects and overdose. The obtained water-solubility of vitamins A and E makes them easier to be digested and the body, especially in pathology, accompanied by metabolic disorders. Based on the above, the composition of the developed product should simultaneously enhance the potential of each other and increase positive effect on the body, immune system and cause antioxidant effect, as well as other action inherent to these vitamins. However, since the vitamin complex DFA BAA 'Aquacarotin' is water soluble, it is necessary to study all directions of its impact on the body and immune system components.

The aim of the research was to determine the influence of new DFA BAA 'Aquacarotin' on physiological indicators, such as body weight, body weight gain, and peripheral blood leukogram, which is a complex indicator of the body condition, in model experiments on animals.

Materials and methods. The research was conducted at the Department of Medical Biology, Microbiology, Virology and Immunology of the Donetsk National Medical University of the Ministry of Healthcare of Ukraine.

The research was conducted on 60 males of white non-breeding rats that had not reached puberty, and achieved it in the process of research. The rats were grouped into control and main groups of 30 individuals in each. Male rats with an initial weight of 100–140 g were used in this research. Animals were kept according to special requirements for rats' keeping (Zapadnyuk et al., 1983).

The main group of animals received DFA BAA 'Aquacarotin' counting 0.01 g of the product per day in addition to their diet. In this case, the daily dose of the DFA given to the animals was equal to the maximum daily dose of a human. On average, animals received 0.15 g of the DFA in 3 weeks of the DFA course. Control group of animals was kept on regular ration for rats.

The effect of DFA BAA 'Aquacarotin' on the physiological body systems was estimated with the indices of body weight, relative increase in body weight and peripheral blood leukogram.

Body weight and relative increase in body weight had been assessed before the start of the research in following dynamics: 2, 3 weeks and 3, 6, 13 weeks after the introduction of the food additive to animals' ratio, i.e. on 6, 9 and 16 weeks of the study.

Body weight and body weight gain were determined by weighing of animals on laboratory scales. Animal weight was determined by the formula (1):

$$A_G = W_T - W_O, \quad (1)$$

where A_G is absolute body weight gain of the animals; W_T — animal body weight at the end of reported period; W_O —animal body weight at the beginning of reported period (Stefanov, 2001).

Blood leukogram indices were evaluated with commonly used methods in examined animals of the main and control groups before start of the research, 3 weeks after use and 13 weeks after termination of taking DFA BAA 'Aquacarotin' (Bazarnova and Morozova, 1988).

Results were statistically analyzed using Statistica 6.0 software (StatSoft, USA) and Student's *t*-criterion. Significant differences were found between control and experiment at $p < 0.05$ (Lakin, 1990).

Results and discussion. The assessment of animals' status showed that the main group, which diet was amended with DFA BAA 'Aquacarotin' during 3 weeks, had differences by various physiological indicators, such as body weight and body weight gain, the content of leukocytes and elements of peripheral leukogroup, compared to the control group of animals which did not receive the DFA.

The data on changes in body weight in the control and main groups of animals in the dynamics of research are shown in Fig. 1.

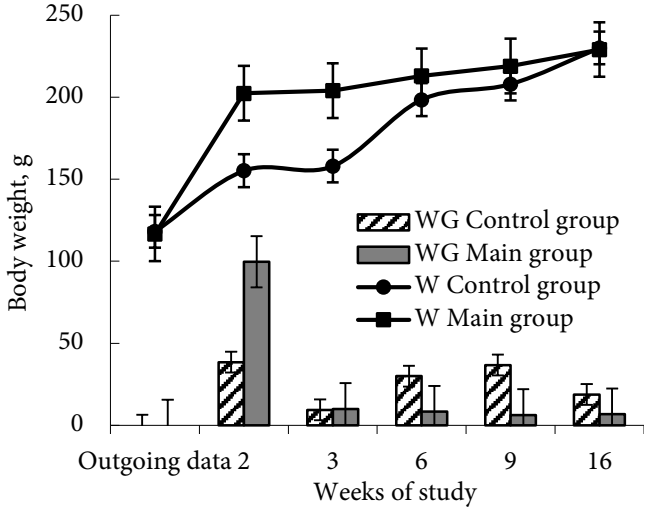

Figure 1. Changes in body weight (W) and body weight gain (WG) of in the main group of animals affected by DFA BAA 'Aquacarotin' and in the control group in the dynamics of research

As it can be seen from the given data, the initial animal body mass in the control and main groups almost did not differ before the experiment was started, and amounted 118.2 ± 2.5 g and 116.7 ± 5.3 g respectively, and the average amount was 117.5 ± 4.1 g. Fluctuations in the initial mass before the experiment in both studied groups were recorded within 100–140 g.

As for 2 weeks of study, the increase in the body weight in the main group was 99.7 ± 19.4 g. It was significantly and credibly higher than in control group 38.5 ± 5.4 g ($p < 0.05$). Fluctuations in body weight in the main group of animals after 2 weeks of the DFA inclusion to the diet was 150–260 g and was greater than that in the control group of animals (150–206 g). Animals' weight in the main group receiving DFA BAA 'Aquacarotin' increased from 116.7 ± 5.3 to 202 ± 19.4 g. Animals' weight in the control group increased from 118.2 ± 2.5 to 155.2 ± 5.7 g and was significantly and credibly less than in the main group ($p < 0.05$).

After 3 weeks, the body weight gain in animals in the main group was 10.0 ± 5.7 g, and probably decreased, compared to the data for 2 weeks of research ($p < 0.05$) and almost did not differ from that in the control group of animals (9.4 ± 3.4 g), which also was possibly reduced compared to those registered for 2 weeks. Fluctuations of body weight in the main and control groups were amounted 170–220 g and 150–205 g respectively. The weight of animals in the main group receiving DFA BAA 'Aquacarotin' for 3 weeks had no significant difference from indices of the previous research reference point amounted to 204.0 ± 9.6 g. Thereby, the animals' weight in the control group had only a slight increase trend and amounted to 158.0 ± 15.38 g, compared to the results obtained for 2 weeks of research. At the same time, animals' body weight in the main group during the third week of study remained significantly and credibly higher than in the control group ($p < 0.05$).

Since the possibility to control the effect is important for dietary food additives, and long influence of food additives on the body without control may be a negative factor that can, in particular, lead to obesity, we have continued the research on weight changes in animals after terminating the consumption of DFA BAA 'Aquacarotin' for next 13 weeks.

As can be seen from the data in Fig. 1, after 6 weeks of study (1.5 months) and 3 weeks after stopping adding DFA BAA 'Aquacarotin' to the diet, the animals' body weight gain considerably slowed down in the main group. The body weight gain in the main group was only 8.3 ± 4.8 g. Animals' body weight gain in the control group was conversely in trend of a significant increase at 30.0 ± 11.5 g ($p < 0.05$) and thereby, individual fluctuations in body weight in the main and control groups were very significant and amounted, 170–300 g and 150–270 g respectively. The body weight in the main group had slight increase trend and amounted to 213.0 ± 8.4 g. However, as at the previous reference research point, the body weight of the animals in the main group was bigger than that in the control group of animals 198.5 ± 9.47 g. At the same time, if in comparison to the results obtained during 3 weeks of research, the weight of animals in the main group during 6 weeks had only a tendency to increase, animals' weight in the control group during 6 weeks of research was significantly higher than at the previous reference point ($p < 0.05$).

After 9 weeks of animal monitoring and 6 weeks after termination of the DFA consumption, in comparison to previous research, the body weight gain in the main group decreased to 6.3 ± 2.5 g (2.81%), and the body weight gain of the control group of animals was 36.7 ± 12.3 g (5.03%), and conversely had significant increase trend. At the same time, on the 9th week of the research, the body weight gain in the control group significantly and probably outpaced such in the main group at $p < 0.05$. Individual fluctuations in body weight in the control group of rats, as during the previous research stage were 170–300 g, and were higher in animals of the control group (175–280 g). Animal body weight in the main group was 219.3 ± 89.4 g after 6 weeks upon the termination of the DFA course and hardly changed compared to previous figures.

Body weight in the control group significantly increased in comparison to previous research stage, and amounted to 208.5 ± 10.1 g. Possible differences between obtained and previous examinations' data for animals from the main and control groups for 9 and 6 weeks' observation period, as well as for 6 weeks after the termination of the DFA consumption have not been detected ($p > 0.05$).

After 16 weeks (4 months) from the beginning of experiment and 13 weeks after the DFA termination to animals of the main group, the body weight increase in it was almost at the level of the previous reference research point and amounted 6.8 ± 2.9 g (4.5%). Body weight gain in the control group at 18.69 ± 2.77 g (10%) was almost twice lower than previously, but significantly higher than in the main group during the last survey. Fluctuations in the body weight c in the main group of animals amounted to 200–310 g, and was 190–290 g for the control group. The body weight in the main and control groups, 13 weeks after the termination of the DFA for the animals in the main group and 16 weeks of research was 230.0 ± 9.1 g and 229.3 ± 8.4 g, respectively, almost had no difference between each other and reached the age norm for mature animals of this species.

Thus, the performed research shows that animals in the main group, which consumed DFA BAA 'Aquacarotin' with the diet only during the course of the additive consumption for up to 3 weeks, had probable and significant increase in body weight, both in relation to the initial data and control indices for the observation period, then, 3 weeks after its introduction, it slowed down, and was further regulated.

Data on changes of blood leukogram indices in the main group of animals under the influence of DFA BAA 'Aquacarotin' compared to the control animals that did not receive this food additive are given in Table 1.

Table 1 — Comparison of peripheral blood leukogram indices in the control group which were kept on the regular diet and the main group which received DFA BAA 'Aquacarotin'

Indices, g/l	Terms of study						Normal indices, g/l
	Before the DFA consumption		After 3 weeks upon adding the DFA to diet		After 13 weeks upon adding the DFA to diet		
	Groups of animals						
	Control (n = 30)	Main (n = 30)	Control (n = 30)	Main (n = 30)	Control (n = 30)	Main (n = 30)	
Leukocytes	9.87 ± 0.22	9.75 ± 0.90	11.24 ± 1.30	14.76 ± 1.90*			11.67 ± 0.37
Metaemyelocytes	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.017 ± 0.005
Stab neutrophils	0.06 ± 0.02	0.04 ± 0.01	0.11 ± 0.01	0.22 ± 0.15	0.04 ± 0.02	0.09 ± 0.05	0.13 ± 0.01
Segmented neutrophils	1.23 ± 0.57	1.10 ± 0.8	2.40 ± 0.69	3.32 ± 0.90	1.96 ± 0.4	1.99 ± 0.90	2.79 ± 0.16
Eosinophils	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	1.02 ± 0.28*	0.12 ± 0.05	0.18 ± 0.08	0.25 ± 0.02
Monocytes	0.08 ± 0.02	0.06 ± 0.01	0.09 ± 0.03	0.48 ± 0.15*	0.12 ± 0.06	0.21 ± 0.09	0.14 ± 0.02
Monocytes	8.47 ± 0.28	8.53 ± 0.15	8.60 ± 2.75	9.42 ± 1.27	8.50 ± 1.27	9.78 ± 0.92	8.10 ± 0.25
Natural killers	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.15	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.01

Note: * — $p < 0.05$ compared to the outgoing data.

As it can be seen from data in Table 1, the baseline indices for the content of leukocytes and peripheral blood elements leukogram did not differ among the animals in the main and control groups. After 3 weeks upon the consumption of DFA BAA 'Aquacarotin' in the food diet, the contents of leukocytes in the animals of the main group increased significantly, compared to the baseline ($p < 0.05$). Animals in the control group demonstrate only a tendency to increase the content of leukocytes. 25% of the animals out of the main group registered young forms of neutrophils in the peripheral blood.

The average number of young neutrophil cells does not exceed the upper limit standards. At the same time of studies on the animals in the main group, there was an increase trend in the number of stab neutrophils, and their content significantly increased in 50% of animals.

The average content of neutrophils reached the upper limit of the species norm, but increasing of stab neutrophil pool was not probable on benchmarks and indices for animals of the control group for a period of study ($p > 0.05$).

The pool of segmented neutrophils increased significantly, but not likely in relation to raw data and control group data.

In comparison to raw data of the main and control groups of animals, eosinophil and monocyte pool increased significantly and likely during examination period ($p > 0.05$). Increase trend for lymphocytic pool of the peripheral blood was also detected in the main group of animals.

The contents of leukocytes and peripheral blood leukogram are complex indices of the body status, and represent hematopoiesis. Therefore, this data demonstrates a positive impact of DFA BAA 'Aquacarotin' on a circulatory system and a whole organism. Herewith, it is important to underline the exact influence of the DFA on the activation of myelocytic and monocytic hematopoiesis, enhancement of non-specific resistance, as evidenced by increasing of monocytic pool, namely macrophages and monocytes, as well as natural killers.

13 weeks after the termination of the DFA addition to the diet, the content of peripheral blood leukocytes in animals of the main group had a downward trend, but remained higher than raw data and control group indices in terms of research. The content of stab neutrophils, segmented neutrophils, eosinophils, monocytes and lymphocytes in the main group of animals was higher than before adding the DFA to the ration and indices for a period of study, but decreased in comparison with these data, which were determined after completion of the DFA course. Trends related to change the leukogram, remained the same as to check them immediately after termination of the DFA. That is, for 13 weeks after stopping the use of DFA BAA 'Aquacarotin', its stimulating effect on peripheral blood remained, but decreased.

The obtained data suggest positive effect of DFA BAA 'Aquacarotin' to these physiological parameters in animals, like body weight gain and body weight, as well as the condition of blood formation, represented with peripheral blood. The biological activity of DFA BAA 'Aquacarotin' on body weight will give significant and express increase and relation to the volume of body weight of animals in the process of growth. Active gain of body weight stopped upon termination of DFA BAA

'Aquacarotin'. It is valuable that after the termination of additive's use, the animals' body weight gain slowed down and got normal, i.e. it did not continue after termination of the DFA, which indicates the control action on the DFA and safety of its consumption. DFA BAA 'Aquacarotin' more significantly influences myelocytic and monocytic hematopoiesis, and represents significant tendency to increase neutrophil pool, the pool of eosinophilic granulocytes, as well as monocytic pool of peripheral blood against the backdrop tendency to increase the pool of lymphocytes. Enhancing functional capacity of neutrophils influenced by DFA BAA 'Aquacarotin', namely the activation of biochemically caused bactericidal activity of neutrophils we detected earlier work (Raksha-Slusareva et al., 2017).

Conclusions. 1. DFA BAA 'Aquacarotin' is controllable, and therefore safe to influence the physiological parameters and weight, as well as body weight gain during growth, as well as peripheral blood indices.

2. The consumption of DFA BAA 'Aquacarotin' is useful to improve physiological characteristics of organism (rapid body weight gain), especially during growth.

3. DFA BAA 'Aquacarotin' activates myelocyte and monocyte hematopoiesis by increasing of macro- and macrophagic poos, as well as enhances their functional activity.

4. The data can be found interesting for being implemented in medicine, agriculture, military affairs, technology and food industry.

Prospects for further research. It will be used for deeper research of the dietary food additive effects with biologically active influence on cytomorphological changes in non-specific resistance and immunity system elements.

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News

Dear colleagues!

National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' of the NAAS of Ukraine under supporting of **the State Service of Ukraine for Food Safety and Consumer Protection,** and **the Gromashevsky Institute of Epidemiology and Infectious Diseases of the NAMS of Ukraine**

17–19 September 2018 in **Kharkiv, Ukraine** holds

the International Scientific-and-Practical Symposium 'BIOLOGICAL SAFETY AND CONTROL OF TRANSBOUNDARY EMERGENT ANIMAL DISEASE (AFRICAN SWINE FEVER, FOOT AND MOUTH DISEASE, BRUCELLOSIS, LUMPY SKIN DISEASE AND HIGHLY PATHOGENIC AVIAN INFLUENZA)'

which will be **dedicated** to the **95th anniversary of foundation of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine'** and to the **90th anniversary of the birth of Krasnikov Gennadiy Andreevich the prominent Ukrainian veterinary immunologist and morphologist, academician of the National Academy of Agrarian Sciences of Ukraine**

The main directions of the Symposium:

(1) Contribution of the scientific schools of NSC 'IECVM' to the system of veterinary support of livestock in Ukraine;

(2) Problems of biosafety and biosecurity. Ways of improving national strategies for counteracting transboundary biological threats, taking into account international experience.

(3) Epizootology, analysis of biological risks and aspects of control of African swine fever, Lumpy skin disease, Foot and mouth disease, Brucellosis, Highly pathogenic avian influenza and other transboundary emergent infections.

(4) Consolidation of DVMs' and MDs' activities and experience within One Health agenda;

(5) Scientific support of developments and implementation of tools and systems for monitoring, diagnosis and prophylaxis of African swine fever, Lumpy skin disease and Highly pathogenic avian influenza.

The Symposium is designed for a wide audience of experts from the State Service of Ukraine on Food Safety and Consumer Protection, laboratory workers and practitioners of veterinary medicine of livestock farms. The program of the Symposium includes lectures, presented by researchers from the USA, Canada, Great Britain, Sweden, Poland, Turkey, and other countries, concerning the epizootic analysis and the development of innovative means of controlling African swine fever and Lumpy skin disease. Moreover, information regarding to the main scientific achievements of the NSC 'IECVM' for veterinary support and solving current problems of livestock in Ukraine will be also offered to the practitioners of veterinary medicine.

Materials of research papers on the conference theme (requirements for registration of scientific articles are included) will be published in the Interdepartmental scientific thematic collection 'Veterinary Medicine', which is included in the list of specialized publications of Certified Staff Evaluation Department of The Ministry of Education and Science of Ukraine, and is a part of the international scientific-and-metric database of RSCI — eLibrary and Google Scholar. Publication cost is **50 UAH/page.**

Materials for publication should be e-mailed to inform@vet.kharkov.ua or nsc.iecvm.conference@gmail.com or send to the NSC 'IECVM', Pushkinska str., 83, Kharkiv, Ukraine, 61023 no later than **June 01, 2018**.

Sincerely,

Symposium Organizing Committee.

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