Dear colleagues!

The modern trends of biological threats growing, emergent diseases (Lumpy skin disease, Foot-and-mouth disease, African swine fever, Avian influenza and other in Europe and Asia) determine the necessarily to pay the extremely high attention to the biosafety issues and biological hazards control.

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.

New journal 'Journal for Veterinary Medicine, Biotechnology and Biosafety", discovered in 2015, aimed to consolidate and share the new developments and achievements in the area of biological science. This was recognized as the profile edition for veterinary medicine doctors and biologists in Ukraine. Our journal promotes the research of Ukrainian institutions, publishing their achievements in English, and sharing it among the scientific community. It includes cooperative veterinary and medical aspects, fitting to One Health Approach declared by WHO, OIE, and FAO. It was included in Index Copernicus and eLibrary scientific databases.

The Editorial board hopes, that our issue will be interesting for wide auditorium of scientists and practical specialists in veterinary medicine, biology, biotechnology and biosafety. We invite new authors for fruitful collaboration and joint development.

Sincerely yours,
Editors-in-Chief

Prof. Borys STEGNIY

Prof. Anton GERILOVYCH

GUIDELINES FOR THE PREPARATION OF THE PAPERS SUBMITTED FOR PUBLICATION AT THE 'JOURNAL FOR VETERINARY MEDICINE, BIOTECHNOLOGY AND BIOSAFETY'

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INFLUENCE OF DIFFERENT DOSES OF FEED ADDITIVE BASED ON SILKWORM PUPAE ON CLINICAL INDICATORS OF RAT BLOOD

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Summary. The paper presents data on the effect of different doses of feed additive based on silkworm pupae. A positive correlation between the number of leukocytes and erythrocytes, as well as an increase in the number of agranulocytes compared with granulocytes in the analysis of leukocyte formula, indicate the activation of hematopoiesis, which in turn affects the natural resistance of animals. Under the conditions of the vivarium of the NSC 'IECVM' the experimental part of the work on studying the effect of different doses of feed additive based on silkworm pupae was carried out on male Wistar rats (n = 49) aged four months, weighing 175–190 g. Three groups of rats were formed following the principle of analogs. Rats of the control group received a standard diet with free access to water, rats of group I were fed a standard diet with the addition of the feed additive at a dose of 8.0 ml/kg body weight, group II — 12 ml/kg body weight. On days 21 and 41 of the experiment, blood samples were taken from rats during light chloroform anesthesia. The level of hemoglobin in the blood was determined using reagent kits manufactured by PJSC 'Reagent' (Ukraine), the total number of leukocytes and erythrocytes was performed according to the generally accepted methods of counting in Goryaev's chamber. The calculation of the leukocyte formula of the blood of rats was performed on blood smears. Smears were stained by Romanowsky–Giemsa method. Based on the obtained data, integrated hematological parameters and color index were calculated following the methodological recommendations. There was a positive correlation between the number of leukocytes by 13.4% ($p \le 0.05$) and erythrocytes by 39.2% ($p \le 0.05$), as well as an increase in the number of agranulocytes to 50.0% ($p \le 0.05$) than granulocytes in the analysis of leukocyte formula — this indicates the activation of hematopoiesis, which in turn affects the natural resistance of animals. In addition, the index of immunoreactivity according to Ivanov et al. (2002) in the group II of animals had an increase of 61.4% ($p \le 0.05$) relative to control. The obtained results allow us to expand the current knowledge about the effect of the drug based on silkworm pupae on the body of animals, in particular on the clinical parameters of the blood. A positive correlation between the number of leukocytes and erythrocytes, as well as an increase in the number of agranulocytes than granulocytes in the analysis of the leukocyte formula, indicates the activation of hematopoiesis, which in turn affects the natural resistance of animals

Keywords: feed additive, clinical indicators, blood, rats

Introduction. Modern medicine uses a wide range of organic drugs for the treatment and prevention of diseases such as pathologies caused by abnormal hemodynamics, they are also used as metabolic activators, antihypoxants, antioxidants, and neuroprotectors. However, today the demand for organic drugs is constantly growing despite the development of traditional medicine. It determines the need to develop and create new drugs. This is evidenced by the rapidity of their implementation (Ohar and Chernykh, 2013; Anon., 2010; Rolik, 2004; Sergienko et al., 2009b).

In this regard, the safety of such drugs deserves special attention. It is known that a quarter of all drugs of biological origin, approved in the United States and Europe, cause side effects, including immune system disorders, post-injection reactions, cancer, etc. (Theurer, 2007).

Our work aimed to study the effect of different doses of feed additive based on silkworm pupae on the clinical parameters of the blood of rats.

Materials and methods. Experimental studies in rats were approved by the Bioethics Commission of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (NSC 'IECVM') and conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 86/609/EEC (CEC, 1986), standards of maintenance, care, and feeding.

Under the conditions of the vivarium of the NSC 'IECVM' the experimental part of the work on studying the effect of different doses of feed additive based on silkworm pupae was performed on male Wistar rats (n = 49) aged four months, weighing 175–190 g. By the principle of analogs, three groups of rats were formed.

After keeping experimental animals of all groups on a standard diet for 15 days (equalization period), rats of the control group received a standard diet with free access to water, rats of group I were fed a standard diet with the addition of the feed additive with silkworm pupae at a dose of 8.0 ml/kg body weight, animals of group II — 12 ml/kg body weight.

Every 7 days experimental and control groups of animals were weighed on an empty stomach. On days 21 and 41 of the experiment, four rats were removed from each group, respectively, to take blood samples from the carotid artery under mild chloroform anesthesia.

The level of hemoglobin in the blood of rats was determined using reagent kits manufactured by PJSC 'Reagent' (Ukraine), the total number of leukocytes and erythrocytes was performed according to the generally accepted methods of counting in Goryaev's chamber (Prystupa, 2019). The calculation of the leukocyte formula of the blood of rats was performed on blood smears. Smears were stained by the method of Romanowsky–Giemsa (Prystupa, 2019; Levchenko, 2010). Based on the obtained data, integrated hematological parameters and color index were calculated, according to the methodical recommendations (Horalskyi, Radzykhovskyi and Dyshkant, 2018).

Digital data were processed biometrically using conventional statistical methods using Student's *t*-test and computer programs Statistics 6.0 (StatSoft Inc., USA) and Microsoft Excel 2007.

We used an experimental sample of additive from silkworm pupae which was made by the staff of the Laboratory of Clinical Biochemistry of the NSC 'IECVM' by extraction and filtration and has the following chemical composition: protein — 5.55 g/l, vitamin A (retinol acetate) — 43.2 mg/l, vitamin E (α-tocopherol acetate) — 200 mg/l; microelement composition of the drug: zinc — 1.318 mg/l, copper — 0.781 mg/l, iron — 1.018 mg/l, manganese — 0.382 mg/l, nickel — 0.011 mg/l, strontium — 0.119 mg/l, bromine — 0.019 mg/l.

Results and discussions. Analyzing the clinical data of rat blood (Table 1) on the 21st day of the study, the following changes were noted: decrease in the number of erythrocytes and leukocytes in group I (8 ml/kg) by 17.6% and 13.5% in group II (12 ml/kg) — by 35.7% ($p \le 0.05$) and 9.0%, as well as a decrease in hemoglobin concentration by 19.5% ($p \le 0.05$) and 27.6% ($p \le 0.05$) respectively relative to the control level.

Table 1 — Clinical parameters of rat blood under different doses of the additive with silkworm pupae (M ± m, n = 3–4)

Indicator	Day of experiment	Experimental groups		Control group
		I	II	III
Erythrocytes, × 10^{12}/l	21	7.31 ± 0.89	5.70 ± 0.17*	8.87 ± 0.05
	41	8.21 ± 0.51	8.31 ± 0.51*	7.33 ± 0.98
Hemoglobin, g/l	21	82.12 ± 4.51*	73.80 ± 3.81*	101.98 ± 4.51
	41	108.30 ± 2.41*	105.89 ± 4.81*	98.67 ± 3.61
Leukocytes, × 10^{9}/l	21	10.72 ± 0.96	11.28 ± 0.80	12.40 ± 0.40
	41	16.64 ± 0.53*	13.55 ± 0.53*	11.95 ± 0.75
Color indicator, conventional units	21	0.94 ± 0.11	1.04 ± 0.06	0.92 ± 0.04
	41	1.05 ± 0.09	1.02 ± 0.03	1.11 ± 0.16

Note. * — $p \le 0.05$ relative to control.

It should be noted that on the 41st day of the study in the blood of rats of these experimental groups there was an increase in the number of erythrocytes and leukocytes in group I (8 ml/kg) by 12.0% and 13.4% ($p \le 0.05$) and in group II — by 39.2% ($p \le 0.05$) and 13.4% ($p \le 0.05$), which led to an increase in the concentration of hemoglobin by 9.7% and 7.3% in the blood of rats of groups I and II respectively. At the same time, the calculated hemoglobin saturation index of erythrocytes — a color indicator on the 21st day of the study increases in the blood of group II rats by 13.0%.

It is known that the leukocyte formula is an integral indicator of the balance of all homeostatic systems of the body. Due to the effect of the additive based on silkworm pupae on the body of rats of group I on the 21st day, there was a decrease in the number of rod-shaped neutrophils to 16.6% and eosinophils to 35.7%, an increase in segment nuclear neutrophils by 8.4%. In the blood of group II rats during this period the number of rod-shaped neutrophils increased by 50.0% and lymphocytes by 10.3% ($p \le 0.05$), while the number of segment nuclear neutrophils and eosinophils reduced by 33.7% and 35, 7%, respectively, relative to control (Table 2).

On the 41st day of the study, the leukocyte formula of rat blood changed by groups: in group I, we observed an increase in rod-shaped neutrophils by 11.1% and monocytes by 72.7%, a decrease in segment nuclear neutrophils by 24.0% ($p \le 0.05$) and eosinophils by 30.2% ($p \le 0.05$) relative to control. In the blood of rats in group II — a decrease in rod-shaped neutrophils by 14.8%, segment nuclear neutrophils by 35.0% ($p \le 0.05$), eosinophils by 53.5% ($p \le 0.05$), and monocytes by 30.3%, instead, lymphocytes increased by 16.7% ($p \le 0.05$).

The dynamics of the leukocyte composition of the blood reflects the activity of the cellular immune response with the participation of innate resistance factors and specific immunity both in inflammatory processes and in the action of drugs on the body (Sydorchuk et al., 2015). The most important information is the indicators of integrated leukocyte indices, which are presented in Table 3.

Table 2 — Leukocyte formula of rat blood under the action of different doses of the feed additive with silkworm pupae (M ± m, n = 3–4)

Indicator	Day of experiment	Experimental groups		Control group
		I	II	III
Rod-shaped neutrophils, %	21	1.25 ± 0.25	2.25 ± 0.25	1.50 ± 0.25
	41	3.00 ± 0.66	2.30 ± 0.33	2.70 ± 0.33
Segment nuclear neutrophils, %	21	22.50 ± 1.50	13.75 ± 1.00	20.75 ± 0.75
	41	16.70 ± 1.33*	14.30 ± 1.00*	22.00 ± 0.66
Eosinophils, %	21	2.25 ± 0.25	2.25 ± 0.50	3.50 ± 0.50
	41	3.00 ± 0.00*	2.00 ± 0.66*	4.30 ± 0.33
Monocytes, %	21	3.75 ± 0.50	4.00 ± 0.50	3.75 ± 0.50
	41	5.70 ± 0.66	2.30 ± 0.66	3.30 ± 1.00
Lymphocytes, %	21	70.00 ± 0.75	77.75 ± 0.50*	70.50 ± 1.75
	41	71.70 ± 0.33*	79.00 ± 0.66*	67.70 ± 1.33

Note. * — $p \leq 0.05$ relative to control.

Table 3 — Integral indices of rat blood under the action of different doses of the feed additive with silkworm pupae (M ± m, n = 3–4)

Indicator	Day of experiment	Experimental groups		Control group
		I	II	III
Lymphocyte to monocyte ratio	21	18.66 ± 0.16	19.44 ± 0.15	18.80 ± 0.10
	41	12.58 ± 0.12*	34.35 ± 0.17*	20.51 ± 0.14
Immunoreactivity index by Ivanov et al. (2002)	21	19.27 ± 0.08	20.00 ± 0.10	19.73 ± 0.06
	41	13.10 ± 0.24*	35.22 ± 0.20*	21.82 ± 0.12
Allergization index	21	3.73 ± 0.10	5.51 ± 0.12*	4.44 ± 0.11
	41	4.39 ± 0.09	5.77 ± 0.11*	4.31 ± 0.10

Note. * — $p \leq 0.05$ relative to control.

The ratio of lymphocytes and monocytes reflects the relationship of affector and effector parts of the immunological process (Glushko and Fedorov, 2014). This indicator on the 41st day of the study was reduced by 38.6% ($p \leq 0.05$) in rats of group I, while in group II was reliably higher than control by 67.5%.

Additionally, we determined the index of immunoreactivity by Ivanov et al. (2002), which on the 41st day of the study in rats of the group II was reduced by 40.0% ($p \leq 0.05$), and in group II of animals, in contrast had an increase of 61.4% ($p \leq 0.05$) regarding control. According to the results of our studies on days 21 and 41 of the experiment, the index of allergization increased only in the body of group II rats (additive at a dose of 12 ml/kg body weight) by 24.1% ($p \leq 0.05$) and 33.87% ($p \leq 0.05$), respectively.

The effect of the additive on the body of rats at a dose of 12 ml/kg body weight had an advantage over the effect of this additive at a dose of 8 ml/kg body weight. It consisted of an adaptogenic effect, namely: immunomodulatory effect, manifested by an increase in the ratio of lymphocytes and monocytes, because of the increase in the number of blood lymphocytes, and a high level of allergization index by reducing the number of neutrophils. Note that according to the literature tissue drugs began to disappear from the arsenal of drugs, and recently they are rarely mentioned. The market of drugs for veterinary medicine is represented mainly by foreign synthetic drugs and antibiotics. The additive developed by us on the basis of silkworm pupae and the obtained data on its effect on the body of laboratory animals were consistent with the results of research by many scientists.

Thus, Sergienko et al. (2009a) developed a technology for obtaining a finely dispersed powder based on silkworm caterpillars, the chemical composition of which (per 100 g of powder) contains 53.5 g of protein, 6 g of lipids, vitamins B and A, C, E; enriched with minerals (sodium, potassium, calcium, magnesium, copper, iron, zinc), and contains chitin-melanin complex.

They studied the general condition of the body of rats, the functional state of the basic physiological systems, biochemical and morphological parameters of blood and internal organs. Prolonged administration (28 days) of a dry mixture of caterpillars *per os* provided an increase in hemoglobin, erythrocytes, reticulocytes, hematocrit, eosinophils, osmotic resistance of erythrocytes, and a decrease in the total number of leukocytes.

There was a decrease in glucose levels, a slight increase in total cholesterol and antiatherogenic lipoproteins, decreased urea. The average water consumption of the experimental group of rats was

3.87 times higher than that of animals on the standard diet, feed use decreased by 22.1%.

The body weight of rats receiving a mixture of caterpillars was 34.8% higher than in the comparison group, which received tap water. The indicators of organ indices adequately changed. They noted the stimulation of physical and mental activity of experimental rats in the first week with decrease by the end of the fourth week to the initial level. An experimentally proven pharmacological substantiation of the use of silkworm larvae in a short course as an anabolic and actoprotective agent, without general toxic action, which will allow the use of a mixture of caterpillars for therapeutic and sports nutrition.

It is known from foreign scientific sources (Baimishev et al., 2018; Wang, Wang and Zhang, 2014; Wattanathorn et al., 2012), that drugs based on silkworm pupae are used in Alzheimer's disease to improve the condition of patients with memory impairment and neurodegenerative processes. In experiments on rats, it was proved that the protein hydrolysates from silkworm pupae with a molecular weight of the peptide less than 5,000 Da reduces systolic blood pressure in 1.5 h to 21.5 mm Hg. In physiologically healthy rats, the hydrolyzate did not cause pressure changes. In the study of acute toxicity, it was found that the hydrolyzate is harmless and does not have side effects.

Ukrainian scientists obtained and patented the hydrophilic extract from the pupae of Chinese oak tussar silkworm (*Antheraea pernyi* G.-M.) and studied its effectiveness (Trokoz et al., 1997, 2018). However, studies on the dynamics of hematological and biochemical parameters of the blood of experimental animals had unidirectional character — increase within the physiological norm. Thus it was necessary to study the blood parameters of experimental animals under the influence of different doses of extract from silkworm pupae on laboratory animals.

The dynamics of clinical, hematological, biochemical and productive parameters in pigs under the influence of hydrophilic extract from Chinese oak tussar silkworm pupae was studied.

Animals in the control group were given one subcutaneous injection of saline at a dose of 0.1 ml/kg, and the experimental group — according to the same scheme was injected with native hydrophilic extract from the pupae of Chinese oak tussar silkworm. In all pigs, body temperature, heart rate and respiration were recorded 1, 2, 5 h and 1 and 10 days after injection.

Before injection of the extract, 1 and 10 days after, the number of erythrocytes and leukocytes, the content of hemoglobin, total protein, and its fractions in serum were determined in the blood. The color index of blood, the average content of hemoglobin in one erythrocyte, and protein ratio were calculated.

It has been established that the effect of hydrophilic extract from Chinese oak tussar silkworm pupae on the body of productive animals is to stimulate hematopoiesis (increase in hemoglobin content in the blood), protein synthesis (increase in total serum protein, mainly due to increased content of γ-globulins), which contributes increase weight gain. The introduction of a complex of biologically active substances of the extract does not have a reaction from the cardiovascular and respiratory systems of the body, as well as a pyrogenic effect.

Hydrophilic extract of Chinese oak tussar silkworm pupae, when administered subcutaneously to productive animals, stimulates hematopoiesis (increase in hemoglobin in the blood by increasing its concentration in one erythrocyte at normal values of color), and proteinaceous processes (increase by 7.5% of total protein in blood serum mainly by increasing the content of γ-globulins by 29.6% in 10 days after administration of the extract.

As the additive from silkworm pupae belongs to tissue drugs, the received data were consistent with the results of other researchers in this direction. Thus, Baimishev et al. (2018), by analysis of morphological parameters of cow blood depending on the use of drugs STEMB and Uteromastin, has found that the hemoglobin content in the blood of animals of the first experimental group was 112.42 g/l, which is 2.19 g/l higher than in animals of the second experimental group and 3.86 g/l lower than in animals of the third experimental group ($p < 0.01$).

The number of erythrocytes in the blood of animals of the third experimental group, which used drugs Uteromastin and STEMB in combination, was 6.75×10^{12}/l, which is 0.7×10^{12}/l higher than when using the drug Uteromastin alone, and 0.46×10^{12}/l more than when using the drug STEMB. The number of leukocytes in the blood of animals of the second experimental group on the 15th day after calving is less by 0.17×10^{9}/l than in the blood of animals of the first experimental group, and 0.40×10^{9}/l less than in the blood of animals of the third experimental group.

Analysis of the leukocyte formula showed that, depending on the drugs used, there are significant differences between groups of animals between individual forms of leukocytes. According to the content of basophils and eosinophils, no significant differences between groups of animals were found.

The number of segment nuclear neutrophils in the blood of animals of the third experimental group is 1.27% higher than in the blood of animals of the second experimental group and 0.54% higher than in the blood of animals of the first experimental group. The number of lymphocytes in the blood of animals of the third experimental group is less by 2.07% than in the blood of animals of the second experimental group, and 1.22% less than in the blood of animals of the first experimental group. However, the number of monocytes, important for the function of phagocytosis, in animals of the third experimental group was higher than those in the first and second experimental groups by 0.4% and 0.63%.

Bagdanova (2013) studied the effect of the drug 'Biostim', which was administered subcutaneously to cows of the second experimental group at a dose of 20 ml/cow twice with an interval of 7 days on the

background of the accepted method of treatment of infertile animals (vitaminization with 'Tetravit' at a dose of 5 ml/cow twice, active regimen, rectal massage of the uterus and ovaries). Cows of the first experimental group received a tissue drug in the same dose without the use of vitamins. Control animals did not receive 'Biostim'.

At the end of the experiment, under the influence of the drug 'Biostim', the number of leukocytes in the blood of cows of the first experimental group significantly increased by 1.06×10^9/l and 1.17×10^9/l in the second experimental group, where the tissue drug was used on the background of vitamin fortification. The content of erythrocytes in the blood of cows increased by 0.62×10^{12}/l in the first experimental group and 0.71×10^{12}/l in the second experimental group. The hemoglobin level increased by 11.07 and 11.46 g/l in the first and second experimental groups, respectively, compared with the control.

Tiutiun et al. (2018) studied the effect of the STP drug on pigs. The results of hematological studies in piglets treated with the STP tissue drug before vaccination with the inactivated vaccine 'Hemofilosan' against swine hemophilia indicate its positive effect on animals and more intensive stimulation of the cellular immune system, as the absolute number of lymphocytes increased by 13.7% ($p < 0.05$), compared with their content in animals of the control group.

In experiments on cows using the tissue drug 'Fetoplacentant', an increase in the number of erythrocytes in the blood of experimental animals by 5.2%, hemoglobin content by 3.8%, and a decrease in leukocytes by 19.8% was determined (Hryshchuk, 2013).

Thus, based on our research and the research of other scientists, it can be argued that under the influence of modern tissue drugs, including our preparation based on silkworm pupae, there is an increase in some components of the blood. And this fact may indicate that under the drug activates some biochemical processes without general toxic effects occurs.

Conclusions. Thus, our results allow us to expand modern knowledge about the effect of the feed additive based on silkworm pupae on the body of animals, particularly on the clinical parameters of the blood.

A positive correlation between the number of leukocytes and erythrocytes, as well as an increase in the number of agranulocytes than granulocytes in the analysis of the leukocyte formula, indicates the activation of hematopoiesis, which in turn affects the natural resistance of animals.

The next stage of work will be an in-depth study of the effect of feed additive based on silkworm pupae on the factors of nonspecific humoral immunity of birds.

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COMPARATIVE EVALUATION OF THE APPLICATION OF MODERN ECTOPARASITICIDES

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Summary. Despite the success achieved in the control of parasitic animal diseases, there are still some issues that need to be scientifically sound. The issue of prevention and treatment of animals affected by ectoparasites is especially relevant. The study aimed to establish and experimentally confirm the effectiveness of innovative ectoparasiticides for dogs and cats for prevention and therapy in parasitic infections. Innovative antiparasitic drugs with the main active ingredient imidacloprid were used in the experiments: 'MegaStop for dogs' (drops for external use, spot application), 'Golden Defence for dogs spot-on', 'MegaStop for cats' (drops for external use, spot application), 'Golden Defence for cats spot-on'. Following the objectives of the study, we used visual and microscopic methods in accordance with existing practical manuals and current guidelines. According to the results of the research, a stable infection of experimental dogs with fleas was established at the mean intensity of 7.5 ± 2.0 parasite individuals per 10 cm^2 of animal skin, and otodectosis, sarcoptosis, and notoedrosis were diagnosed separately in some animals. Experimental cats were diagnosed with otodectosis, notoedrosis, sarcoptosis, demodicosis by clinical signs. Parasitism of fleas on the animal bodies was detected, and in one animal heartworm disease was diagnosed. The mean intensity of flea infection in cats ranged from 8 to 12 parasite individuals per 10 cm^2 of skin, and the mean intensity of mite infection was 2–3 mites in the field of view of the microscope. High activity of 'MegaStop for dogs', 'Golden Defence for dogs spot-on', 'MegaStop for cats', 'Golden Defence for cats spot-on' as agents with a broad spectrum of action against fleas (*Ctenocephalides* spp.), acariform mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes sapis*), mites of the genus *Demodex*; heartworm (effective against L3 and L4 larvae of *Dirofilaria immitis*) has been established

Keywords: antiparasitic drugs, dogs, cats, fleas, acariform mites, *Demodex* spp., microfilariae

Introduction. Despite the success achieved in the control of animal diseases, there are still many veterinary issues related to the control and prevention of infectious and parasitic pathologies (Pereira et al., 2016; Lappin et al., 2017; Paliy et al., 2020). The fight against ectoparasites of animals remains a particularly important issue today (Xhaxhiu et al., 2009; Colella et al., 2020).

Ectoparasites are not only pathogens of a particular group of diseases, but also carriers of viral, bacterial, protozoan, rickettsial diseases and mycoses of animals and humans (Spinage, 2012; Fong, 2017). The spread of ectoparasitosis among animals is facilitated by the uncontrolled increase in the number of stray animals on the streets (Becker et al., 2012; Szwabe and Blaszkowska, 2017; Otranto et al., 2017).

The most common ectoparasites of domestic animals are fleas (Siphonapteridae: *Ctenocephalus canis*) (Iannino et al., 2017). They can be mechanical and biological vectors of pathogens, as well as intermediate hosts of cestode *Dipylidium caninum* and filariasis of dogs *Dipetalonema reconditum* (Abdullah et al., 2019). Flea bites are painful, cause itching, inflammation of the skin, which causes weight loss in animals (Coşkun and Çetin, 2018). In young animals, there is progressive depletion, anemia. Puppies can die in the event of a high intensity of infection. Animals affected by fleas gnaw fur and injure the skin. The skin is covered with ulcers, hair falls out, the skin spreads an unpleasant odor (Bond et al., 2007; Farkas et al., 2009; Rust, 2017).

Scabies mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes sapis*, *Demodex* spp.) cause skin inflammation, sometimes severe itching, partial alopecia, irritability in animals, and decreased immunity (Moog et al., 2021). Animals affected by parasites grow poorly, do not gain weight with a good diet, have disorders of the digestive tract and respiratory system (Khalil et al., 2017; Arlian and Morgan, 2017).

Along with ectoparasites, pets can be affected by pathogens of endoparasitic diseases, which requires appropriate attention in the prevention and control of them (Jones and Garcia, 2019; Paliy et al., 2021a).

Successful control of invasive animal diseases is possible only in the presence of highly effective veterinary drugs (Pink et al., 2005; Paliy et al., 2021d). The market in Ukraine is provided with expensive imported veterinary drugs. Recommendations for their use are often developed without taking into account the current epizootic situation, animal resistance and environmental impact of local factors.

Providing animal owners with the necessary range of effective and inexpensive means of combating ectoparasite diseases, in convenient forms — the path to well-being for these diseases (Woods and Williams, 2007; Mukherjee et al., 2016).

The therapeutic efficacy of chemotherapeutic agents for the treatment of invasions depends primarily on the chemical activity of the active substance. However, it has been found that the pharmaceutical form and technology

of manufacture of drugs, their physical condition, properties of constituent components, application methods are also important (Mäser et al., 2012; Paliy et al., 2021c).

Antiparasitic drugs belong to different classes of compounds. They are usually effective against a narrow range of pathogens, which encourages pet owners to use dozens of drugs for the treatment and prevention which are not perfect in action on the animal and are dangerous in environmental terms. Therefore, specialists have always been interested in the possibility of creating and using drugs with a broad spectrum of action (Martin and Robertson, 2010; Paliy et al., 2021b).

In recent years, manufacturers have proposed several tools that are used for therapeutic and prophylactic purposes in animal ectoparasitosis at all stages of parasite development and prevention and treatment of helminthiasis (Rajput et al., 2006).

The effectiveness of the drug can be estimated by the spectrum of its action. In addition, the effectiveness of the drug is characterized by improving the clinical condition of sick animals, the speed of their recovery, tolerance to the drug, the manifestation of adverse reactions (Geary, Conder and Bishop, 2004; Molento, 2009).

Thus, timely diagnosis, treatment and prevention of ectoparasitosis among homeless and domestic animals, especially in cities, are of great sanitary and epidemiological importance (Wall, 2007).

The aim of the study was to establish and experimentally confirm the effectiveness of innovative ectoparasiticides for dogs and cats for prophylactic and therapeutic purposes in parasitic infections.

Material and methods. Studies to determine the effectiveness of insecticides on dogs and cats were conducted in the Laboratory of Veterinary Sanitation and Parasitology of the NSC 'Institute of Experimental and Clinical Veterinary Medicine' and at the animal shelter (Balakliia, Kharkiv Region).

Innovative antiparasitic drugs were used in the experiments:

— 'MegaStop for dogs' (drops for external use, spot application) (Ukraine). 1 ml of the drug contains active substances: imidacloprid — 100 mg, ivermectin — 25 mg; excipients: N-methylpyrrolidone, dimethyl sulfoxide, PEG-400;

— 'Golden Defence for dogs spot-on' (Ukraine). The drug contains active substances: imidacloprid — 12%, moxidectin — 3%; excipients: benzyl alcohol, H-methyl pyrrolidone, caprylic triglyceride, PEG-400;

— 'MegaStop for cats' (drops for external use, spot application) (Ukraine). 1 ml of the drug contains active substances: imidacloprid — 100 mg, ivermectin — 10 mg; excipients: N-methyl pyrrolidone, dimethyl sulfoxide, PEG-400;

— 'Golden Defence for cats spot-on' (Ukraine). The drug contains active substances: imidacloprid — 11%, moxidectin — 1.2%; Excipients: benzyl alcohol, H-methyl pyrrolidone, caprylic triglyceride, PEG-400.

Research scheme:

— clinical examination of animals in the shelter, establishment of a preliminary diagnosis, sampling of ectoparasites and skin scrapings for laboratory examination, continuous clinical monitoring of the physiological condition of experimental animals;

— microscopic studies of samples to determine of pathogens of parasitic diseases in the biological material, their identification, determination of mean intensity of infection in dogs and cats;

— formation of experimental and control groups of animals;

— application of drugs externally, individually, directly on the skin, keeping animals in the shelter, taking samples of scrapes for laboratory testing 5, 10, 30, and 45 days after the last application of the drug. Determination of the effectiveness of the drug.

— daily clinical examination of the health of the experimental animals throughout the experiment.

Experimental animals: the study involved 18 purebred dogs of different ages with a body weight from 3 to 20 kg, 16 purebred cats of different ages. Animals were kept in typical aviaries at an air temperature of 24.0 ± 1.5°C, relative humidity 40–70%, air movement 0.2–0.5 m/s. Animals were fed according to the ration approved by the shelter.

In accordance with the objectives, the study was conducted by visual and microscopic methods in accordance with practical guidelines (Vasil'kova, 1955; Yuskiv, 1998; Halat et al., 2009).

Intravital diagnosis of ectoparasitosis was performed and the number of ectoparasites was determined. Identification of ectoparasitic pathogens was performed by microscopic method. The mean intensity was determined by counting ectoparasites per 10 cm^2 area of the animal's skin. Intravital diagnosis of helminthiasis was also performed. For this faeces and blood samples were taken. Identification of pathogens was performed by microscopic method (Kuzmin, 1998). The mean intensity was determined by counting the number of helminth eggs in 1 g of feces and the number of microfilariae in the blood smear.

Sick dogs were divided into two groups, which were separately applied the drug 'MegaStop for dogs' (drops for external use, spot application) (n = 10) and the drug 'Golden Defence for dogs spot-on' (n = 8). Clinical examination of animals was performed before, during, and after treatment. The external examination included an assessment of the general appearance, condition of the skin and coat, measurement of body temperature, pulse and respiratory rate, examination of eyes, mouth, and ears, palpation of the skin and peripheral lymph nodes.

From cats diagnosed with ectoparasitic infection we formed experimental groups: Group 1 (n = 4) — fleas, demodicosis; Group 2 (n = 2) — fleas, otodectosis, notoedrosis, sarcoptosis; Group 3 (n = 2) — fleas, heartworm disease (*Dirofilaria immitis* larvae stage L3 and L4); Group 4 (n = 6) — fleas, demodicosis; Group 5 (n = 2) — fleas, otodectosis, sarcoptosis.

In 5th, 10th, 20th, and 30thday after treatment, the results of studies were recorded on the basis of examinations of treated animals, counting of live ectoparasites on them and determined the prevalence and effectiveness of the drug after treatment.

To collect ectoparasites from animal skin, they were fixed in a supine position. Examination of the skin of the animals began with the head. Then we examined the neck, back, sides, abdomen, and limbs. The fur was parted and combed during the inspection. We examined first with the naked eye, and then — with a magnifying glass.

Detected ectoparasites were removed from the skin of animals with tweezers or by hand in a rubber glove. Removed ectoparasites were placed in a glass vessel filled with Barbagalo liquid (3% aqueous formalin solution in saline) or 70% ethanol.

Some ectoparasites were delivered alive to the laboratory in test tubes or containers with moist filter paper inside. The strips of filter paper were moistened with boiled water. Tubes and jars were covered with a layer of cloth and tied. A label was to each test tube and jar.

When sampling for acariform mites, scrapings from animals were taken both from the affected areas and from the inner surface of the ear with a blunt scalpel on the border of healthy and affected areas of skin. The skin was scraped until capillary bleeding was observed (no more than 0.5 cm^3), skin peels were placed in a tightly closed test tube and labeled.

The selected material was examined no later than 72 h after scraping. The material was studied by mortal methods (detection of dead mites) and biotic methods (detection of live mites, larvae, and eggs) (Vodianov, Lutsuk and Tolokonnikov, 2009).

The diagnosis of heartworm disease was established by microscopic examination for the detection of microfilariae in a thick drop of peripheral blood and serum (modified Knott method), by enriched smear method, by cytological examination of punctures obtained from pseudotumor and ulcerative lesions of the skin and soft tissues, as well as ascitic fluid. In addition, a test was performed for antibodies produced in response to the presence of L4, immature and mature helminths.

To examine dust and debris from the enclosures where the treated animals were kept, a sample weighing 30 g was placed in a cylinder or conical flask, filled with water and mixed thoroughly. The particles that surfaced were removed and the water was carefully drained, leaving a sediment. The sediment was mixed with 20 parts of saturated salt solution and precipitated for 20 min.

Later, a sample (three drops) was removed from the surface of the flotation film with a wire loop, placed on a glass slide, covered with a cover glass and examined under a microscope

Prevalence (P) was defined as the ratio of the number of infected animals to the number of examined animals, expressed as a percentage:

$$P = \frac{X}{Y} \times 100,$$

where: X — number of animals with detected ectoparasites, microfilariae;
Y — total number of animals.

The mean intensity (MI) was determined by the number of ectoparasites per 10 cm^2 of animal skin, the number of nymphs and adults in skin scrapings, microfilariae in blood smears.

Effectiveness (E) of the drug was calculated by the number of treated animals in the percentage that were completely free of parasites.

To determine the acaricidal action of 'MegaStop for cats' and 'Golden Defence for cats spot-on' against fleas, two experimental and one control groups of fleas from cats were formed. Experimental groups of fleas were treated separately with the studied agents, the control group was not treated. The experiments were performed in glasses of 250 cm^3. Fleas were immobilized by carbon dioxide. The drug was applied on fleas using a microsyringe. The glasses were covered with gauze and rubber rings. The glasses were left at a temperature of 20–25°C indoors.

Determination of 'knockdown effect' in fleas in each glass was performed after 6, 12, and 24 h.

Experiments on animals were conducted following the recommendations of the 'European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes' (CE, 1986) and Council Directive 86/609/EEC (CEC, 1986), and in accordance with Art. 26 of the Law of Ukraine No. 3447-IV of 21.02.2006 'About protection of animals from cruel treatment' (VRU, 2006) and basic bioethical principles (Simmonds, 2017).

The research program was reviewed and approved by the Bioethics Commission of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' in the current order.

Results and discussions. As a result of a clinical examination of sick dogs, redness, inflammation of the skin, a well-marked itching reflex, papules and scales were found. Visible areas of alopecia. The skin in the affected areas was rough and cracked. The animals were exhausted.

The results of laboratory studies of biological material selected from dogs are given in Table 1.

Visual examination revealed persistent flea infection in all dogs, demodicosis was detected in two dogs along with fleas, otodectosis was detected in two animals, sarcoptosis in two dogs, and notoedrosis in one animal. The mean intensity of flea infection in dogs was 7.5 ± 2.0 individuals per 10 cm^2 of animal skin area.

A clinical examination of 16 cats kept in an animal shelter revealed that fleas parasitized on all animals, and the inner surfaces of the ears were affected. Animals were constantly itching, combing their ears, sitting with their heads down.

Table 1 — Prevalence and mean intensity of parasite infection in dogs (n = 18)

Species of parasites	P, %	MI
Ectoparasites		
Ctenocephalus canis	100.0	7.5 ± 2.0 per 10 cm^2
Sarcoptes canis	11.1	4.5 in sight
Otodectes cynotis	16.7	3.3 in sight
Notoedres cati	9.7	1.2 in sight
Demodex canis	11.1	3.5 in sight
Endoparasites		
Dipylidium caninum	100.0	13.1 ± 1.0
Ancylostoma caninum	50.0	3.5 ± 1.5
Toxocaris leonina	10.0	3.5 ± 2.5
Toxocara canis	100.0	3.5 ± 1.5
Toxocaris leonina	20.0	2.5 ± 1.5
Dirofilaria spp.	11.1	2.5 ± 1.5 in a smear

In 6 cats, purulent exudate is secreted from the ears. According to clinical signs, otodectosis (*O. cynotis*), notoedrosis (*N. cati*), sarcoptosis (*S. canis*), demodicosis, fleas (*C. felis*) were preliminarly diagnosed.

One animal was diagnosed with cough and dyspnea, as well as non-food vomiting. This gave us a reason to establish a preliminary diagnosis of heartworm disease. Cat fleas (*C. felis*) and mites (*O. cynotis*, *N. cati*, *S. canis, Demodex* spp.) were found in cats.

The intensity of flea infection ranged from 8 to 12 fleas per 10 cm^3 of skin area. The prevalence was 100%. The mean intensity of mite infection was 2–3 mites in the field of view of the microscope. One animal was diagnosed with heartworm disease — 1–2 microfilariae in a smear.

The result of determining the rate of the 'knockdown effect' of fleas under the action of the veterinary drug 'MegaStop for cats' and 'Golden Defence for cats spot-on' with topical application is given in Table 2.

Table 2 — Effectiveness of drugs with topical application

Species of parasites	Number of parasites	'Knockdown effect', after		
		6 h	12 h	24 h
'MegaStop for cats'				
C. felis	10	9	1	–
'Golden Defence for cats'				
C. felis	10	8	2	–
Control				
C. felis	10	–	–	–

The 'knockdown effect' in all fleas began 6 h after exposure, and 12 h later there was a complete 'knockdown' of all experimental fleas.

Subsequently experimental groups of animals were applied with veterinary drugs individually, point wise according to the instructions.

Thus, from the 2nd to the 7th day after treatment, dead fleas were found on treated dogs. On day 10, no fleas were found on the animal bodies. On the 30th day after the application of the drugs, fleas were not detected on the animal bodies (Tables 3, 4).

Table 3 — Insecticidal action of 'MegaStop for dogs' and 'Golden Defence spot-on for dogs' against fleas on dogs

Group of animals	Before treatment		After treatment, day							
			5		10		15		30	
	P, %	MI, average	P, %	MI	P, %	MI	P, %	MI	P, %	MI
Experimental group I (n = 10)	100	8.25	20	2.5	0	0	0	0	0	0
Experimental group II (n = 8)	100	7.75	12.5	2.0	0	0	0	0	0	0

Table 4 — Results of the study of skin scrapings from dogs with acarosis after treatment with 'MegaStop for dogs' and 'Golden Defence for dogs spot-on'

Group of animals	Before treatment		After treatment, day							
			5		10		20		30	
	P, %	MI, average	P, %	MI	P, %	MI	P, %	MI	P, %	MI
Experimental group I (n = 10)	100	3.8	20	2.5	10	1.5	10	0.5	0	0
Experimental group II (n = 8)	100	3.75	12.5	2,0	0	0	0	0	0	0

Effectiveness of 'MegaStop for dogs' and 'Golden Defence for dogs spot-on' in industrial trials for flea infection of dogs was 100%.

Effectiveness of 'MegaStop for dogs' and 'Golden Defence for dogs spot-on' in industrial trials in dogs with demodicosis, otodectosis, notoedrosis, and sarcoptosis was 100%.

The results of determining the therapeutic efficacy of the studied drugs in cats are presented in Table 5.

According to the results of studies on the use of 'MegaStop for cats' and 'Golden Defence for cats spot-on' against ectoparasites of animals, it has been found that on the 2nd day after application of the drug on animals dead fleas were detected. Dead ectoparasites on animals were detected up to the 7th day, and on the 10th day, fleas were not observed on the bodies of animals.

No fleas were detected on the animal bodies during observation for 60 days.

When using drugs against otodectosis, notoedrosis, sarcoptosis, demodicosis, one mite was detected in the field of view of the microscopeafter the first treatment, after the second treatment, mites were not detected.

Table 5 — Effectiveness of the drug against pathogens of parasitic diseases

Group of animals	Species of ectoparasites	Before treatment		After treatment
		P, %	MI	E, %
Experimental group 1 (n = 4)	*C. felis*, *Demodex* spp.	100	8–12 2–3	100 (not found)
Experimental group 2 (n = 2)	*C. felis*, *O. cynotis*, *N. cati*, *S. canis*	100	8–12 2–3	100 (not found)
Experimental group 3 (n = 2)	*C. felis*, *D. immitis*	100	8–11 1–2	100 (not found)
Experimental group 4 (n = 6)	*C. felis*, *Demodex* spp.	100	8–11 2–3	100 (not found)
Experimental group 5 (n = 2)	*C. felis*, *O. cynotis*, *S. canis*	100	8–11 2–3	100 (not found)

No complications or changes in animal clinical status were observed during treatment and during clinical observation of experimental and control animals.

Effectiveness of 'MegaStop for cats' and 'Golden Defence for cats spot-on' when used to treat cats with ectoparasites (fleas, scabies mites) is 100%. The results of the study of bedding samples from aviaries where treated animals were kept for the presence of larvae and adults of ectoparasites are presented in Table 6.

Table 6 — The results of analyzes of bedding samples from aviaries for the presence of larvae and adults of ectoparasites

Test method	Number of larvae and adults of ectoparasites in the bedding	
	Before treatment	On the 10th day after treatment
Flotation	14.5 ± 1.5	not found

As can be seen from Table 6, after treatment of animals on the 10th day in the bedding larvae and adults of ectoparasites were not found.

The results of determining the effectiveness of the studied drugs in heartworm disease are presented in Table 7.

As can be seen from Table 7, after treatment of animals for 20 days in the studied smears from sick animals microfilariae were not detected.

No complications or changes in animal clinical status were observed during treatment and during clinical observation of experimental and control animals.

Table 7 — The results of blood tests in dogs after treatment with 'MegaStop for dogs' and 'Golden Defence for dogs spot-on' in heartworm disease

Group of animals	Before treatment		After treatment, day							
	P, %	MI, average	5		10		20		30	
			P, %	MI	P, %	MI	P, %	MI	P, %	MI
Experimental group I	100	2.5±1.5 in a smear	100	2.0	100	1.0	0	0	0	0
Experimental group II	100	2.5±1.5 in a smear	100	2.0	100	1.0	0	0	0	0
Negative control	0	0	0	0	0	0	0	0	0	0

The use of veterinary drugs for the prevention and control of parasitic animal diseases has integrated into practical veterinary medicine. This is due to the widespread of parasitic pathogens in the environment (Paliy et al., 2019; Bogach et al., 2020). According to the epizootic situation and the type of disease, various manufacturers have proposed to use many drugs. However, their effectiveness is not always satisfactory, and new drugs must undergo extensive pre-clinical and clinical trials. Common drugs in the control of animal ectoparasites are the drugs with the active substance imidacloprid (Larsen, Siggurdsson and Mencke, 2005). Thus, after 3 months of imidacloprid use, the number of fleas in domestic animals decreased by 99.5% (Dryden, Denenberg and Bunch, 2000). The use of Seresto® collars significantly reduces the risk of infection of cats with *Bartonella* spp. (Greco et al., 2019) and also has a high (> 98.2%) efficacy against flea in cats during an 8-month study (Dryden et al., 2016). Imidacloprid collars are 95% effective against fleas and 99% effective against their larvae for 8 months. In addition, the stable acaricidal effectiveness of this tool for 8 months is 100% (Stanneck et al., 2012). Therefore, our results are consistent with the results of other researchers on the high anti-parasitic efficacy of drugs with the active substance imidacloprid.

Conclusions. On the basis of the conducted researches, preparations 'MegaStop for dogs' (drops for external use, point drawing), 'Golden Defence for dogs spot-on', 'MegaStop for cats' (drops for external use, point drawing), 'Golden Defence for cats spot-on' have been found to be well tolerated by animals and give no side effects or changes in the clinical status of the animals.

Clinical studies have shown high activity of 'MegaStop for dogs', 'Golden Defence for dogs spot-on', 'MegaStop for cats', 'Golden Defence for cats spot-on' as veterinary drugs with a broad spectrum of action against fleas (*Ctenocephalides* spp.), acariform mites (*Otodectes cynotis*, *Notoedres cati*, *Sarcoptes sapis*), mites of the genus *Demodex*; heartworms (effective against L3 and L4 stage larvae of *Dirofilaria immitis*).

According to the results of research, it has been established that veterinary drugs 'MegaStop for dogs', 'Golden Defence for dogs spot-on', 'MegaStop for cats', 'Golden Defence for cats spot-on' can be used for prevention and treatment of pets affected by fleas, acariform mites, *Demodex* mites, heartworms.

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Part 2. Biotechnology

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DEVELOPMENT OF RECOMBINANT POSITIVE CONTROL FOR DETECTION OF PORCINE CIRCOVIRUS TYPE 3 BY POLYMERASE CHAIN REACTION

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Summary. This work aimed to obtain positive control using recombinant DNA technology for detection by PCR of a new poorly studied pathogen — porcine circovirus type 3. Recombinant positive control was designed using Clone Manager Basic. As a vector in the creation of recombinant control we used plasmid pTZ57R/T, as an insert — a fragment of the gene *rep* PCV-3 with the length of 418 nucleotide pairs, obtained by classical PCR. Transformation of competent cells of *E. coli* strain DH5a was carried out by chemical poration, followed by plating on LB-medium with the addition of ampicillin at a final concentration of 100 μg/ml. The selection of *E. coli* cell colonies was performed by the marker of antibiotic resistance to ampicillin. The presence of a specific insert was checked by PCR with electrophoretic visualization of the results. The developed recombinant positive control can be used for the monitoring of biological samples from pigs for the presence of genetic material PCV-3 using molecular technologies

Keywords: PCV-3, PCR, plasmid pTZ57R/T, gene *rep*, *E. coli* strain DH5a

Introduction. One of the branches of animal husbandry that is actively developing all over the world, including Ukraine, is pig breeding. The level of development of this industry and the quality of the products obtained depend on the welfare of pig breeding concerning infectious animal diseases. One of the most common diseases among pigs today is circovirus infection, which causes significant economic damage to the industry and is characterized by severe immunosuppression and multiorgan pathology. Losses from PCV infection are estimated by the death of piglets at the stages of rearing and fattening; failure to gain weight of piglets; losses due to pathology of reproduction; ineffectiveness of antibiotic therapy in the presence of bacterial coinfections.

The causative agent of PCV disease is mainly porcine circovirus type 2, which belongs to the family Circoviridae, is characterized by pronounced pathogenicity and is the cause of many syndromes and diseases of pigs. In 2016, it was reported about a new type of porcine circovirus — PCV-3 in sows with clinical signs of dermatitis and nephropathy, as well as with reproductive problems (Palinski et al., 2017).

Currently known data indicate the prevalence of PCV-3 among pigs and wild boar populations in many countries in different geographical regions of the world (Bera et al., 2020; Hayashi et al., 2018; Serena et al., 2021; Saraiva et al.., 2019; Saporiti et al., 2020b; Yuzhakov et al., 2018; Franzo et al., 2018; Souza et al., 2021).

PCV-3 is associated with cardiac and multisystem inflammations, dermatitis and nephropathy syndrome, abortion and reproductive disorders in pigs, respiratory diseases; it is able to cause intrauterine infections in the absence of obvious reproductive disorders (Jiang et al., 2019; Ouyang et al., 2019; Phan et al., 2016; Saporiti et al., 2020c).

At present, for the detection of PCV-3 and rapid assessment of the epizootic situation, methods based on amplification technologies, including the use of different formats of polymerase chain reaction (PCR), is mainly used (Ji et al., 2019; Liu et al., 2019; Wang et al., 2019; Kim et al., 2020; Yuan et al., 2020; Zheng et al., 2020).

An important component that ensures the high quality of molecular genetic tests based on PCR and the level of reliability of the results obtained regarding the detection of PCV-3 is a positive control, which often uses material containing the virus. The use of such control is caused by the need to periodically obtain the appropriate material with a limited shelf life.

Therefore, the **aim of this work** was to construct a recombinant positive control containing an insert of gene *rep* PCV-3 with a length of 418 bp.

Materials and methods. Virtual design of recombinant positive control was performed using Clone Manager Basic v. 9 (Sci Ed Software, USA).

Plasmid vector pTZ57R/T, which is part of the commercial kit 'InsTAclone PCR Cloning Kit' (Fermentas, Latvia), was used to create *in vitro* plasmid control.

A fragment of the *rep* PCV-3 gene with a length of 418 bp was used as an insert. It was obtained by classical PCR using the commercial 'Maxima Hot Start Green PCR Master Mix' (Thermo Scientific, Lithuania) and the PCV-3 primer system F (5'-TTGTGGTGCTACGAGTG TCC-3'); PCV-3 R (5'-CGTCTCCGTCAGAATCCGAG-3') (Saporiti et al., 2020a).

Integration of the plasmid molecule into the culture of competent cells of *E. coli* strain DH5a was performed

by chemical poration followed by plating on LB medium (Sigma-Aldrich, USA) with the addition of ampicillin at a final concentration of 100 μg/ml.

A commercial kit 'Plasmid Miniprep Kit' (GeneJET, Lithuania) was used to extract the plasmid DNA.

Measurement of DNA concentration and evaluation of DNA quality was performed using a NanoDrop spectrophotometer (DeNovix, USA).

Electrophoretic analysis of amplification products was performed by horizontal electrophoresis (horizontal electrophoresis chamber Biorad (USA) in 1.5% agarose gel.

For electrophoretic analysis we used agarose produced by Biozym (Germany), ethidium bromide manufactured by Sigma-Aldrich (USA), molecular weight markers manufactured by Invitrogen (USA), Promega (USA) and Fermentas Gene ruler (Latvia) with a discreteness of 100 bp.

Results. In order to obtain a recombinant positive control sample for the detection of PCV-3 genetic material by PCR, we constructed a virtual model of a vector molecule based on plasmid pTZ57R/T with an embedded fragment of the *rep* PCV-3 gene with a length of 418 bp The total length of the theoretically modeled plasmid molecule was 3,304 bp (Fig. 1).

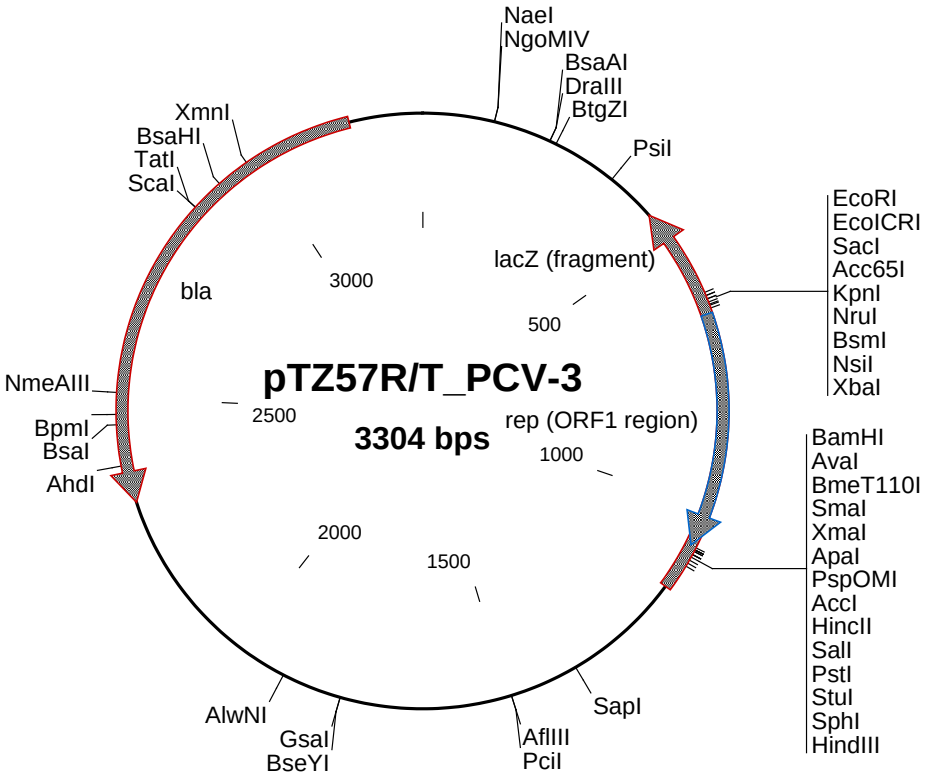

Figure 1. Scheme of recombinant plasmid pTZ57R/T_PCV-3

To create a plasmid control *in vitro* in the first stage of our work, a fragment of the *rep* PCV-3 gene with a length of 418 bp was developed. To do this, we used a DNA sample obtained from pig liver homogenate, which we had previously described as positive for the presence of PCV-3 genetic material. The analysis by horizontal gel electrophoresis confirmed the presence of an amplicon of the required length — 418 bp.

The resulting amplicon was purified and ligated to the plasmid vector pTZ57R/T, which transformed competent *E. coli* DN5a cells.

Because this vector contained an ampicillin resistance gene, it was a marker of selective traits during subsequent cloning of strain DN5a in *E. coli* culture.

Therefore, after the transformation, six white single colonies of *E. coli* with signs of acquired resistance to ampicillin were selected (Fig. 2).

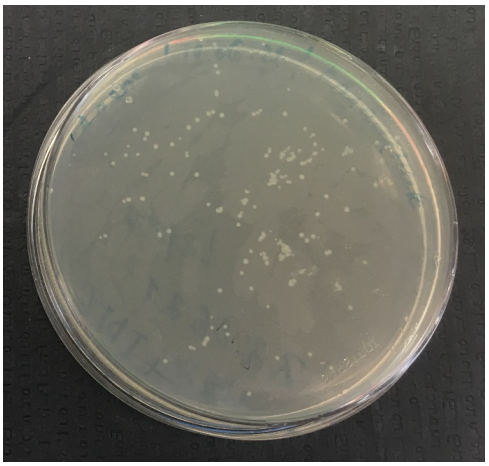

Figure 2. Selection of colonies of *E. coli* cells by the marker of antibiotic resistance to ampicillin

PCR screening of selected colonies showed the presence of a specific insert with the length of 418 bp in each of them (Fig. 3).

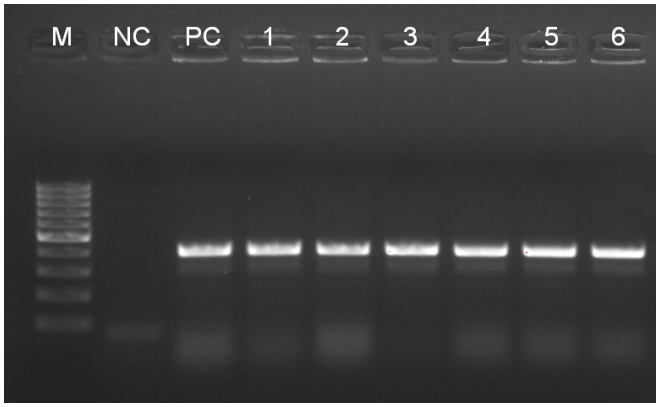

Figure 3. Visualization of the result of PCR screening of selected colonies by electrophoretic analysis in 1.5% agarose gel (M — molecular weight marker, NC — negative control, PC — positive control, 1–6 — samples positive for the presence of PCV-3 DNA)

Three colonies of *E. coli* were selected for cultivation in liquid nutrient medium, after which the resulting bacterial biomass was used for extraction of plasmid DNA. The presence of fragments about 3 thousand bp in length when performing electrophoretic analysis in 1.5% agarose gel of the obtained samples testified to the successful transformation of *E. coli* cells by the recombinant plasmid constructed by us (Fig. 4).

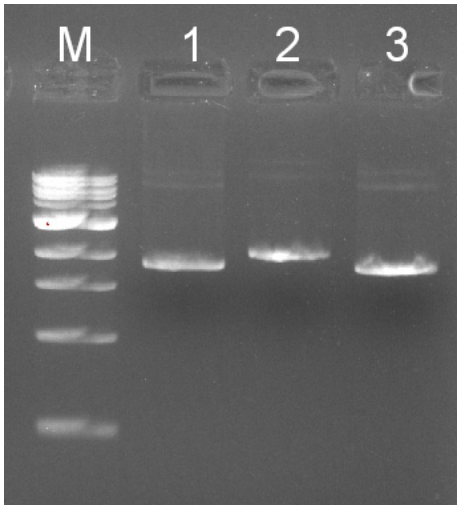

Figure 4. Visualization of the result of electrophoretic analysis of purified plasmid DNA (M — molecular weight marker; 1–3 — positive for the presence of plasmid DNA samples)

The concentration of DNA in the first sample was 106.15 ng/μl, in the second — 132.39 ng/μl, in the third — 83.08 ng/μl (Fig. 5).

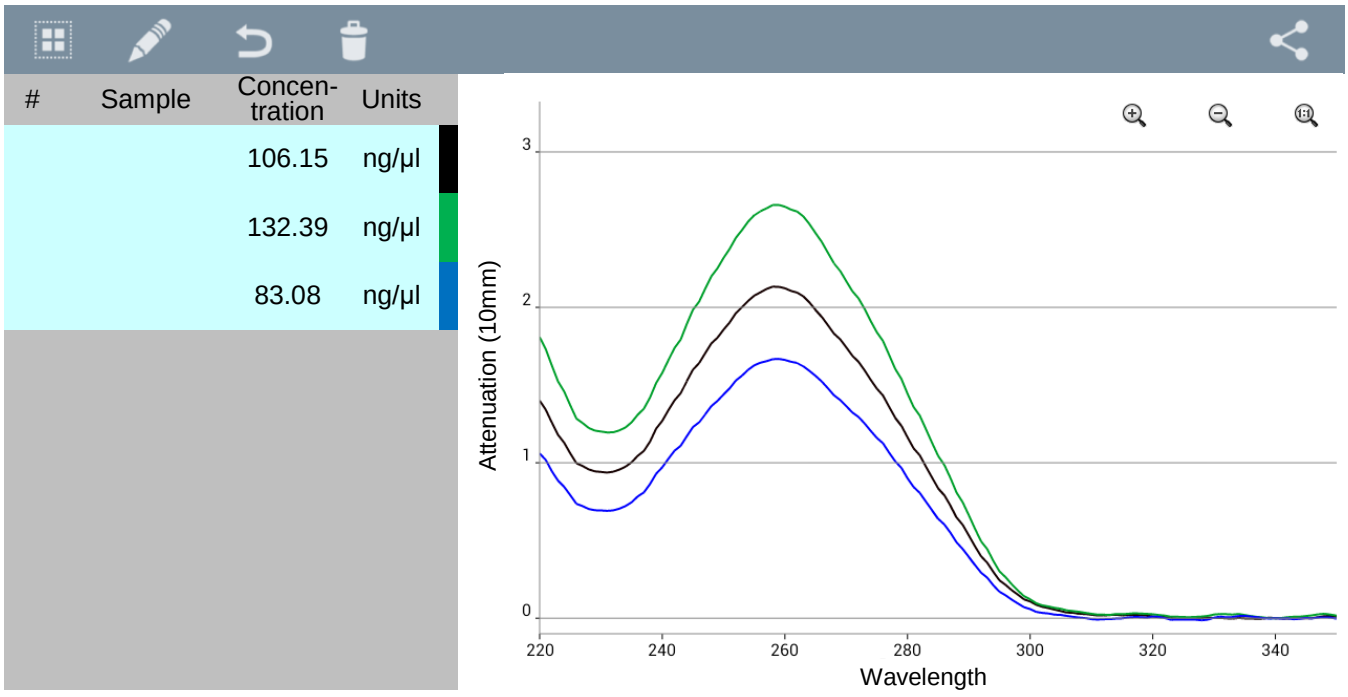

Figure 5. Results of plasmid DNA concentration measurement

The sample with the highest concentration of plasmid DNA was selected for our further studies.

Thus, we have developed a recombinant sample that contains a fragment with a length of 418 bp porcine circovirus type 3 *rep* gene. It can be used as a positive control in the detection of PCV-3 by PCR.

Discussion. Issues related to the selection and design of adequate positive controls for different PCR formats have always received considerable attention, primarily due to the potential risk to biosafety during the study (Chan, Jiang and Tan, 2016; Caasi et al., 2013; Chen et al., 2006; Lion, 2001).

Considerable factors in ensuring the high accuracy and efficiency of molecular genetic analysis based on PCR are, in particular, the conditions and form of storage of biological material used as a positive control.

At present, the use of positive control in the form of a recombinant plasmid containing a fragment of certain genomic DNA has become widespread in the detection of infectious diseases in farm animals, poultry (Yao et al., 2019; Das et al., 2017) and humans (Camacho et al., 2016), food pathogens (Gokduman et al., 2016), food allergens (Miyazaki et al., 2019), determination of GMOs (Taverniers, Van Bockstaele and De Loose, 2004) due to the long shelf life of such a structure, high copy capacity, the possibility of recovery (Matange, Tuck and Keung, 2021).

An equally important advantage of recombinant positive controls is that they do not require the permits provided by the Cartagena Protocol on Biosafety to the Convention on Biological Diversity, which was signed by Ukraine in 1992. Therefore, the recombinant positive control developed by us can be used to monitor biological samples obtained from pigs for the presence of PCV-3 genetic material using molecular technologies.

Conclusions. Thus, we obtained an ampicillin-resistant clone of *E. coli* DN5α, transformed with the constructed plasmid pTZ57R/T_PCV-3 with insertion of the *rep* gene with a length of 418 bp, which can be used as a positive control sample for the detection of the PCV-3 genetic material by PCR method.

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DEVELOPMENT OF RECOMBINANT ANTIGEN EXPRESSION AND PURIFICATION FOR AFRICAN SWINE FEVER SEROLOGICAL DIAGNOSTICS

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Summary. The paper reports the purification and its optimization of recombinant proteins p10, p32, p54, p54ΔTM, DNA ligase and DNA ligaseΔDBD of African swine fever virus. The corresponding coding sequences were subcloned into pASG-IBA105 and pASG-IBA103 vectors, multiplied and used for transformation of competent *E. coli* expression strain. Expressed proteins were purified using Strep-Tactin XT purification system under native and denaturing conditions, as well as using detergents according to the optimized protocol for recombinant proteins solubilization from inclusion bodies. Among all expressed and purified proteins p32 and p54 were found to be immunoreactive and specific. Although p54 was unstable during long-term storage, after further storage condition optimization, the protein can be used for indirect ASF ELISA development. Recombinant p32 was shown to be an effective antigen for ASF ELISA providing detection of antibodies against ASFV with low background signal

Keywords: ELISA, p10, p32, p54, DNA ligase

Introduction. African swine fever (ASF) is a viral contagious disease of domestic pigs and wild boars. An acute form of the disease causes up to 100% mortality of infected animals (Beltrán-Alcrudo et al., 2017). The causing agent, African swine fever virus (ASFV), is one of the most complex livestock viruses (Dixon et al., 2012; Oura, 2017; Alonso et al., 2018). For this reason, the virus is highly resistant to a variety of chemical and physical factors and remains stable in the environment for months and even years while being kept at a temperature below 0°C in a suitable reach in proteins environment (Mazur-Panasiuk, Żmudzki and Woźniakowski, 2019). The virus is transmitted from animal to animal via direct contact with blood, saliva, feces, urine, tears, secretions from genital tract and nose (Beltrán-Alcrudo et al., 2017). Soft ticks of the genus *Ornithodoros* (especially *O. moubata* and *O. erraticus*) have been identified as both specific arthropod vectors for ASFV transmission and natural reservoir. Transmission from pig to tick and *vice versa* occurs during feeding (Beltrán-Alcrudo et al., 2017; Dixon et al., 2020). ASF is widely spread in Africa, Eastern and Western Europe, the Caucasus region, Asia and the Pacific (Alonso et al., 2018). The first case of ASF in Ukraine was confirmed in 2012 and more than 550 cases have been registered so far in all regions of the country (SSUFSCP, 2021; OIE, 2021b).

Depending on the situation and the aim of the test, different diagnostic techniques can be used for ASF diagnostics. The laboratory tools used for this purpose are divided into two groups: aimed at causing agent identification (virus isolation, conventional and qPCR, antigen detecting ELISA, fluorescent antibody test) or immune response detection (antibody detecting ELISA, immunoperoxidase test, indirect fluorescent antibody test, immunoblotting). For an outbreak confirmation, using both virus and antibodies detection is recommended (Beltrán-Alcrudo et al., 2017; OIE, 2021a).

In the case of infection with a highly virulent strain of ASFV, animals die before the antibodies formation, whereas in the case of infection with a low virulent virus, animals survive. Antibodies to ASFV start forming at 7–10 dpi and persist in blood for months and sometimes years (Beltrán-Alcrudo et al., 2017; OIE, 2021a). Since there are no commercial vaccines against the disease available, the antibodies in a test sample always indicate the ASFV infection. Mutant attenuated variants of the virus are known to occur in areas where the ASF virus has long been established. Thus, such isolate has been detected during ASF serological surveillance study among wild pigs in Estonia (Zani et al., 2018). In addition to surveillance studies, ASF ELISA is used for eradication strategies improvement, as well as for conformation ASF-free status of populations and territories, which is important for animal movement and international trade (OIE, 2021a).

According to the OIE Terrestrial Manual 2021, the use of the antigen prepared from ASFV infected MS culture is recommended for ELISA (OIE, 2021a). However, since work with live ASFV requires the conditions of BSL-3 laboratory, obtaining such antigens for in-house diagnostics is impossible at laboratories with limited resources.

Therefore, the **aim of the study** was to express recombinant ASFV proteins, which could be used as antigens for ASF serological diagnostics.

Materials and methods. *Sera samples.* ASFV positive and negative sera samples (n = 15) were used for Western blot analysis of recombinant proteins and ASF ELISA development. The samples were kindly provided by PD Dr. S. Blome (Friedrich Loeffler Institute, Institute of Diagnostic Virology, Germany; ASF Serumpanel Charge:11/2018) and Dr. H. von Buttlar (Bundeswehr Institute of Microbiology, Germany, sera described in Al Dahouk et al. (2005) was used as negative sera sample).

Molecular cloning. ORFs coding ASFV proteins p10, p32, p54 and DNA ligase, as well as two truncated proteins with deletions of the transmembrane domain

(p54ΔTM) and DNA binding domain (ligaseΔDBD), have been selected for the study. The PCR primers flanking sequences of interest and including sites for Star Gate combinatorial cloning are shown in Table 1. The PCR products were generated using AmpliTaq Gold DNA Polymerase (Applied Biosystems, USA) according to manufacturer instruction and the data in Table 1. Using *Esp3*I restriction enzyme and T4 DNA ligase, purified and quantified PCR products were subcloned into vectors pASG-IBA103 and pASG-IBA105 (IBA Lifesciences, Germany), which include Twin-Strep-tag coding sequence for target protein purification at C- and N-termini respectively.

Table 1 — Primer sets used for the recombinant proteins development

Primer name	Primer sequence, 5'⟶3'	Encoded gene	Amplicon length, bp	Annealing temperature, °C
p10_F	AGCGCGTCTCCAATGCCTACAAAAGCTGGC	*K78R*	237	59
p10_R	AGCGCGTCTCCTCCCTTTTGACCGTTTAATTTTTTTCTCC			
p32_F	AGCGCGTCTCCAATGGATTTTATTTTAAATATATCCATG	*CP204L*	615	52
p32_R	AGCGCGTCTCCTCCCAAACATTAAATGTAGGTGAG			
p54_F	AGCGCGTCTCCAATGGATTCTGAATTTTTTCAACCGG	*E183L*	552	52
p54_R	AGCGCGTCTCCTCCCCAAGGAGTTTTCTAGGTC			
p54ΔT_F1	GCCGCGTCTCGAATGGATTCTGAATTTTTTCAAC	*E183LΔ*	115	50
p54ΔT_R1	CACCACACGTCTCGAACCGCCACCGAAGAAGCTCGG			
p54ΔT_F2	GCCGCGTCTCAGGTTCGATTATTATCATCG		461	
p54ΔTM_R2	CGGCCGTCTCATCCCCAAGGAGTTTTCCAGGTC			
ligase_F	AGCGCGTCTCCAATGCTAAATCAATTTCCTGGG	*NP419L*	1260	52
ligase_R	AGCGCGTCTCCTCCCAATGATTTCTAAAACATTTATCGG			
ligaseΔDBD_F	TAATCGTCTCAAATGAGAGGAATGATCCCCCCTATG	*NP419LΔ*	928	60
ligaseΔDBD_R	CGGCCGTCTCATCCCAATGATTTCTAAAAC			

E. coli NEB Turbo (NEB, USA) competent cells were transformed with the resulting vector. After blue-white screening and carbenicillin selection, amplified plasmids were extracted from bacterial culture using Monarch Plasmid Miniprep Kit (NEB, USA). The plasmids with correct insertion confirmed by sequencing (Eurofins Genomics, Germany) were used for transformation of expressing *E. coli* BL21 Lemo (D3) (NEB, USA) competent cells.

Expression and purification of recombinant proteins. Overnight culture of transformed *E. coli* BL21 Lemo (D3) was diluted 1:50 in fresh LB medium with carbenicillin and grown at 37°C until OD was 0.4–0.6. Protein expression was induced with anhydrotetracycline (200 ng/ml) followed by 3 h incubation at 37°C. The cells were harvested by centrifugation at 6,700 *g* for 10 min and stored at –20°C until use.

In case of native and denaturing protein purification, cells were disrupted by homogenization for 30 s at 6 m/s. Protein purification was performed using Strep-TactinXT 4Flow gravity-flow columns and Strep-TactinXT 4Flow resins (IBA Lifesciences, Germany). Purification under native conditions was performed according to columns manufacturer instruction. Briefly, buffer W (100 mM Tris-HCL, pH 8.0, 150 mM NaCl) was used for the column equilibration and washing, elution was performed using buffer BXT (100mM Tris-HCL, pH 8.0, 150 mM NaCl, 50 mM biotin). Purification under denaturing conditions was also performed according to manufacturer instructions. In this case, both buffers used for purification included 6 M urea. The eluted protein fractions were dialysed against buffer W and refolded using Slide-A-Lyzer G2 Dialysis Cassette 3.5 K MWCO (Thermo Scientific, USA) according to manufacturer instruction.

Non-denaturing purification with protein solubilization from inclusion bodies was performed as followed. The pellet from 100 ml culture was resuspended in 2 ml buffer W additionally containing 150 mM NaCl and 0.1% sodium deoxycholate. 10 µl lysozyme (50 mg/ml), 2 µl benzonase (25–29 U/µl), 2 µl 2 M $MgCl_2$ were added to the suspension and incubated for 20 min at room temperature and for 15 min on ice. After 20 cycles of sonication (30 s on, 20 s off) the suspension was centrifuged for 20 min at 11,000 *g*. The supernatant was removed and the pellet was resuspended in 1 ml of buffer W containing 150 mM NaCl and 0.3% sarcosyl. After 4 cycles of freeze-thawing (–80°C for 15 min, 37°C for 10 min), the suspension was sonicated and centrifuged as mentioned above. The supernatant was incubated overnight at 4°C rotating mixed with 1 ml of equilibrated with buffer W+0.3% sarcosyl Strep-TactinXT 4Flow resins. The pellet was resuspended in 2 ml buffer W+0.3% sarcosyl and incubated overnight at 4°C in a rotator. The suspension was centrifuged again and the supernatant was added to the resins. Protein purification was performed according to the resins manufacturer instructions. Dialysis of proteins from detergent was performed directly during purification at the stage of washing by stepwise reducing the sarcosyl concentration in the buffer W from 3 to 0%. Briefly, 1 ml of buffer W with sarcosyl concentration reduced by 0.5%

was used for each washing step, three last washing steps were performed by buffer W without the detergent. For the long-term storage, glycerol was added to the purified proteins to the final concentration of 5%.

Detection of recombinant proteins. The concentration of recombinant proteins was measured with spectrophotometer DeNovix DS-11 (DeNovix, USA). The purity of the eluted fractions was verified by SDS-PAGE electrophoresis and protein transfer to the nitrocellulose membrane followed by reversible protein staining with Pierce Reversible Protein Stain Kit (Thermo Scientific, USA). Detection of Tween-Strep-tagged proteins was performed by Western blot with Strep-MAB-Classic HRP IgG (IBA Lifesciences, Germany) according to manufacturer instruction.

The recombinant proteins specificity testing was performed by Western blot with ASF positive and negative swine sera samples as primary antibodies. The purified proteins were separated by SDS-PAGE and transferred to Nitrocellulose Membrane (Invitrogen, CIIIA). After overnight blocking with 10 ml of PBS containing 5% skimmed milk powder and 0.05% Tween 20, the primary antibodies were added directly to the blocking buffer in dilution 1:500. The membrane was washed 3 times for 10 min with washing buffer (PBS+0.05% Tween 20) and incubated for 1 h with secondary Rabbit-anti-Pig IgG HRP (Thermo Scientific, USA) diluted 1:5,000 in washing buffer. After washing twice with washing buffer and once with PBS the membrane was incubated in dark with TMB SeramunBlau precipitate solution (Seramun Diagnostica, Germany) for 10 min. The enzymatic reaction was stopped by washing the membrane with ultrapure water.

ELISA development. Nunc MaxiSorp 96 well plates (Thermo Scientific, USA) were coated overnight at 4°C with recombinant antigen titrated in carbonate buffer (pH 9.6) from 10 μg/ml to 0.1563 μg/ml from A to G. After discarding coating buffer, the wells were blocked with 10% skimmed milk powder and 1% rabbit serum in PBS for 1 h at 26°C followed by blocking buffer removal. ASF positive and negative swine sera samples were titrated from 1:50 to 1:400 in the dilution buffer (10% skimmed milk powder and 1% rabbit serum in PBS with 0.2% Tween 20). The final volume of the sera dilution was 50 μl per well. After 2 h incubation at 26°C, the buffer was removed and the wells were washed 4 times with 300 μl washing buffer (PBS with 0.2% Tween 20). The secondary Rabbit-anti-Pig IgG HRP-coupled antibodies (Invitrogen, USA) were also titrated in dilution buffer from 1:10,000 to 1:30,000 (50 μl of a dilution per well) and incubated for 1 h at 26°C. After washing as described above, 50 μl of TMB SeramunBlau slow solution substrate (Seramun Diagnostica, Germany) were added to each well followed by 10 min incubation in dark. The reaction was stopped by addition 50 μl of 0.25 M sulfuric acid. The OD was measured at a wavelength of 450 nm with a reference wavelength of 620 nm. When testing the reference sera samples, the OD threshold was established at the level of average OD value for negative sample duplicates (cut-off = average (native sera samples) + (3×SD (average (native sera samples))).

Results and discussion. *Molecular cloning.* Four proteins were selected for this study: p10 — a DNA binding protein (8.3 kDa), p32 — a structural protein responsible for the virus entry into a host cell (22.4 kDa), p54 — an inner envelope protein (20 kDa) and a 48 kDa DNA ligase. These proteins were described among 14 the strongest ASFV antigens (Kollnberger et al., 2002). Truncated proteins with deletions of transmembrane (p54ΔTM, 18.3 kDa) and DNA binding (ligaseΔDBD, 34.6 kDa) domains were developed in order to potentially simplify the purification process.

Although coding sequences of all the proteins were subcloned into two vectors including either C- or N-terminal Tween-Strep-tag, not for all of them the cloning process was successful. Thus, cloning to both types of vectors was successful for truncated and full-length DNA ligase, as well as for p54ΔTM, whereas for other proteins only one construct was obtained: pASG-IBA103_p10, pASG-IBA105_p32, pASG-IBA105_p54. All the plasmids were used for *E. coli* transformation and were shown to successfully express target proteins after induction (data not show).

Protein purification. In the first stage, the purification of recombinant proteins was performed under physiological conditions. However, Western blot of purified proteins with antibodies against Twin-Strep-tag revealed that the proteins were insoluble. As a result, only a small amount of the synthesized protein was present in the lysate (the concentration of pooled eluted fractions for each protein did not exceed 70 μg/ml), and most of the protein remained in the cell debris pellet (Fig. 1A). The only exception was the p10, which was successfully purified under native conditions and the concentration of the pooled purified fractions was 3.5 mg/ml (data not shown).

One reason for the unsuccessful purification could be the formation of inclusion bodies, which are dense spherical aggregates of proteins of the same type that are formed in bacterial cells during the expression of heterologous proteins. Twin-Strep-tag is known not to affect the folding and aggregation of tagged proteins, therefor, most often inclusion bodies are formed when the expression level of heterologous protein exceeds 2% of the total amount of cell proteins (Zhao, Li and Liang, 2013; Singh et al., 2015). About 70% of recombinant proteins overexpressed in *E. coli* form inclusion bodies (Yang et al., 2011). This can be caused by high temperature during protein expression, high concentration of the inducer, expression under the regulation of a 'strong' promoter, a large copy number of the target gene. As a result of a large amount of expressed target protein accumulation, aggregates consisting of misfolded and not completed folding molecules, as well as proteins of native structure, are formed. Proteins can also be inversely disaggregated from inclusion bodies and fold to native conformation (Singh et al., 2015).

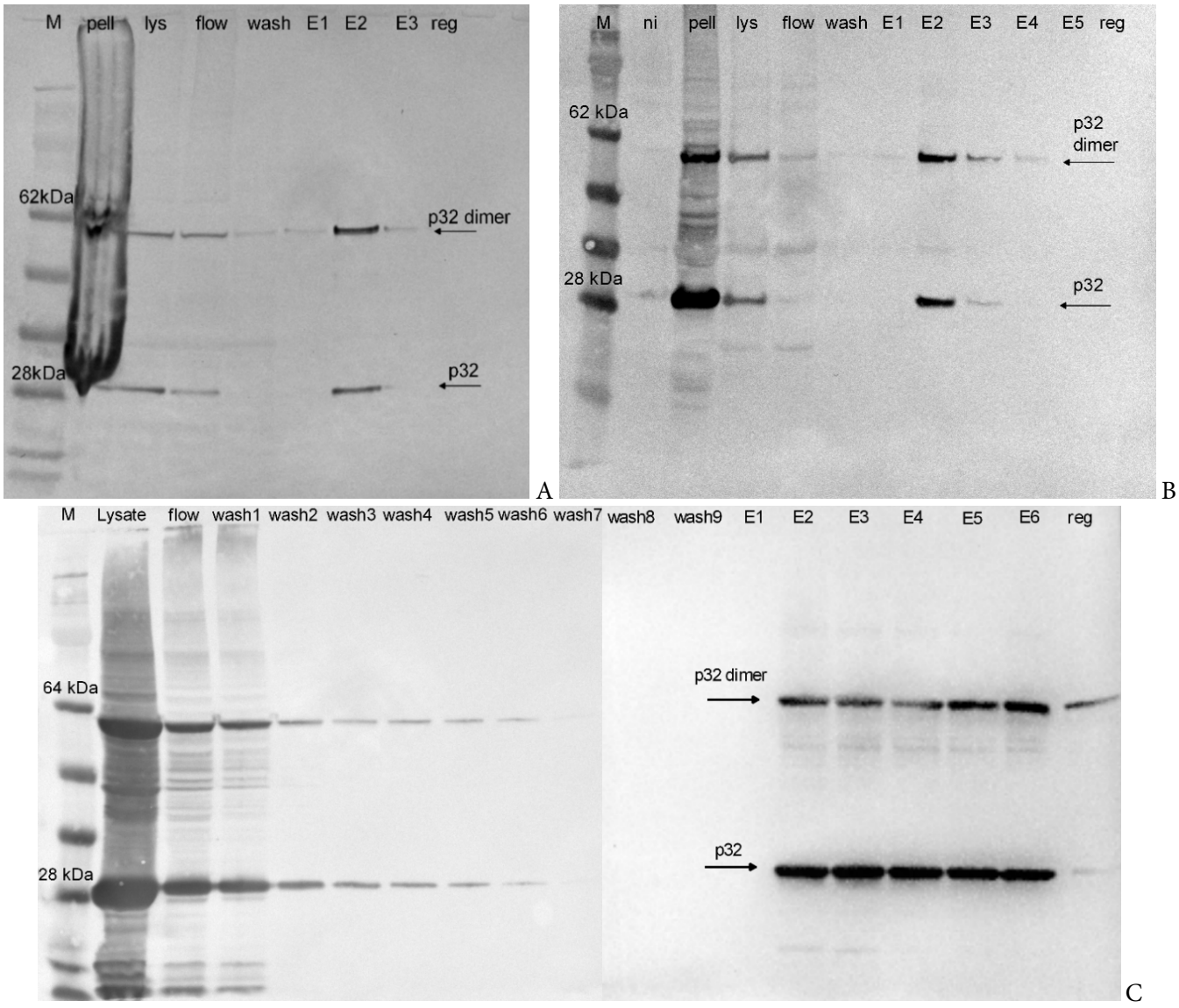

Figure 1. Western blot with Twin-Strep-tag antibodies of the fractions obtained during p32 purification under physiological conditions (A), denaturing conditions (B), non-denaturing purification with inclusion bodies solubilization (C) (M — SeeBlue Plus2 Pre-stained Protein Standard, ni — bacterial culture control before induction, pell — cell debris pellet, lys — lysate before application to the column, flow — flowthrough, wash — column washing fraction, E — eluted protein fractions, reg — column regeneration fraction)

One of the most common ways to solubilize recombinant proteins from inclusion bodies is bacterial culture lysate preparation and further protein purification under denaturing conditions in the presence of urea. By this approach, it was possible to purify a larger amount of soluble protein. In Fig. 1B the purification of p32 under denaturing condition is shown as an example. Similar results have been obtained for the proteins p54, p54ΔTM, DNA ligase and DNA ligaseΔDBD (data not shown). However, the largest part of the synthesized proteins still remained in the pellet, which indicated the need for further improvement of the purification protocol. A general disadvantage of the denaturing protein purification is the need for dialysis of the obtained fractions to free them from urea. Since proteins are in denatured form, dialysis and refolding should be performed gradually and slowly to prevent re-aggregation of proteins after urea removal.

It is known that the use of detergents in low concentrations results in mild protein solubilization, which allows obtaining active recombinant proteins without refolding, and the combination of different detergents can increase the efficiency of solubilization and further purification (Ma, Lee and Park, 2020; Singh et al., 2015; Tao et al., 2010; Burgess, 1996). Thus, based on an in-house method from the Bundeswehr Institute of Microbiology and a protocol for purifying His-tagged proteins (Heimerman et al., 2018) the two anionic detergents: sodium deoxycholate and sodium lauryl sarcosinate (sarcosyl) were used for further purification optimization steps. The sodium deoxycholate is used for lysis of membranes and solubilization of proteins from

inclusion bodies, is particularly effective for the disruption of protein-protein interactions (Burgess, 1996; Lau and Othman, 2019; Johnson, 2013). The use of sarcosyl in the purification of recombinant proteins is well established. Its advantages include the non-denaturing effect, the lack of interference in spectrophotometric measurement of protein concentration, the inhibitory effect on proteases and the low cost (Burgess, 1996; Johnson, 2013). Despite its widespread use in protein studies, sarcosyl has been used for the first time to solubilize Twin-Strep-tagged proteins. Sodium deoxycholate has a mild denaturing effect, while sarcosyl helps protein refolding (Johnson, 2013; Coligan, 1998). Despite the treatment with a denaturing agent, the proteins do not require an additional refolding phase after solubilization due to a 16 h incubation phase in the presence of sarcosyl. It was found that the supernatant contained only a very small amount of target protein after pellet treatment with sodium deoxycholate buffer. However, this step was crucial for purity since this treatment let to the removal of major part of native bacterial proteins from the cell lysate (Burgess, 1996). Subsequently, the treatment with sarcosyl effective enough for further protein solubilization of our target protein. As shown in Fig. 1C, the use of the solubilization protocol allowed to obtain a large amount of purified p32 with a low content of contaminating protein fractions.

Specificity test of the recombinant proteins. After purification, the antigenic properties and specificity of the obtained recombinant proteins were tested by Western blot with ASF positive and negative reference swine sera. The p32 and p54 proteins were proved to have antigenic properties and did not show a background signal in a study with ASF negative serum, while the p10 protein as well as the truncated and full-length DNA ligases did not show any difference between the reaction with ASF positive serum nor negative serum (Fig. 2). This might be due to the lack of necessary posttranslational modifications that *E. coli* expression system could not ensure. Since ASFV native proteins are expressed in eukaryotic cells, immunoreactive recombinant proteins are likely required to be expressed in eukaryotic systems such as HEK-293, insect cell culture, or *Leishmania tarentolae* (Basile and Peticca, 2009; Ikonomou, Schneider and Agathos, 2003; Ma, Lee and Park, 2020; Thomas and Smart, 2005).

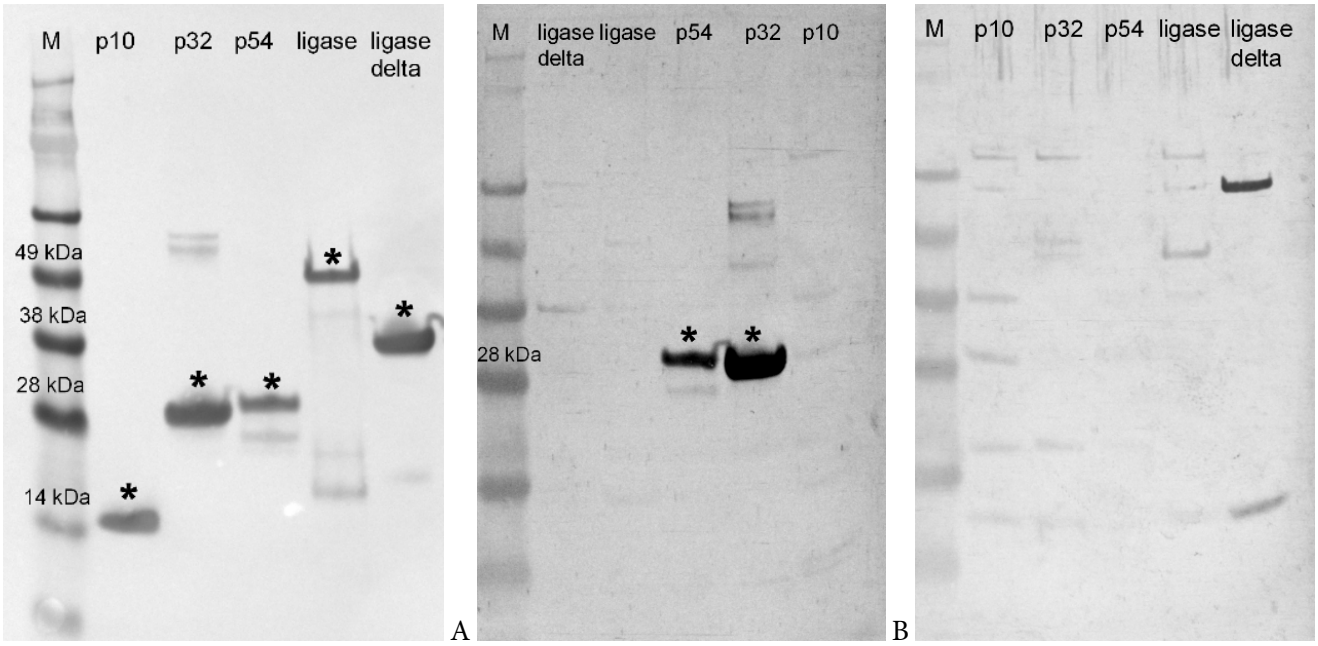

Figure 2. Western blot of purified recombinant proteins with Twin-Strep-tag antibodies (A), ASF positive (B) and negative (C) swine sera (M — SeeBlue Plus2 Pre-stained Protein Standard, p10, p32, p54, ligase, ligase delta — purified recombinant proteins. Recombinant proteins of the target size are marked with asterisks)

The antigenic properties and specificity of the p54ΔTM protein were tested separately. The results of Western blot with both ASF positive and negative sera were positive, indicating nonspecific binding of the antigen to antibodies in swine sera. Therefore, recombinant p54ΔTM protein cannot be used as an antigen for serological diagnosis of ASF. The loss of p54ΔTM protein specificity can be explained by the fact that deletion of the transmembrane domain could affect the folding and tertiary structure of the protein, potentially altering the structure of epitopes which bind to swine antibodies. Thus, p32 and p54 proteins were selected for further ELISA development with the obtained proteins as antigens.

Pilot ELISA development. When choosing the optimal antigens and antibodies concentration, the ratio of OD values of positive and negative samples and the dynamics of signal reduction was considered. Thus, after a sharp

nonlinear decrease of the OD value, the previous antigen concentration was chosen, because it provided the maximum saturation of the well surface with antigen without its redundant use. After selecting the optimal antigen concentration, the optimal serum dilution was chosen. The serum dilution providing high OD values for the positive samples and OD value about 0.2 for negative samples was considered optimal.

After titration of p32 as coating antigen, its optimal concentration was determined to be 5 µg/ml as it was the closest concentration before nonlinear OD decrease (Fig. 3).

The optimal OD values ratio for positive and negative samples was observed with 1:100 sera dilution. In case of establishing optimal dilution of secondary antibodies, a nonlinear OD decrease was observed after a dilution of 1:10,000, so this concentration was selected for further work as optimal (Fig. 4).

A panel of swine ASF reference sera was tested by the developed ELISA based on the use of recombinant p32 protein. The obtained data met the expected criteria. The ASF sera panel included as internal control a negative sample. This sample was #7 which was clearly identified. All other samples have been ASFV positive (Fig. 5).

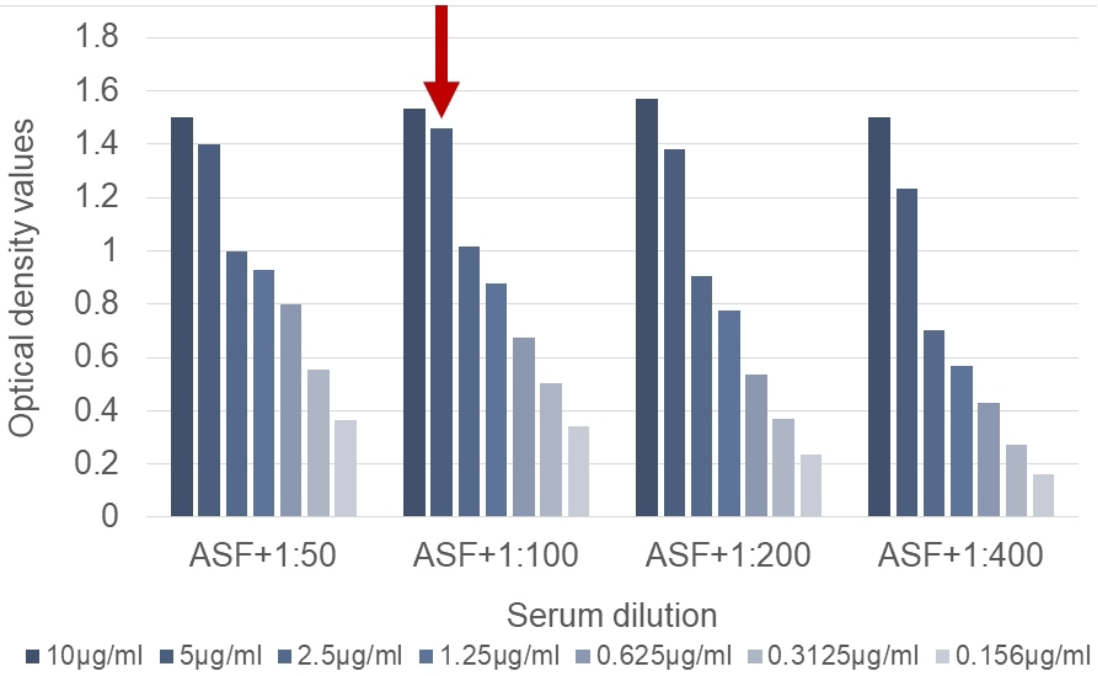

Figure 3. Recombinant p32 protein titration with ASF positive serum (the chosen concentration of the antigen and serum is indicated with the arrow)

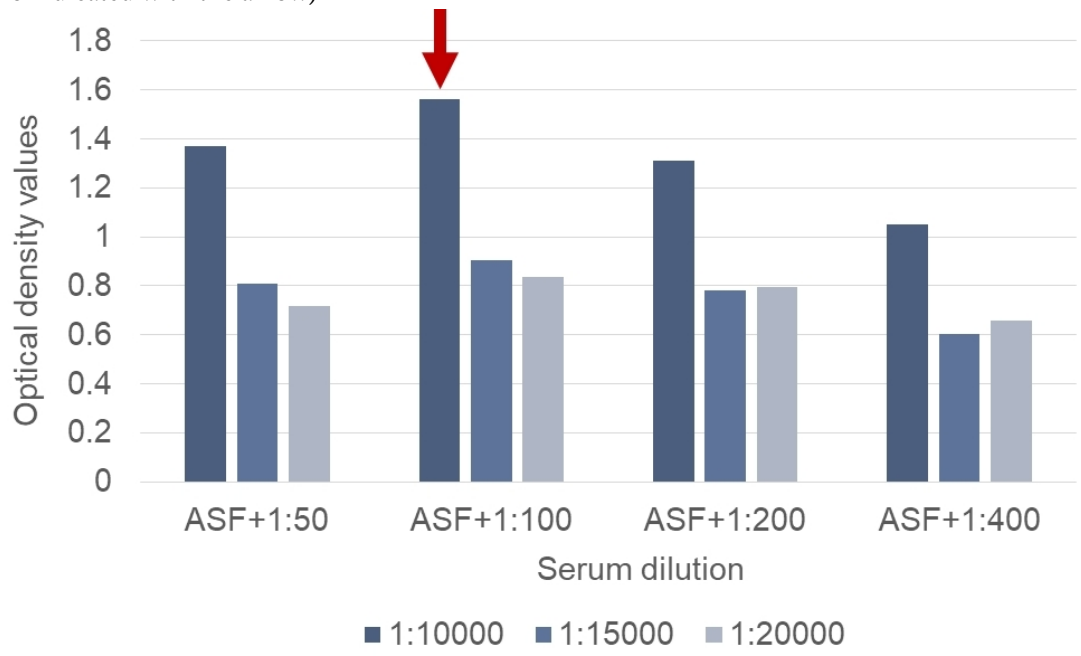

Figure 4. Secondary antibodies titration with ASF positive serum (the chosen concentration of the conjugate and serum is indicated with the arrow)

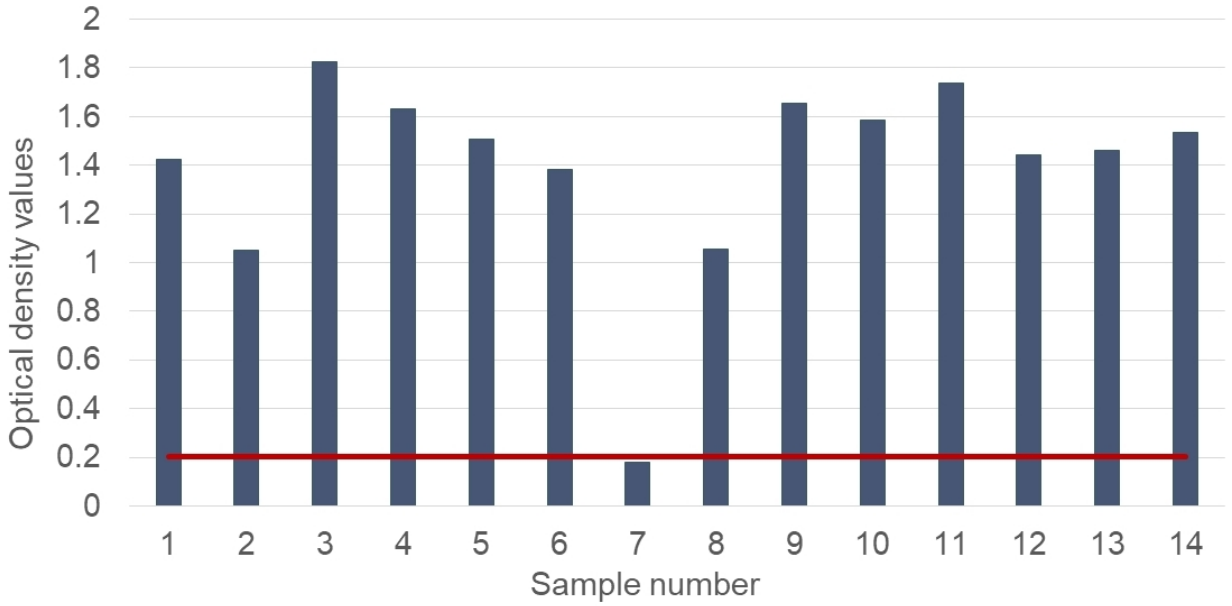

Figure 5. Reference swine sera testing with the developed p32 based ELISA (the red line indicates the average OD threshold for negative results (0.2))

Titration of p54 protein was performed several times, but despite a multiple increase of the antigen introduced into the wells, the maximum saturation of the surface could not be achieved. After storage at –20°C, a sharp drop in the protein concentration was observed and ELISA development with p54 as antigen was suspended for the moment. The concentration decrease can be explained by protein degradation, binding to the storage tubes and re-aggregation of molecules after removal of the solubilizing agent. It is known that proteins in a solution with a concentration less than 1 mg/ml can lose stability (Pierce Biotechnology, 2009; Simpson, 2010) and this could be prevented by concentrating of protein fractions, for example by filtration. Glycerol was added to all aliquots of proteins for long-term storage to a final concentration of 5%, but in the case of p54 samples, it did not lead to any stability. The use of other cryoprotectants (ethylene glycol, polyethylene glycol, Tween 80), protease inhibitors, reducing agents (DTT, 2-mercaptoethanol) could improve the protein stability (Pierce Biotechnology, 2009; Simpson, 2010). Another possible way to solve the problem is to use special protein low bind tubes. Reaggregation of protein molecules can be avoided by optimizing the purification protocol and adding a separate step of dialysis using cassettes. Nevertheless, after identifying the reason for the concentration decrease and optimization of storage conditions, p54 can be used as an antigen for ELISA due to its specificity and low background signal in the study with negative sera.

Conclusions. Immunoreactive recombinant ASFV proteins p32 and p54 were successfully expressed and purified using the optimised protocol for solubilization of Twin-Strep-tagged proteins. After establishing optimal long-term storage conditions, p54 can be used for indirect ASF ELISA development.

For p32 based ELISA, the optimal concentration of antigen, serum and secondary antibodies were determined, which sets the basis for the further development and validation of the assay. Additional studies with weak-positive samples use are necessary to evaluate the sample-to-noise ratio. Testing of the sera samples positive to other swine viral diseases are required for assessment of the assay specificity.

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Part 3. Biosafety

UDC 619:636.52/.58.085.1/.3:577.16:546.23:546.47:546.56 **DOI 10.36016/JVMBBS-2021-7-3-5**

MONITORING OF FEED FOR CHICKENS BY THE CONTENT OF VITAMINS AND MICROELEMENTS

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Summary. The paper presents the results of monitoring of compound feeds for different types of chickens for the period 2017–2021 by the indicators of the content of vitamins (A, E, B_2) and trace elements (Zn, Cu, Se). Vitamin A content did not show significant deviations from the norm in compound feeds for productive chickens and for reproductive meat poultry, and in compound feeds for reproductive egg hens, samples with low vitamin A content (5.7%) were found. By the content of vitamin E in compound feeds for commercial chickens, the percentage of samples with reduced content was 75.2%, in compound feeds for reproductive egg hens — 63.9%, and in compound feeds for reproductive meat hens — 12.7%. By the content of vitamin B_2 in compound feeds for reproductive egg chickens, samples with reduced content of vitamin B_2 were not observed, while in compound feeds for reproductive meat chickens, reduction of vitamin B_2 was found in 4.0% of samples, and in compound feeds for commercial laying hens in 5.1%. The average zinc content in compound feeds for productive laying hens and reproductive egg and meat hens was within normal limits, but reduced zinc levels were observed in 11.0, 2.5, and 17.3% of samples, and above the MAL — in 11.1, 16.9, and 47.1% of the samples, respectively. The average content of copper in compound feeds for commercial laying hens and reproductive egg and meat hens was within the norm, but the reduced level of the element was registered in 1.4, 0.0, and 18.0% of samples, and above the MAL — in 1.4, 8.3, and 16.9% of samples, respectively. The average content of selenium in compound feeds for commercial laying hens and reproductive egg and meat hens was within the norm, but the reduced level of the element was registered in 52.8, 29.3, and 26.8% of samples, the exceeding of the maximum allowable level was not detected

Keywords: vitamins A, E, B_2, zinc, cooper, selenium

Introduction. Modern chicken crosses have a potential productivity of more than 330 eggs per year. Over the past 20 years, the live weight of 42-day-old broilers has increased 2.3 times (2.4 kg), and the fattening period to a weight of 2 kg has decreased by 26 days (from 63 to 37 days). At the same time, the efficiency of compound feed use increased, the costs per 1 kg of weight gain decreased from 2.5 to 1.65 kg of feed.

In the near future, broilers are expected to reach a live weight of 2 kg by 36 days of age with the conversion of compound feed 1.4 kg (Makarynska and Vorona, 2019).

Given the above, the increase of the load on the body in terms of sensitivity to various stresses would be logical, so to realize the inherent genetic potential the bird's need for vitamins and trace elements will increase, especially those involved in antioxidant protection and affect reproductive function: vitamins B_2, A, and E (Abd El-Hack et al., 2019; Lauridsen, 2019; Idamokoro et al., 2020), trace elements selenium, zinc, copper (Huma et al., 2019; Sobolev et al., 2019; Ghasemi et al., 2020).

Monitoring of nutrients in feed is one of the elements of a timely correction of the measures for the optimal provision of vitamin and mineral nutrition of chickens in modern conditions (Kutsan and Orobchenko, 2011, 2015; Alagawany et al., 2021).

Therefore, the **work was aimed** to monitor feed for chickens with indicators of vitamins and trace elements.

Materials and methods. As part of achieving the goal of this work, we monitored feed for chickens of different areas of productivity in terms of vitamins (A, E, B_2) and trace elements (zinc, copper, selenium) for the period 2017–2021.

Compound feed samples were examined in the Laboratory of Toxicological Monitoring of the National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (Kharkiv) and in the Scientific Chemical and Toxicological Department of the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (Kyiv).

The study of the content of vitamins in compound feeds was carried out according to Kutsan and Orobchenko (2009) and using liquid chromatography following DSTU 4687:2006 (DSSU, 2007); trace elements — using the methods of atomic absorption spectrometry following DSTU EN 14082:2019 (SE 'UkrNDNC', 2019) and X-ray fluorescence analysis (Kutsan, Orobchenko and Kocherhin, 2014).

In total for the period 2017–2021 there were examined:

— for the content of vitamin A — 678 samples of compound feeds (281 samples for commercial laying hens, 228 samples for reproductive population of laying hens, and 169 samples for reproductive meat chickens);

— for the content of vitamin E — 305 samples of compound feeds (129 samples for commercial laying hens, 97 samples for reproductive population of laying hens, and 79 samples for reproductive meat chickens);

— for the content of vitamin B_2 — 531 samples of compound feeds (237 samples for commercial laying hens, 168 samples for reproductive population of laying hens, and 126 samples for reproductive meat chickens);

— for the content of zinc — 725 samples of compound feeds (298 samples for commercial laying hens, 236 samples for reproductive population of laying hens, and 191 samples for reproductive meat chickens);

— for the content of copper — 713 samples of compound feeds (288 samples for commercial laying hens, 242 samples for reproductive population of laying hens, and 183 samples for reproductive meat chickens);

— for the content of selenium — 588 samples of compound feeds (233 samples for commercial laying hens, 191 samples for reproductive population of laying hens, and 164 samples for reproductive meat chickens).

The obtained results have been calculated by the methods of variation statistics using the software package StatPlus v. 5.9.8.5. Data are presented as mean values with standard deviation at a confidence level of 95%, the reliability of research results was evaluated by Fisher's test.

Results and discussion. Monitoring of feed for chickens (commercial and reproductive population of egg and meat chickens) in 2017–2021 by the content of vitamins (A, E, B_2) and trace elements (zinc, copper, selenium), which affect the reproductive function of poultry, has been carried out.

For the content of vitamin A 678 samples of compound feeds have been tested over the years (281 samples for commercial laying hens, 228 samples for reproductive population of laying hens, and 169 samples for reproductive meat chickens): the average content was respectively 11,510.59 ± 62.88 IU/kg (10,009.98–14,238.5 IU/kg), 14,749.57 ± 175.75 IU/kg (7,912.14–17,622.87 IU/kg) and 18,240.04 ± 36.94 IU/kg (17,366.59–19,631.89 IU/kg) (Fig. 1).

The content of vitamin A in all terms of research was reliably higher ($p < 0.05$) in compound feeds for reproductive population of meat chickens relative to the rate of feed for commercial poultry: in 2017 — by 47.6%, in 2018 — by 59.2%, in 2019 — by 53.2%, in 2020 — by 69.0%, in 2020 — by 68.4%, and exceeding the overall figure for 5 years was 58.5%.

A similar pattern was observed in compound feeds for reproductive population of egg hens relative to the indicators of compound feeds for commercial poultry: in 2017, the excess ($p < 0.05$) of vitamin A content was 38.7%, in 2018 — 46.7%, in 2019 — 32.8%, in 2020 and 2021 — 5.7 and 1.0%, respectively (but the value was not reliable), and the excess of the overall figure for 5 years was 28.1% (Fig. 1).

It should be noted that the average content of vitamin A in compound feeds for commercial laying hens was within normal limits (10,000.0–12,000.0 IU/kg) (Melnyk et al., 2013), while in compound feeds for reproductive population of chickens of both directions of productivity, the content of vitamin A was higher than the upper limit of normal (Melnyk et al., 2013) by an average of 43.3%. In addition, there is a tendency to reduce the content of vitamin A in feed for reproductive population of egg chickens starting from 2020 (Fig. 1).

Dynamics of the norm and lack of vitamin A, the in feed for chickens of different types of productivity is shown in percentage in Fig. 2. Thus, in compound feeds for commercial chickens and reproductive meat poultry during the whole period of research no samples with reduced content of vitamin A were observed.

Samples with reduced vitamin A content were detected in compound feeds for reproductive hens: in 2020 — 14.0% (norm — 86.0%), and in 2021 — 21.2% (norm — 78.8%), that was reflected in the average for 5 years: 5.7% of samples were below normal (Fig. 2).

305 samples of compound feeds were investigated for the content of vitamin E over the years (for commercial laying hens — 129 samples, for reproductive population of laying hens — 97 samples, and for reproductive meat chickens — 79 samples): the average content was 119.16 ± 2.77 mg/kg (62.72–181.03 mg/kg), 165.10 ± 4.68 mg/kg (75.32–247.76 mg/kg), and 219.93 ± 2,02 mg/kg (180.6–267.02 mg/kg) (Fig. 3).

The content of vitamin E in all terms of research was reliably higher ($p < 0.05$) in compound feeds for reproductive meat chickens relative to the rate in feed for commercial poultry: in 2017 — by 85.4%, in 2018 — by 86.7%, in 2019 — by 78.7%, in 2020 — by 95.4%, in 2021 — by 76.9%, and exceeding the overall figure for 5 years was 84.6%.

A similar pattern was observed in compound feeds for reproductive population of egg hens relative to the indicator of compound feeds for commercial poultry: in 2017 the excess ($p < 0.05$) of vitamin E content was 68.4%, in 2018 — 39.6%, in 2019 — 35.8%, in 2020 — by 22.3%, in 2021 — by 24.7%, and the excess of the overall figure for 5 years was 38.6% (Fig. 3).

It should be noted that the average content of vitamin E in compound feeds for commercial laying hens and reproductive population of egg hens was less than normal (150.0 and 200.0 mg/kg, respectively) (Kutsan and Orobchenko, 2009) by 20,6% and 17.5%, while in compound feeds for reproductive population of meat chickens the content of vitamin E was higher than the upper norm (Kutsan and Orobchenko, 2009) by 10.0%. In addition, there is a tendency to reduce the content of vitamin E in feed for reproductive population of egg hens, especially pronounced in 2020 (Fig. 3).

In percentage, the dynamics of the norm and lack of vitamin E in compound feed for chickens of different directions of productivity is shown in Fig. 4.

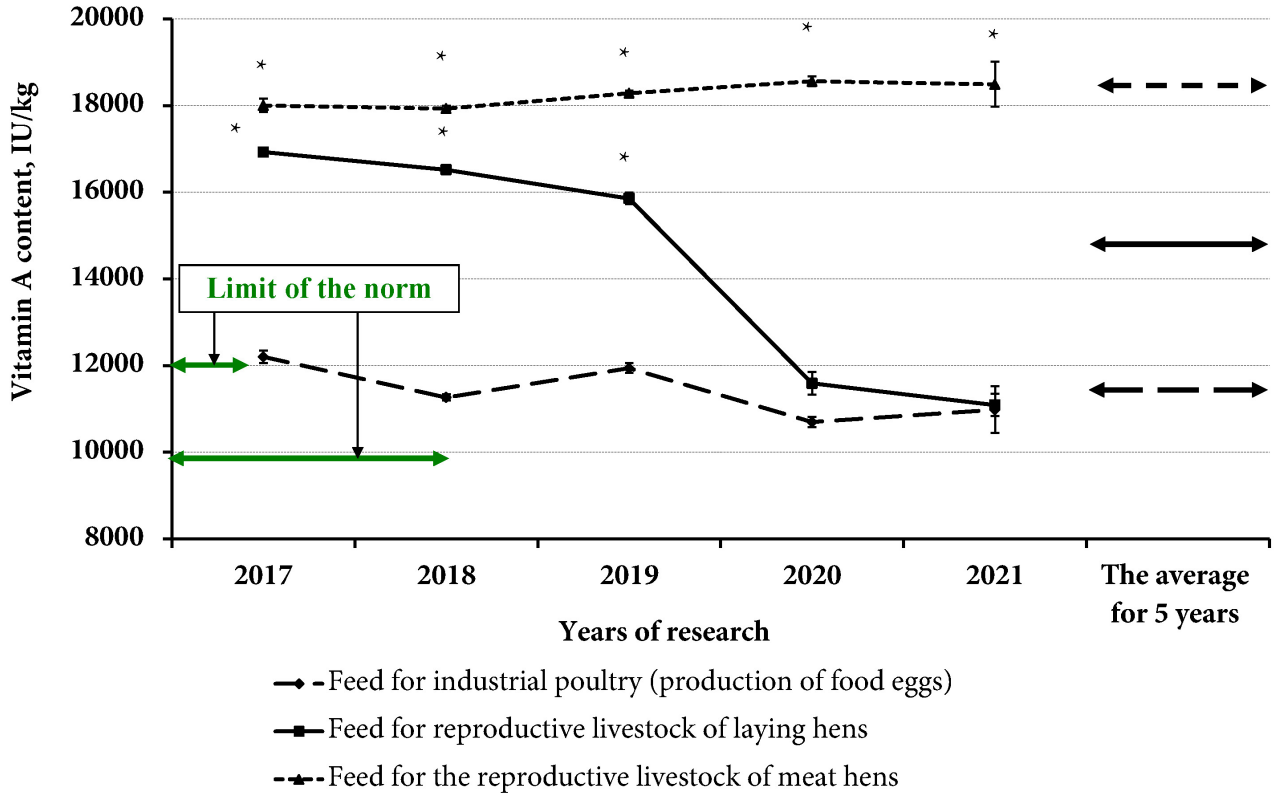

Figure 1. The content of vitamin A in compound feed for chickens of different directions of productivity in the dynamics of 2017–2021 (M ± m, n = 678, * — $p < 0.05$ relative to the figure of feed for commercial chickens)

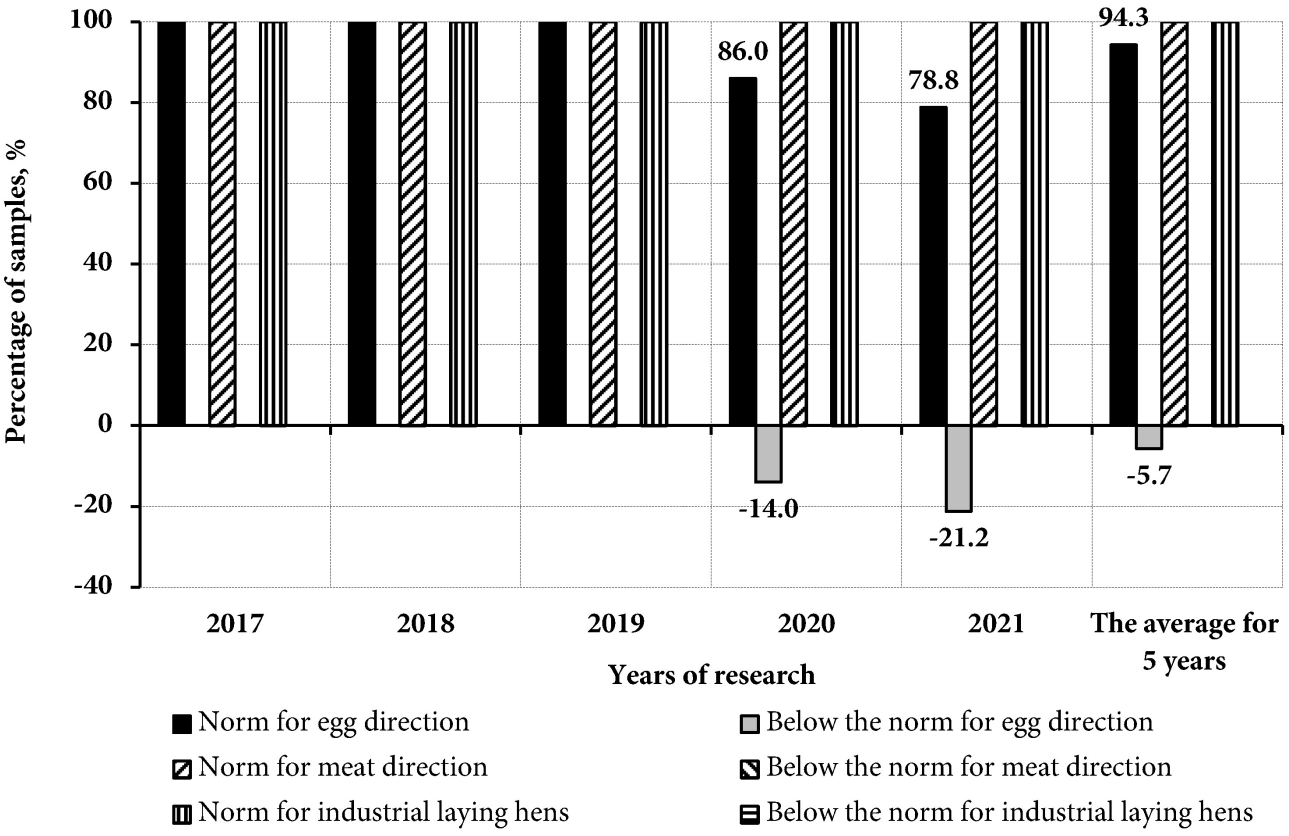

Figure 2. The ratio of vitamin A norm and deficiency in feed for chickens of different areas of productivity in the dynamics of 2017–2021 (M ± m, n = 678)

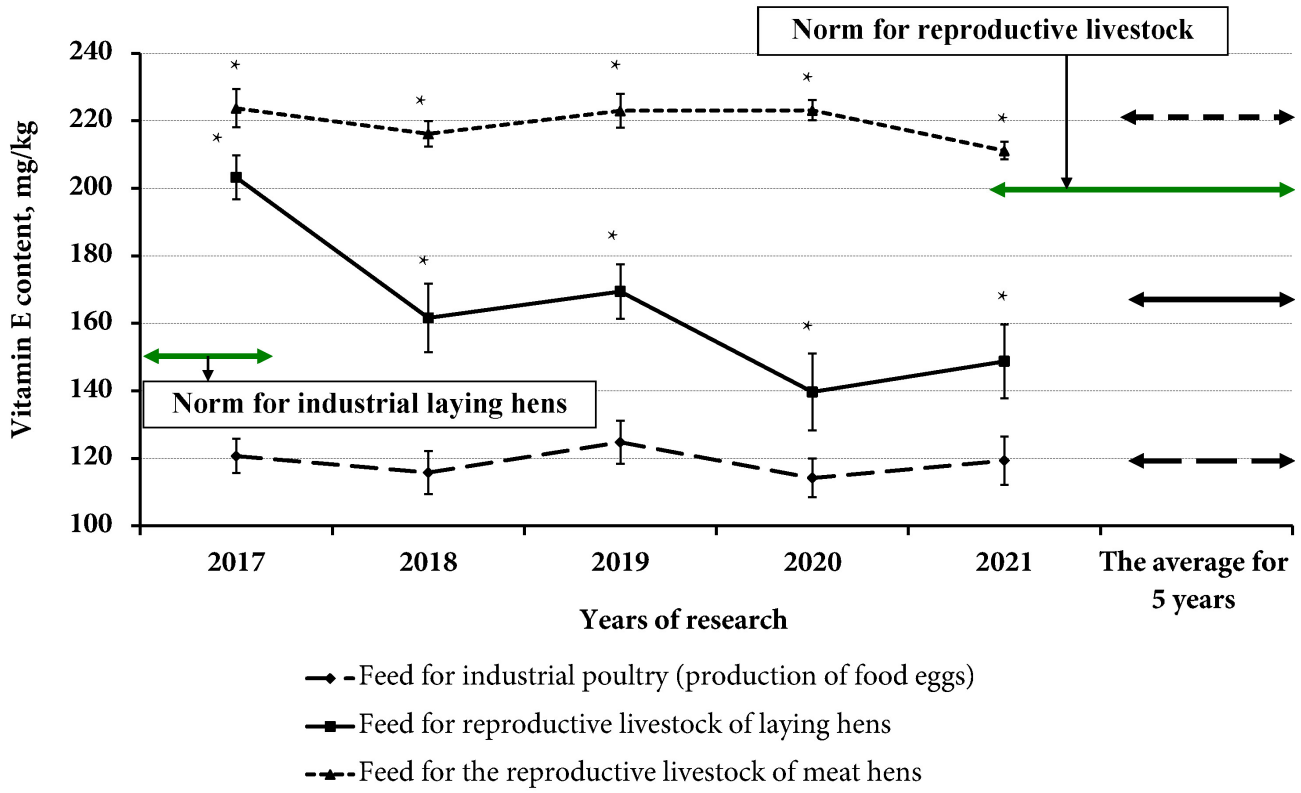

Figure 3. The content of vitamin E in feed for chickens of different directions of productivity in the dynamics of 2017–2021 (M ± m, n = 305, * — $p < 0.05$ relative to the rate in feed for commercial chickens)

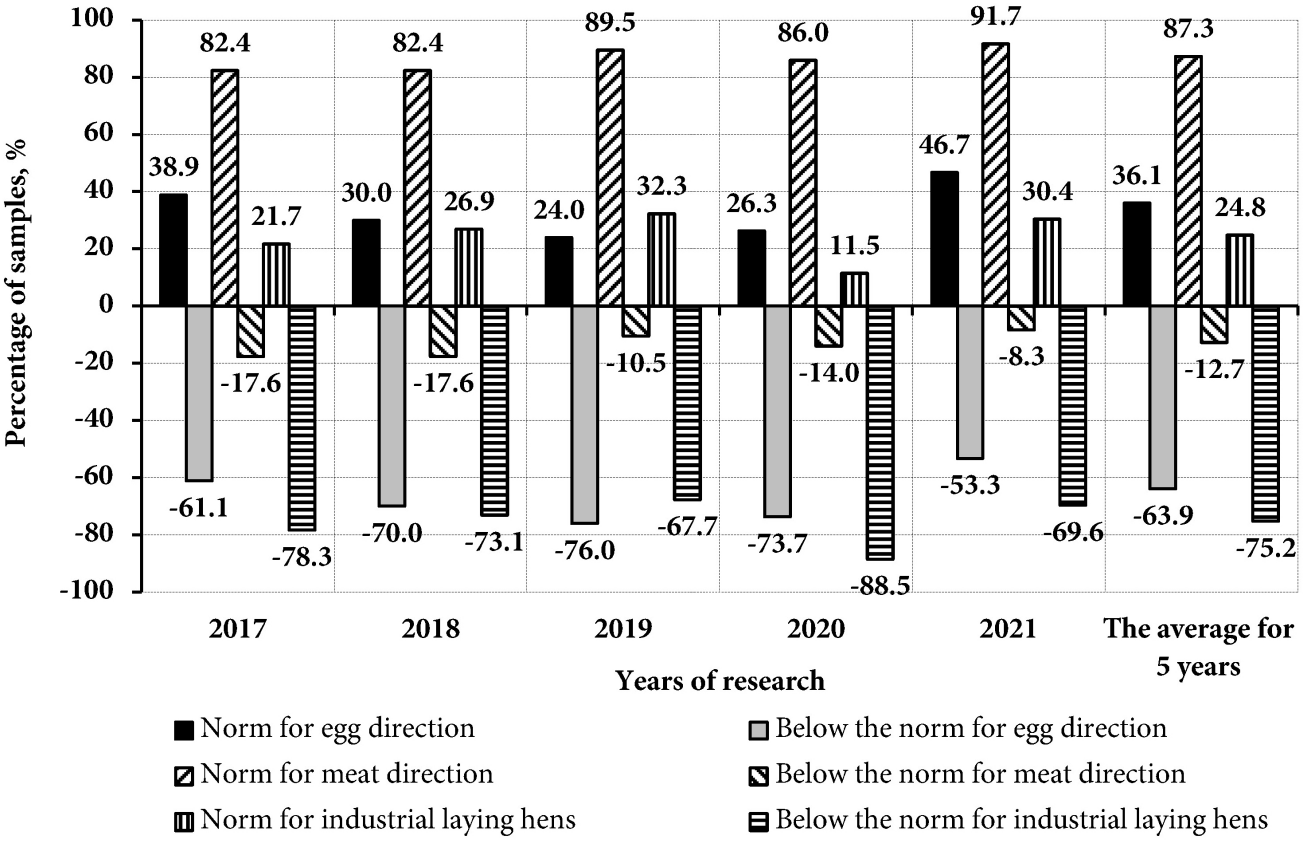

Figure 4. The ratio of vitamin E norm and deficiency in compound feed for chickens of different directions of productivity in the dynamics of 2017–2021 (M ± m, n = 305)

Thus, in compound feeds for commercial chickens at all terms of research we found a significant percentage of samples with low vitamin E content: in 2017 — 78.3% (norm — 21.7%), in 2018 — 73.1% (norm — 26.9%), in 2019 — 67.7% (norm — 32.3%), in 2020 — 88.5% (norm — 11.5%), and in 2021 — 69.6% (norm — 30.4%), which was reflected in the average indicator for 5 years: 75.2% of samples were below the norm.

A similar pattern was observed in compound feeds for the reproductive population of egg hens: the percentage of samples with low vitamin E content in 2017 was 61.1% (norm — 38.9%), in 2018 — 70.0% (norm — 30.0%), in 2019 — 76.0% (norm — 24.0%), in 2020 — 73.7% (norm — 26.3%), and in 2021 — 53.5% (norm — 46.7%), which was reflected in the average for 5 years: 63.9% of samples were below normal .Samples with a reduced content of vitamin E were also found in compound feeds for the reproductive population of meat chickens, but their percentage was not significant: in 2017 — 17.6% (norm — 82.4%), in 2018 — 17.6% (norm — 82.4%), in 2019 — 10.5% (norm — 89.5%), in 2020 — 14.0% (norm — 86.0%), and in 2021 — 8.3% (norm — 91.7%), which was reflected in the average for 5 years: 12.7% of samples were below the norm (Fig. 4).

531 feed samples were tested for vitamin B_2 content over the years (for commercial laying hens — 237 samples, for reproductive egg hens — 168 samples and for reproductive meat hens — 126 samples): the average content was 5.84 ± 0.04 mg/kg (4.79–7.78 mg/kg), 7.06 ± 0.08 mg/kg (5.14–9.78 mg/kg), and 7.92 ± 0.13 mg/kg (4.60–10.61 mg/kg) (Fig. 5). The content of vitamin B_2 in all terms of research was reliably higher ($p < 0.05$) in compound feeds for reproductive meat chickens relative to the rate of feed for commercial poultry: in 2017 — by 32.3%, in 2018 — by 28.9%, in 2019 — by 22.5%, in 2020 — by 55.0%, in 2021 — by 49.9%, and exceeding the overall figure for 5 years was 35.6%. A similar pattern was observed in compound feeds for reproductive population of egg hens relative to the rate of feed for commercial poultry: in 2017, the excess ($p < 0.05$) was 30.2%, in 2018 — 13.6%, in 2019 — 21.3%, in 2020 — 15.1%, in 2021 — by 26.4%, and exceeding the overall figure for 5 years was 20.9%. It should be noted that the average content of vitamin B_2 in compound feeds for commercial laying hens was within normal limits (5.0–6.0 mg/kg) (Melnyk et al., 2013), while in compound feeds for reproductive population of both meat and egg chickens, the vitamin content was higher than the upper limit of normal (Melnyk et al., 2013) by an average of 28.3% (Fig. 5).

In percentage terms, the dynamics of the norm and lack of vitamin B_2 in the compound feed for chickens of different directions of productivity is shown in Fig. 6.

Thus, in the compound feed for reproductive egg hens throughout the study period, no samples were observed with low levels of vitamin B_2. In compound feeds for reproductive population of meat chickens samples with the reduced rate of the content of vitamin B_2 were found: in 2017 — 10.7% (norm — 89.3%), in 2018 — 6.5% (norm — 93.5%), and in 2019–2021 there were no samples with low vitamin B_2 content, which was reflected in the average for 5 years: 4.0% of samples were below normal.

Samples with reduced content of vitamin B_2 were also found in compound feeds for commercial laying hens: in 2017 — 4.7% (norm — 95.3%), in 2018 — 3.5% (norm — 96.5%), in 2020 — 4.2% (norm — 95.8%), and in 2021 — 16.2% (norm — 83.8%), in 2019 there were no samples with low vitamin B_2 content, that was reflected in the average for 5 years: 5.1% of samples were below normal (Fig. 6).

725 samples of compound feeds were studied for the content of zinc in the dynamics of years (for commercial laying hens — 298 samples, for reproductive population of egg hens — 236 samples and for reproductive meat chickens — 191 samples): the average content was 89.54 ± 1.29 mg/kg (50.49–131.60 mg/kg), 98.06 ± 1.38 mg/kg (52.44–135.88 mg/kg), and 118.32 ± 1.31 mg/kg (80.09–147.62 mg/kg) (Fig. 7).

The content of zinc in all terms of research was reliably higher ($p < 0.05$) in compound feeds for reproductive population of meat chickens relative to the rate of feed for commercial poultry: in 2017 — by 28.6%, in 2018 — by 55.9%, in 2019 — by 38.4%, in 2020 — by 23.0%, in 2021 — by 24.9%, and the excess of the overall figure for 5 years was 32.1%. A slightly different picture was observed in compound feed for reproductive egg hens relative to the rate of feed for commercial poultry: in 2017, 2020, and 2021 — no significant changes in zinc content were registered; in 2018, the excess ($p < 0.05$) of zinc content was 34.0%, in 2019 — 19.6%, and the excess of the overall figure for 5 years was 9.5% (Fig. 7).

It should be noted that the average zinc content in compound feeds for commercial laying hens and reproductive chickens of both directions of productivity was within the norm (approximate indicator, according to the instructions on the operation of poultry crosses) (not less than 60.0 and not less than 100.0 mg/kg, respectively) (Fig. 7).

However, in addition to zinc deficiency in compound feeds, the maximum allowed level (MAL) was exceeded (120.0 mg/kg) (MAPFU, 2012) during the study period: in commercial chickens — 11.1% of samples, in reproductive egg chickens — 16.9% of samples, and in reproductive meat chickens — 47.1% of samples.

In percentage, the dynamics of the norm and zinc deficiency in compound feed for chickens of different directions of productivity is shown in Fig. 8.

Thus, the lowest percentage of samples with reduced zinc content was observed in compound feeds for reproductive population of egg hens during the entire study period: in 2017 — 4.3% (norm — 95.7%), in 2018 — 100.0% samples corresponded to the norm, in 2019 — 4.3% (norm — 95.7%), in 2020 — 3.4% (norm — 96.6%), and in 2021 — 100.0% of samples corresponded to the norm, that was reflected in the average for 5 years: 2.5% of samples were below the norm.

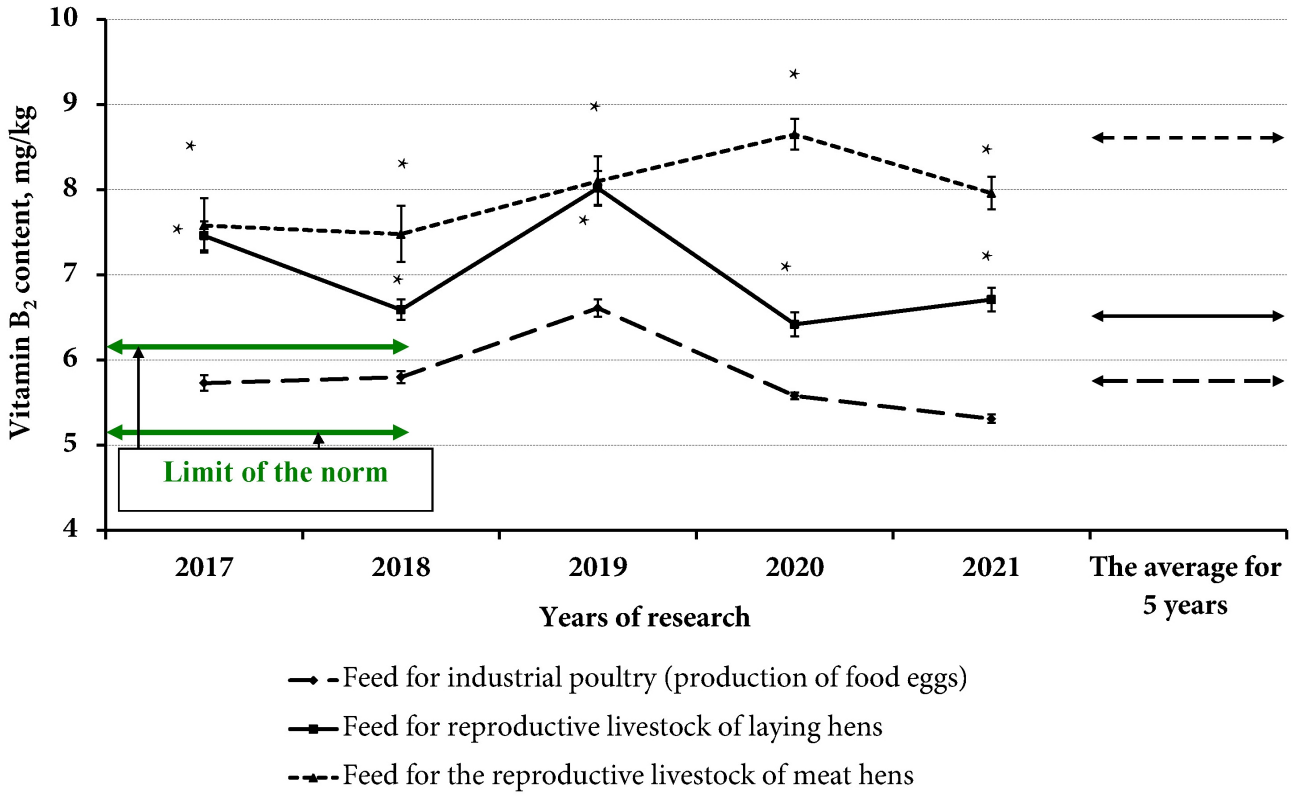

Figure 5. The content of vitamin B_2 in compound feed for chickens of different productivity directions in the dynamics of 2017–2021 (M ± m, n = 531, * — $p < 0.05$ relative to the indicator of feed for commercial chickens)

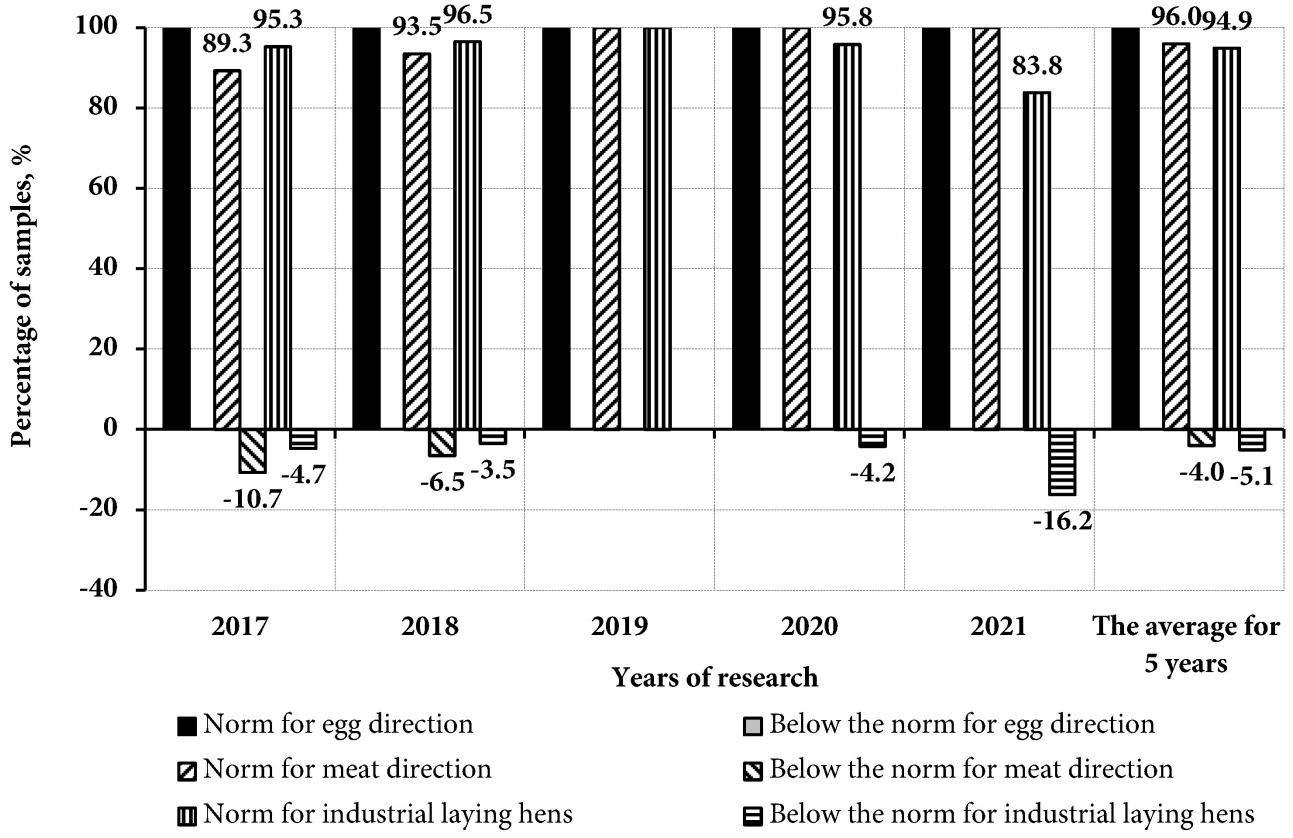

Figure 6. The ratio of vitamin B_2 norm and deficiency in feed for chickens of different areas of productivity in the dynamics of 2017–2021 (M ± m, n = 531)

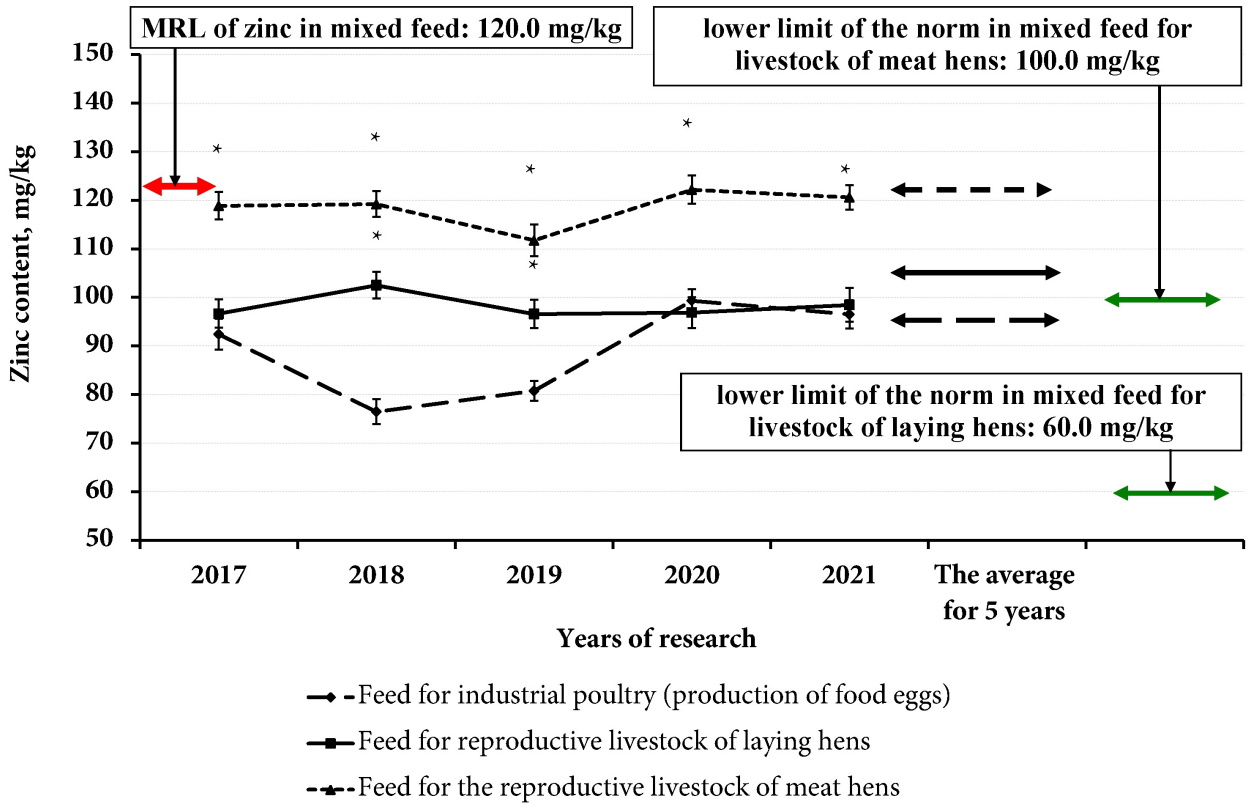

Figure 7. Zinc content in compound feeds for chickens of different directions of productivity in the dynamics of 2017–2021 (M ± m, n = 725, * — p <0.05 — relative to the rate of compound feeds for commercial chickens)

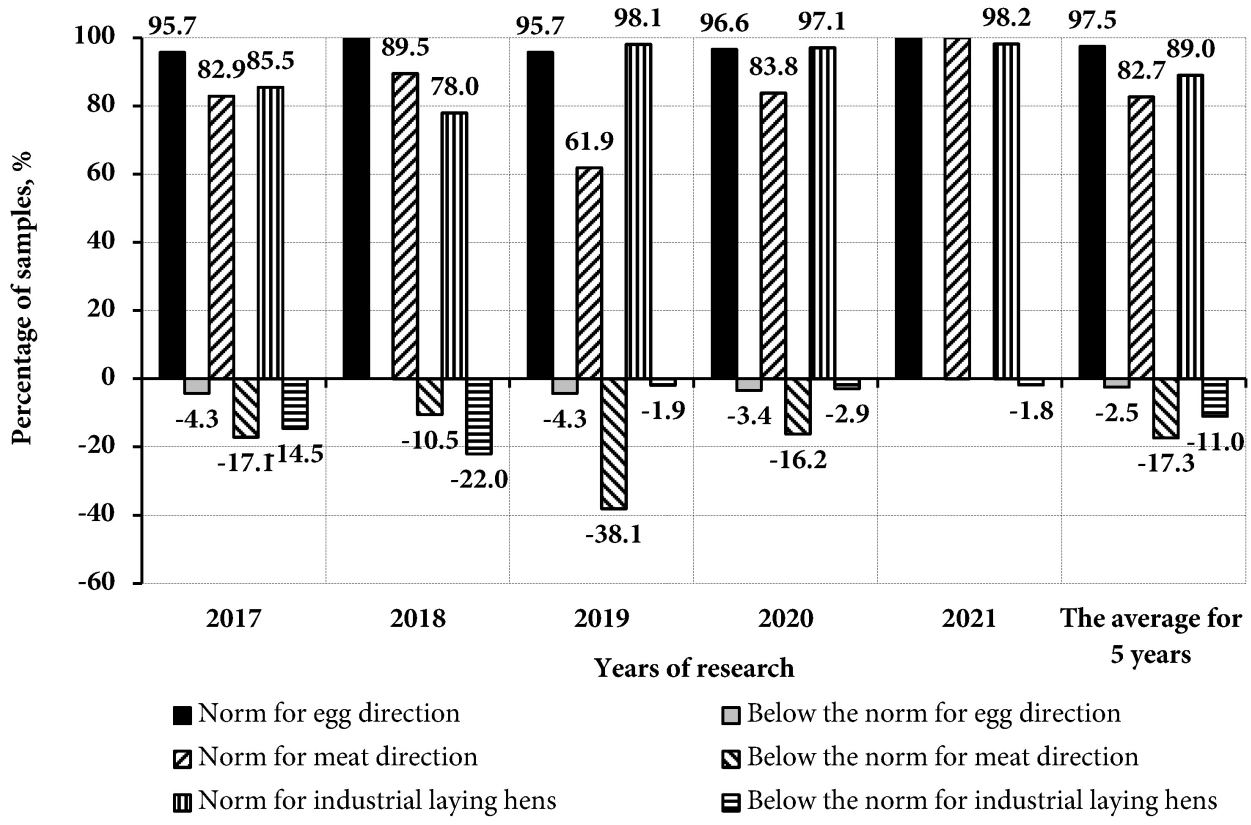

Figure 8. The ratio of norm and zinc deficiency in compound feed for chickens of different directions of productivity in the dynamics of 2017–2021 (M ± m, n = 725)

In compound feeds for the reproductive population of meat chickens the largest number of samples with low zinc content was detected: in 2017 — 17.1% (norm — 82.9%), in 2018 — 10.5% (norm — 89.5%), in 2019 — 38.1% (norm — 61.9%), in 2020 — 16.2% (norm — 83.8%), and in 2021 — 100.0% of samples corresponded to the norm, which was reflected in the average for 5 years: 17.3% of samples were below the norm.

Samples with reduced zinc content were also found in compound feeds for laying hens: in 2017 — 14.5% (norm — 85.5%), in 2018 — 22.0% (norm — 78.0%), in 2019 — 1.9% (norm — 98.1%), in 2020 — 2.9% (norm — 97.1%), and in 2021 — 100.0% of samples corresponded to the norm, that was reflected in the average for 5 years: 11.0% of samples were below the norm (Fig. 8).

713 samples of compound feeds were investigated for the content of copper in the dynamics of years (for commercial laying hens — 288 samples, for reproductive egg hens — 242 samples, for reproductive meat hens — 183 samples): the average content was respectively 10.49 ± 0.25 mg/kg (4.37–26.68 mg/kg), 17.39 ± 0.31 mg/kg (8.03–28.29 mg/kg), and 20.28 ± 0.31 mg/kg (11.53–29.87 mg/kg) (Fig. 9).

The content of copper in all terms of the research was reliably higher ($p < 0.05$) in compound feeds for reproductive meat chickens relative to the rate of feed for commercial poultry: in 2017 — by 98.7%, in 2018 — 2 times, in 2019 — by 39.9%, in 2020 — 2.4 times, in 2021 — 2.4 times, and the excess of the overall figure for 5 years was 93.3%.

A similar pattern was observed in compound feeds for reproductive population of egg hens relative to the rate of compound feeds for commercial poultry ($p < 0.05$): in 2017, the excess of copper content was 65.8%, in 2018 — 87.3%, in 2019 — 36.1%, in 2020 — 62.5%, in 2021 — 91.7%, and exceeding the overall figure for 5 years was 65.8%. (Fig. 9).

It should be noted that the average content of copper in compound feeds for commercial laying hens and reproductive chickens of both directions of productivity was within the norm (approximate indicator, according to the instructions on the operation of poultry crosses) (not less than 5.0 and not less than 16.0 mg/kg, respectively) (Fig. 9).

However, in addition to the lack of copper in compound feeds, it was found that its MAL was exceeded (25.0 mg/kg) (MAPFU, 2012) during the study period: in commercial chickens — 1.4% of samples, in reproductive egg chickens — 8.3% of samples, and in reproductive population of meat chickens — 16.9% of samples.

In percentage, the dynamics of the norm and lack of copper in compound feed for chickens of different directions of productivity is shown in Fig. 10.

Thus, the highest percentage of samples with reduced copper content was observed in compound feeds for reproductive meat chickens in the entire period of research: in 2017 — 35.9% (norm — 64.1%), in 2018 — 25.0% (norm — 75.0%), in 2019 — 19.5% (norm — 80.5%), while in 2020 and 2021 — 100.0% of samples were within the norm, that is reflected in the average for 5 years: 18.0% of samples were lower than norm.

No samples with reduced copper content were detected in the feed for reproductive population of egg hens. A small percentage of samples with reduced copper content was found in compound feeds for commercial laying hens: in 2017, 2018, and 2021 — 100.0% of samples were within the norm, in 2019 — 1.7% (norm — 98.3%) and in 2020 — 1.8% (norm — 98.2%), which was reflected in the average for 5 years: 1.4% of samples were below the norm (Fig. 10).

588 samples of compound feeds were studied for the content of selenium over the years (for commercial laying hens — 233 samples, for reproductive population of egg hens — 191 samples, and for reproductive meat hens — 164 samples): the average content was respectively 0.21 ± 0.005 mg/kg (0.11–0.49 mg/kg), 0.24 ± 0.004 mg/kg (0.12–0.36 mg/kg), and 0.34 ± 0.01 mg/kg (0.17–0.48 mg/kg) (Fig. 11).

The content of selenium in all terms of research was reliably higher ($p < 0.05$) in feed for reproductive population of meat chickens relative to the rate of feed for commercial poultry: in 2017 — by 94.4%, in 2018 — by 77.8%, in 2019 — by 42.3%, in 2020 — by 40.9%, in 2021 — by 70.0%, and exceeding the overall figure for 5 years was 61.9%.

A slightly different picture was observed in compound feeds for reproductive population of egg-laying hens in relation to the indicator of compound feeds for commercial poultry ($p < 0.05$): in 2017, the excess of selenium content was 50.0%, in 2018 — 33.3%, in 2020 — 13.6%, while in 2019 — the figure was reliably lower than the commercial by 23.1%, and in 2021 the excess was not reliable. Exceeding the overall figure for 5 years was 14.3%. (Fig. 11).

It should be noted that the average content of selenium in feed for commercial laying hens and hens of reproductive population in both areas of productivity was within the norm (approximate indicator, according to the instructions on the operation of poultry crosses) (not less than 0.2 and not less than 0.3 mg/kg, respectively) (Fig. 11) while exceeding MAL (0.5 mg/kg) (MAPFU, 2012) during the study period was not detected.

In percentage, the dynamics of the norm and the lack of selenium in feed for chickens of different directions of productivity is shown in Fig. 12.

Thus, the following percentage of samples with reduced selenium content was observed in compound feeds for reproductive population of meat chickens during the entire study period: in 2017 — 23.7% (norm — 76.3%), in 2018 — 45.0% (norm — 55.0%), in 2019 — 10.8% (norm — 89.2%), in 2020 — 9.7% (norm — 90.3%), and in 2021 — 27.8% (norm 72.2%), which was reflected in the average for 5 years: 26.8% of samples were below the norm.

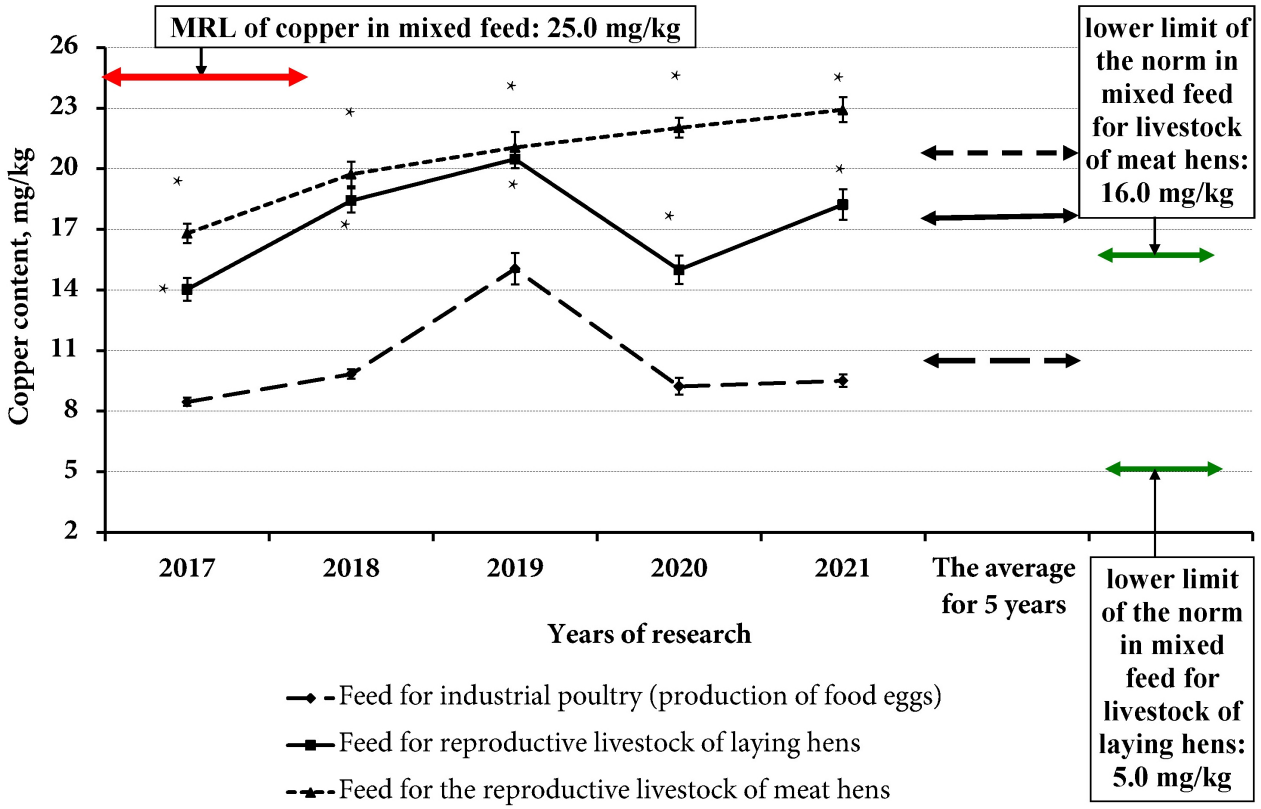

Figure 9. Copper content in compound feeds for chickens of different directions of productivity in the dynamics of 2017–2021 (M ± m, n = 713, * — $p < 0.05$ relative to the rate of compound feeds for commercial chickens)

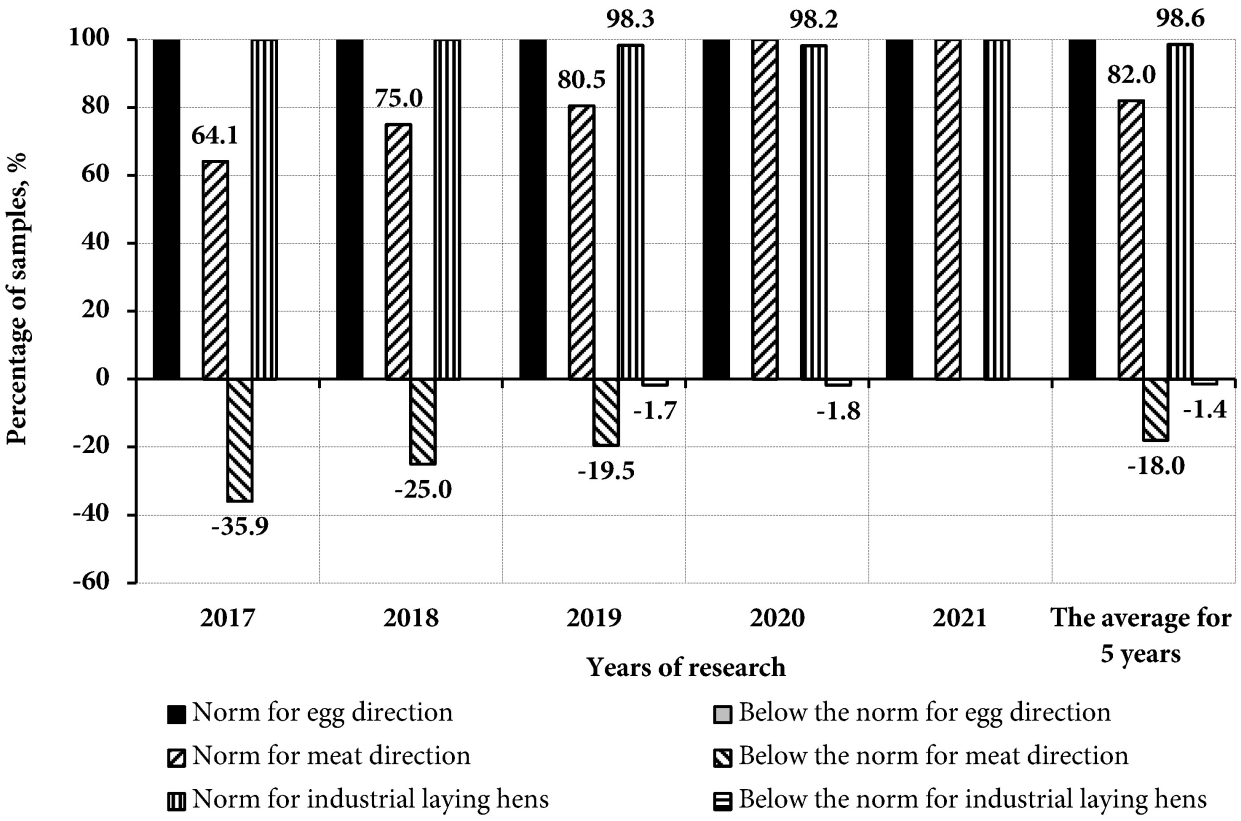

Figure 10. The ratio of the norm and lack of copper in the feed of chickens of different directions of productivity in the dynamics of 2017–2021 (M ± m, n = 713)

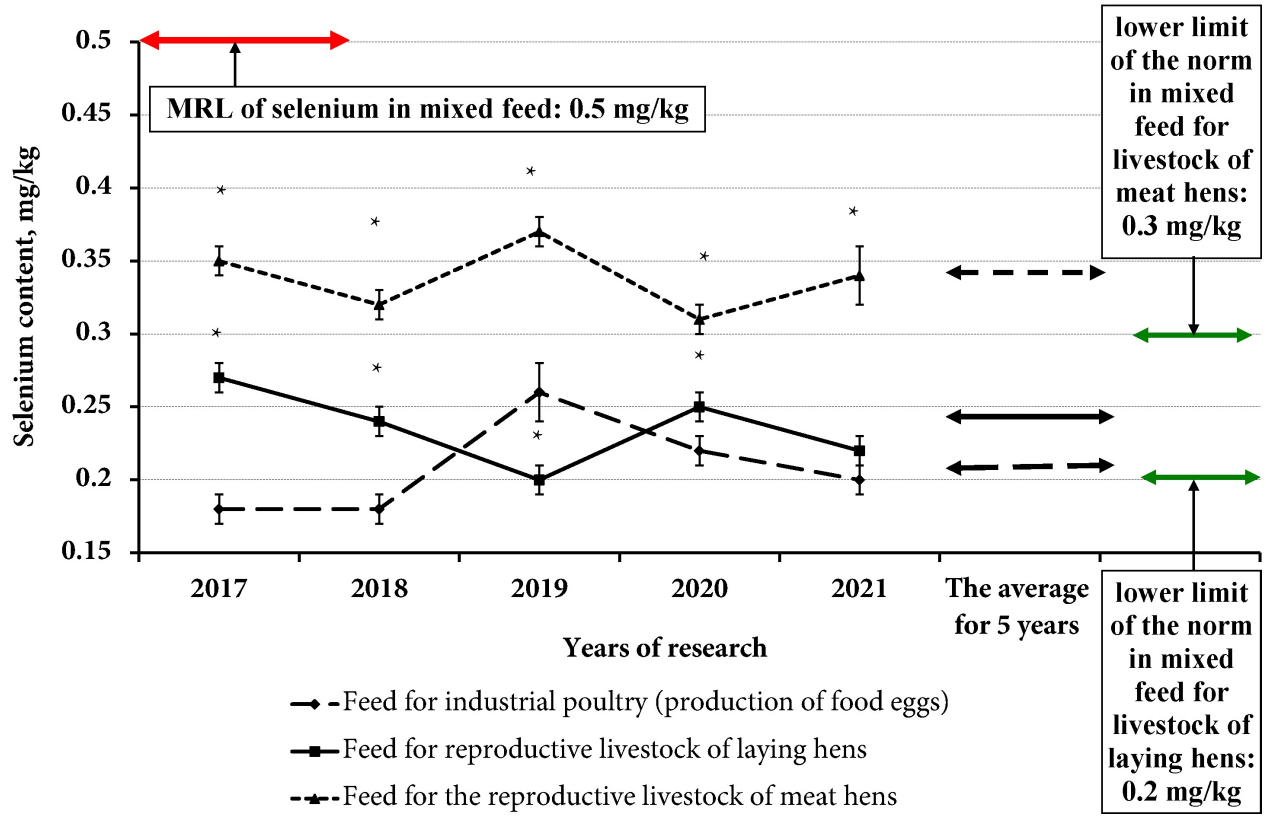

Figure 11. Selenium content in compound feeds for chickens of different directions of productivity in the dynamics of 2017–2021 (M ± m, n = 588, * — $p < 0.05$ relative to the rate of compound feeds for commercial chickens)

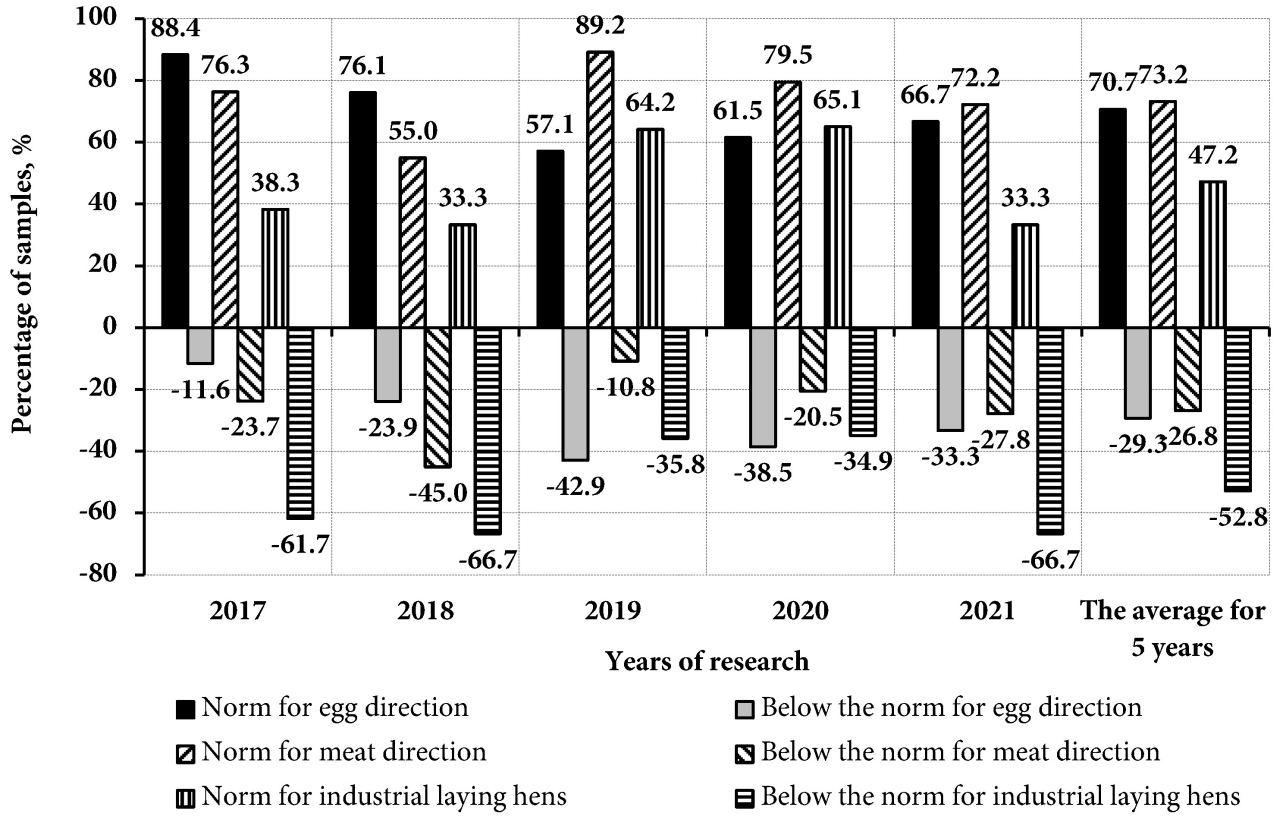

Figure 12. The ratio of the norm and the lack of selenium in the compound feed for chickens of different directions of productivity in the dynamics of 2017–2021 (M ± m, n = 588)

Samples with reduced selenium content were also detected in compound feeds for reproductive population of egg hens: in 2017 — 11.6% (norm — 88.4%), in 2018 — 23.9% (norm — 76.1%), in 2019 — 42.9% (norm — 57.1%), in 2020 — 38.5% (norm — 61.5%), and in 2021 — 33.3% (norm — 66.7%), that was reflected in the average for 5 years: 29.3% of samples were below the norm.

The following percentage of samples with reduced selenium content was found in compound feeds for commercial laying hens: in 2017 — 61.7% (norm — 38.3%), in 2018 — 66.7% (norm — 33.3%), in 2019 — 35.8% (norm — 64.2%), in 2020 — 34.9% (norm — 65.1%), and in 2021 — 66.7% (norm — 33.3%), that was reflected in the average for 5 years: 52.8% of samples were below the norm (Fig. 12).

Therefore, based on the monitoring of feed for chickens, the following can be stated: for the feed for reproductive population of meat chickens there are disproportions in the content of vitamin E and selenium in fee; in feed for reproductive population of egg chickens — disproportions in the content of vitamin E, zinc and selenium; and in compound feeds for commercial chickens — a pronounced deficiency of vitamin E, zinc and selenium.

Vitamin A content did not show significant deviations from the norm in compound feeds for commercial chickens and reproductive population of meat chickens, while in compound feeds for reproductive population of egg hens 5.7% of samples with reduced vitamin A content were found. According to Karkach and Mashkin (2020) lack of vitamin A in feed leads to the hatching of non-viable or weak young chickens, embryos deficient in this vitamin showed signs of abnormal circulatory system, had poor plumage, swollen joints, beak abnormalities and dwarfism.

According to the content of vitamin E in compound feeds for commercial chickens, the percentage of samples with reduced content was 75.2%, in compound feeds for reproductive population of egg hens — 63.9%, and in compound feeds for reproductive livestock of meat hens — 12.7%.

Vitamin E deficiency in birds is manifested in increased embryonic mortality due to a decrease in the content of α-tocopherol in eggs. There is a direct relationship between the content of vitamin E in the diets of breeding birds and the hatchability and viability of broiler chickens.

Exclusion of vitamin E supplements from the diet of laying hens, ducks and quails leads to the rapid disappearance of its reserves in the yolk and significantly reduces the hatchability of chickens. In the process of incubation of eggs obtained from birds with vitamin E deficiency in the diet, the growth and development of embryos slows down and they die in the first 2-4 days of incubation. Chickens obtained from hens with vitamin E deficiency can not independently break the shell during hatching, they have pathology of the cardiovascular system (Kutsan and Orobchenko, 2009).

By the content of vitamin B_2 in compound feeds for hens of reproductive population of egg chickens throughout the study period samples with low content of vitamin B_2 were not observed, while in feed for reproductive population of meat chickens the decreased in vitamin B_2 content was detected in 4.0% of samples, and in compound feeds for commercial laying hens in 5.1%. According to Zon (2013), a decrease in the amount of riboflavin in feed, or its poor absorption, provokes toxicosis, causes the excretion of the amino acids of tryptophan, histidine and phenylalanine in unchanged form. Vitamin B_2 deficiency has the most negative effect on the nervous system, which inhibits the intensity of tissue respiration in the body of birds. Lack of vitamin B_2 in the diet of laying hens negatively affects the quality of hatching eggs, causes increased embryonic death, degrades the quality of hatched young.

Zinc average content in compound feeds for commercial laying hens and reproductive population of egg and meat chickens was within the norm, but a reduced level of zinc was observed in 11.0, 2.5, and 17.3% of samples, and above the MAL — in 11.1, 16.9, and 47.1% of the samples, respectively. Zinc deficiency in poultry is characterized by loss of appetite, growth retardation, impaired feather growth, pigmentation and replacement. In addition, egg fertility reduces, dermatoses, chondrodystrophy occur, all long bones of pelvic extremities are shortened. In embryos, there is curvature of the spine, head injuries, edema, abnormalities in the development of eyes, internal organs (Medvid et al., 2017). Excess zinc causes growth retardation and inhibits the reproductive functions of birds. In addition, zinc is an antagonist of copper, so high doses of zinc lead to a decrease in absorption and assimilation of copper, accompanied by functional and morphological abnormalities of the central nervous system, digestive organs, liver, kidneys (Kutsan and Orobchenko, 2011).

According to the content of copper, its average indicator in compound feeds for commercial laying hens and reproductive population of egg and meat hens was within the norm, but the reduced level of the element was registered in 1.4, 0.0 and 18.0% of samples, and above the MAL — in 1.4, 8.3 and 16.9% of samples, respectively. Copper deficiency leads to the development of anemia in chickens, a decrease in egg weight, deformation of the shell or even its absence (Berwanger et al., 2018). Growth depression, muscular dystrophy, and lesions of the cuticle of the muscular stomach, and liver dystrophy have been observed with long-term intake of high doses of copper in poultry (Kutsan and Orobchenko, 2011).

The average content of selenium in compound feeds for commercial laying hens and reproductive population of meat and egg chickens was within the norm, but the reduced level of the element was registered in 52.8, 29.3, and 26.8% of samples, and no exceeding of the maximum allowable level were detected. The most common diseases of the poultry caused by selenium

deficiency are myopathy, exudative diathesis and encephalomalacia in chickens, arthritis, enteritis, renal autolysis, hemolysis of erythrocytes, decreased visual acuity, etc. Excess selenium in chickens is manifested by a decrease in egg production, growth inhibition, weight loss, deteriorating hatching of chickens, embryonic abnormalities, fragile feathers. In addition, muscle and skin edema and macrocytic anemia develop (Orobchenko, 2011).

Conclusions. 1. 678 samples of compound feeds (for commercial laying hens — 281 samples, for reproductive flocks of egg hens — 228 samples, and for reproductive flocks of meat chickens — 169 samples) have been studied for the content of vitamin A by years: the average content was 11,510.59 ± 62.88 IU/kg (10,009.98–14,238.5 IU/kg), 14,749.57 ± 175.75 IU/kg (7,912.14–17,622.87 IU/kg), and 18,240.04 ± 36.94 IU/kg (17,366.59–19,631.89 IU/kg). The content of vitamin A in all terms of research was reliably higher ($p < 0,05$) in compound feeds for reproductive flocks of meat chickens relative to the indicators of feed for commercial poultry by 58.5%, and in compound feeds for reproductive flocks of egg hens — by 28.1%. In feed for commercial chickens and for reproductive flocks of meat chickens during the whole period of research no samples with reduced content of vitamin A were observed, while in compound feeds for reproductive flocks of egg hens samples with low content of vitamin A were detected in 5.7% of samples.

2. 305 samples of compound feeds were tested for the content of vitamin E by years (for commercial laying hens — 129 samples, for reproductive flocks of egg hens — 97 samples, and for reproductive flocks of meat chickens — 79 samples): the average content was 119.16 ± 2.77 mg/kg (62.72–181.03 mg/kg), 165.10 ± 4.68 mg/kg (75.32–247.76 mg/kg), and 219.93 ± 2.02 mg/kg (180.6–267.02 mg/kg). The content of vitamin E in all periods of research was reliably higher ($p < 0,05$) in compound feeds for reproductive flocks of meat chickens relative to the indicators of feed for commercial poultry by 84.6%, and in compound feeds for reproductive flocks of egg hens — by 38.6%. In compound feeds for commercial chickens, the percentage of samples with low vitamin E content was 75.2%, in compound feeds for reproductive flocks of egg hens — 63.9%, and in compound feeds for reproductive flocks of meat hens — 12.7%.

3. 531 samples were tested for vitamin B_2 content by years (for commercial laying hens — 237 samples, for reproductive flocks of egg hens — 168 samples, and for reproductive flocks of meat chickens — 126 samples): the average content was 5.84 ± 0.04 mg/kg (4.79–7.78 mg/kg), 7.06 ± 0.08 mg/kg (5.14–9.78 mg/kg), and 7.92 ± 0,13 mg/kg (4.60–10.61 mg/kg). The content of vitamin B_2 at all periods of the study was reliably higher ($p < 0.05$) in compound feeds for reproductive flocks of meat chickens relative to feed for commercial poultry by 35.6%, and in compound feeds for reproductive flocks of egg hens — by 20.9%. In compound feeds for reproductive flocks of egg hens during the whole period of research no samples with reduced content of vitamin B_2 were observed, while in compound feeds for reproductive flocks of meat hens reduction of vitamin B_2 content was detected in 4.0% of samples, and in compound feeds for commercial laying hens in 5.1%.

4. 725 samples of compound feeds were studied for the content of zinc by years (for commercial laying hens — 298 samples, for reproductive flocks of egg hens — 236 samples, and for reproductive flocks of meat chickens — 191 samples): the average content was 89.54 ± 1.29 mg/kg (50.49–131.60 mg/kg), 98.06 ± 1.38 mg/kg (52.44–135.88 mg/kg), and 118.32 ± 1.31 mg/kg (80.09–147.62 mg/kg). The content of zinc in all periods of research was reliably higher ($p < 0.05$) in compound feeds for reproductive flocks of meat chickens relative to the indicators of feed for commercial poultry by an average of 32.1%, in compound feeds for reproductive flocks of egg hens — by 9.5%. The average zinc content in compound feeds for commercial laying hens and reproductive flocks of both meat and egg chickens was within the norm, but a reduced level of zinc was observed in 11.0, 2.5, and 17.3% of samples, and above the MAL — in 11.1, 16.9, and 47.1% of the samples, respectively.

5. 713 samples of compound feeds were investigated for the content of copper by years (for commercial laying hens — 288 samples, for reproductive flocks of egg hens — 242 samples, and for reproductive flocks of meat chickens — 183 samples): the average content was respectively 10.49 ± 0.25 mg/kg (4.37–26.68 mg/kg), 17.39 ± 0.31 mg/kg (8.03–28.29 mg/kg), and 20.28 ± 0.31 mg/kg (11.53–29.87 mg/kg). Copper content at all research periods was reliably higher ($p < 0.05$) in compound feeds for reproductive flocks of meat chickens relative to the indicators of feed for commercial poultry on average by 93.3%, and in compound feeds for reproductive flocks of egg hens — by 65,8%. The average content of copper in compound feeds for commercial laying hens and for reproductive flocks of both egg and meat chickens was within the norm, but the reduced level of the element was registered in 1.4, 0.0, and 18.0% of samples, and above the MAL — in 1.4, 8.3, and 16.9% of samples, respectively.

6. 588 samples of compound feeds were studied for the content of selenium by years (for commercial laying hens — 233 samples, for reproductive flocks of egg hens — 191 samples, and for reproductive flocks of meat chickens — 164 samples): the average content was 0.21 ± 0.005 mg/kg (0.11–0.49 mg/kg), 0.24 ± 0.004 mg/kg (0.12–0.36 mg/kg), and 0.34 ± 0.01 mg/kg (0.17–0.48 mg/kg), respectively. The content of selenium at all research periods was reliably higher ($p < 0.05$) in compound feeds for reproductive flocks of meat chickens relative to the indicators of feed for commercial poultry by 61.9%, and in compound feeds for reproductive flocks of egg hens — by 14.3%. The average content of selenium in compound feeds for commercial laying hens and reproductive flocks of both egg and meat chickens was within the norm, but the reduced level of the element was registered in 52.8, 26.8, and 29.3% of samples, and no exceedances of the maximum allowable level were detected.

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RELEVANCE OF FOOD BACTERIAL ZOONOSES IN THE DNIPROPETROVSK REGION (UKRAINE) DURING 2020

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Summary. When the world is facing an unprecedented COVID-19 pandemic, the importance of animal disease surveillance has become apparent. A significant proportion of new and existing human diseases are zoonoses. Microbiological researches remain relevant today. They guarantee the quality of food raw materials and products, as well as identify sources of infection. The aim of our work was to study the spread of foodborne bacterial zoonoses in the Dnipropetrovsk region during 2020. The study used the methods provided by DSTU ISO 6887-2:2005, DSTU ISO 4833:2006, DSTU ISO 4832:2015, DSTU EN 12824:2004, DSTU ISO 11290-1:2003, DSTU 7444:2013, DSTU ISO 6888-2:2003, DSTU ISO 7937:2006. As a result of the study of the epidemic situation regarding outbreaks of acute intestinal infectious diseases and food poisoning in 2020 in Ukraine, 52 cases were investigated, including cases in which children have been affected (43.8%). In the etiological structure, the largest share of outbreaks caused by salmonella (34.6%) and outbreaks of viral etiology (25%) was observed. The registration of outbreaks of human salmonellosis in 82% of regions in Ukraine proves that geographical conditionality, climatic conditions, species structure of farm animals and the level of socio-economic status of the population are important factors in the development of the disease. The presence of positive for animal salmonellosis localities also demonstrates the constant spread of this zoonosis in Ukraine, which causes contamination by these bacteria of food products of animal origin and environmental objects. The dominance of the Dnipropetrovsk Region in 2020 in the number of outbreaks of acute intestinal infectious diseases contributed to a detailed investigation of bacteriological studies of raw materials of animal origin and food products, which revealed 2.56% of non-compliant samples, where the highest percentage of violations of safety criteria was poultry products. Thus, high-risk products included semi-finished products and culinary products from meat, in particular poultry; minced meat and mechanically rolled meat, as well as ready-to-eat meat products — sausages, namely poultry, and eggs. Poultry products of seven types (n = 3,226) were analyzed for the presence of sanitary-indicative microflora (coliform bacteria, mesophilic aerobic and facultative-anaerobic microorganisms), opportunistic microflora (coagulase-positive staphylococci, *Proteus*, sulfite-reducing clostridia), and pathogenic microflora (bacteria of the genera *Salmonella, Listeria).* The percentage of samples that did not meet the requirements of regulatory documentation by indicators was: mesophilic aerobic and facultative anaerobic microorganisms up to 10.92%; coliform bacteria — up to 3.90%. Pathogenic contaminants of poultry products of domestic Ukrainian production were: *Salmonella* Enteritidis (up to 4.20%), *Listeria monocytogenes* — 12.50%. The paper shows problematic in 2020 nosological forms in the structure of pathogens of food bacterial zoonoses and identifies their potential sources. Microbiological studies have shown that the contaminants of poultry products of domestic Ukrainian production in the Dnipropetrovsk Region were sanitary-indicative (coliform bacteria, mesophilic aerobic and facultative-anaerobic microorganisms), opportunistic pathogens (*Staphylococcus aureus*, sulfite-reducing clostridia), and pathogenic microflora (bacteria of the genera *Salmonella, Listeria).* The author has monitored the geographical serotypic predisposition of *Salmonella*, which is due to the prevalence of O-group D in the Dnipropetrovsk Region, and the possibility of their passage through the food chain. It was found that minced meat and meat of mechanical deboning from poultry is one of the factors of transmission of a potentially pathogenic for humans and animals species of *Listeria — L. monocytogenes* in the region

Keywords: poultry products, *Salmonella* Enteritidis, *Listeria monocytogenes*, meat of mechanical deboning

Introduction. At a time when the world is facing an unprecedented pandemic COVID-19, the importance of animal disease surveillance has become apparent. Zoonoses have been widespread since the Bible (Will, 1994). Thus, in Ukraine every year more than 50 nosological forms of infectious diseases are registered among the population (Markovych and Grynevych, 2013).

According to scientists, at least 60% of known human infectious diseases can be transmitted from animals, and 75% of new or emerging human diseases are derived from animals (CDC, 2021). Quite a significant part of the most dangerous zoonotic diseases account for emergent food zoonoses (Grynevych, Markovych and Markovych, 2012) Sources of pathogens of most food poisoning in humans: salmonella, *Escherichia* coli, yersinia, listeria, campylobacter, are farm animals and animal products (Trykhlib, 2018). Among all the acute intestinal infections that occur due to eating poor-quality food, salmonellosis occupies the leading place.

To date, due to the widespread of this disease, it is just a matter of reducing the incidence and containment of pathogen spread among the main sources of this infection (Zarytsky, Hlushkevych and Bubalo, 2016). In recent years, *Salmonella* Enteritidis occupies the leading place in the etiology of salmonellosis.

The main source of alimentary infection is poultry products (Afshari et al., 2018). In addition, currently food products produced by catering enterprises of individual ownership can be added to the above

categories of products. Microbiological monitoring serves as an information base of modern epidemiology and allows to influence the effectiveness of epidemiological surveillance (Druzhaeva, 2014).

In the late twentieth century, listeriosis re-emerged as a foodborne infection, leading to a significant number of outbreaks associated with human consumption of pasteurized milk, cheese, ice cream, meat, vegetables, and other food products (Mengesha et al., 2009).

Listeria is widespread in nature, causing diseases with polymorphism of clinical manifestations and high mortality. Listeriosis also causes significant economic damage to agriculture by morbidity and high animal deaths. The work of recent decades shows that *L. monocytogenes* undergoes adaptive changes.

This occurs mainly under the influence of anthropogenic factors (use of preservatives, uncontrolled use of antibiotics and disinfectants, etc.), which change the biological properties of bacteria, most often it is the emergence of resistance to certain antibacterial drugs, the ability to form biofilms, and the appearance of low virulent mutants (Vovk, 2009).

According to the Public Health Center of the Ministry of Health of Ukraine, from 2007 to 2017, 22 cases of listeriosis have been registered in Ukraine, both in adults and children (PHCU, 2021). Of particular concern is listeriosis in pregnant women, which leads to severe pathology of the fetus and newborns and has mortality rates of 10–12% or more (Tartakovski, 2000).

The lack of an effective system of sanitary and epidemiological surveillance for listeriosis and the unsatisfactory quality of laboratory diagnostics have led to a kind of vacuum between the real role of listeria in human infectious pathology and practical research in clinical microbiology. Not all cases of human listeriosis are detected and registered.

However, we can shortly predict an increase in listeriosis due to the high adaptive properties of listeria, the ability to reproduce in an abiotic environment, increasing the percentage of people with immunodeficiency, the predominance of the food route of infection (Tartakovski, Maleev and Ermolaeva, 2002). Now a new stage in the study of this disease has begun, associated with the widespread introduction into modern medical practice of up to date laboratory methods of specific diagnosis (Yushchuk and Vengerov, 2021).

Thus, microbiological research remains relevant today, because they guarantee the quality of food raw materials and products, as well as reveal sources of infection.

The **aim of our work** was to study the spread of foodborne bacterial zoonoses in the Dnipropetrovsk region during 2020.

Materials and methods. The material for the study was data from annual reports and data from the State Service of Ukraine on Food Safety and Consumer Protection. The compliance of microbiological indicators with the requirements of normative documents in seven types of poultry products in the Dnipropetrovsk Region was determined. A total of 3,226 samples were studied in real-time (Table 1), of which 19.5% were export products, 78.6% — domestic Ukrainian production, 1.27% — state control, and 0.62% — imports.

Table 1 — List of samples of studied poultry food products in the Dnipropetrovsk Region during 2020 (n = 3,226)

Type of poultry products	Total number, samples
Semi-finished and culinary products from poultry meat	1,048
Sausages from poultry	615
Poultry meat	761
Eggs	284
Minced meat and mechanically deboned poultry meat (MDPM)	119
Egg products	344
Poultry by-products	55

Preparation of the tested samples was performed in accordance to DSTU ISO 6887-2: 2005 (DSSU, 2005a).

The methods provided by DSTU ISO 4833:2006 (DSSU, 2008), DSTU ISO 4832:2015 (SE 'UkrNDNC', 2018), DSTU EN 12824:2004 (DSSU, 2005d), DSTU ISO 11290-1:2003 (DSSU, 2005c), DSTU 7444:2013 (MEDTU, 2014), DSTU ISO 6888-2:2003 (DSSU, 2005b), DSTU ISO 7937:2006 (DSSU, 2009) were used in the work.

Results. Analysis of the epidemic situation in Ukraine regarding outbreaks of acute intestinal infectious diseases and food poisoning in 2020, according to the annual report of the State Service of Ukraine on Food Safety and Consumer Protection, shows an investigation of 52 cases with 646 victims, including 283 children (43.8%). The decrease in the number of outbreaks and casualties by almost four times compared to 2019, when 204 outbreaks were recorded, was due to anti-epidemic measures to prevent the spread of acute respiratory disease COVID-19 in Ukraine. Outbreaks were registered in almost all regions of Ukraine, except for Zhytomyr, Luhansk, Khmelnytsky, and Chernihiv regions.

Most outbreaks (n = 6) were registered in the Dnipropetrovsk Region. In the etiological structure, the largest share of outbreaks caused by salmonella was observed — 18 (34.6%) against 74 (36.3%) in 2019 and outbreaks of viral etiology (rota-, adeno-, noroviruses) — 13 (25%) against 42 (20.6%) in 2019. Thus, the monitoring of foodborne infections in Ukraine during 2019–2020 shows that 35.4% of outbreaks were caused by salmonella, which is quite dangerous due to the ease of transmission of the pathogen. The structure of salmonella allows them to experience environmental conditions and continue their life cycle in water and soil (WHO, 2018).

Thus, the registration of outbreaks of human salmonella infection in 82% of regions of Ukraine proves that important factors in the development of the disease are geographical conditionality, climatic conditions, species structure of farm animals and the level of socio-economic status of the population.

To assess the prevalence of salmonellosis in Ukraine, statistical data were analyzed (Fig. 1).

Thus, the annual presence of positive on salmonellosis settlements demonstrates the constant spread of this zoonosis in Ukraine, which causes contamination by these bacteria of food products of animal origin and the environment, and proves not only veterinary but also medical, environmental, and social problems of this infection.

Since the Dnipropetrovsk Region was dominated by the number of acute intestinal infectious diseases outbreaks, a detailed study of quantitative data in this region was conducted. Thus, the analysis of the results of bacteriological studies of raw materials of animal origin and food products in the Dnipropetrovsk Region shows that during 2020, 136 samples with discrepancies were detected, which was 2.56% of the total number of samples (n = 5,320).

Thus, according to the results of microbiological studies, poultry products had the highest percentage of violations of safety criteria (Fig. 2).

It has been established that the number of high-risk products includes semi-finished products and culinary products made of meat, in particular poultry; minced meat and MDPM, as well as ready-to-eat meat products (those that have undergone heat treatment) — sausages, including poultry, and eggs (Table 2).

The results of samples culturing showed that MA&OAMO exceeded the allowable norms in 1.14% of samples from semi-finished and culinary products made of poultry meat; in 0.90% — in sausages; in 10.92% — in minced meat and MDM from poultry.

Coliform bacteria were found in samples from semi-finished products — 3.90%; in sausages — 2.13%.

Bacteria of the genus Salmonella serological group D were isolated in 4.20% of samples of minced meat and MDM from poultry and 0.47% of samples of eggs, which proves the transmission of the pathogen by food.

The presence of staphylococci in semi-finished products is not normalized, but studies have shown that they are present in 12.82% of samples from semi-finished and culinary products from poultry meat.

Clostridia were found in semi-finished products — in 0.48% of samples.

L. monocytogenes were isolated in 12.5% of minced meat and MDM from poultry, which indicates the presence of listeriosis in the regions where the poultry was raised.

Thus, the main source (85.7%) of salmonellosis infection were poultry products of domestic production (Table 3). Sporadic outbreaks of salmonellosis can be supported by the constant supply of salmonella-contaminated meat products that have not been detected during processing, which is a risk factor for zoonosis.

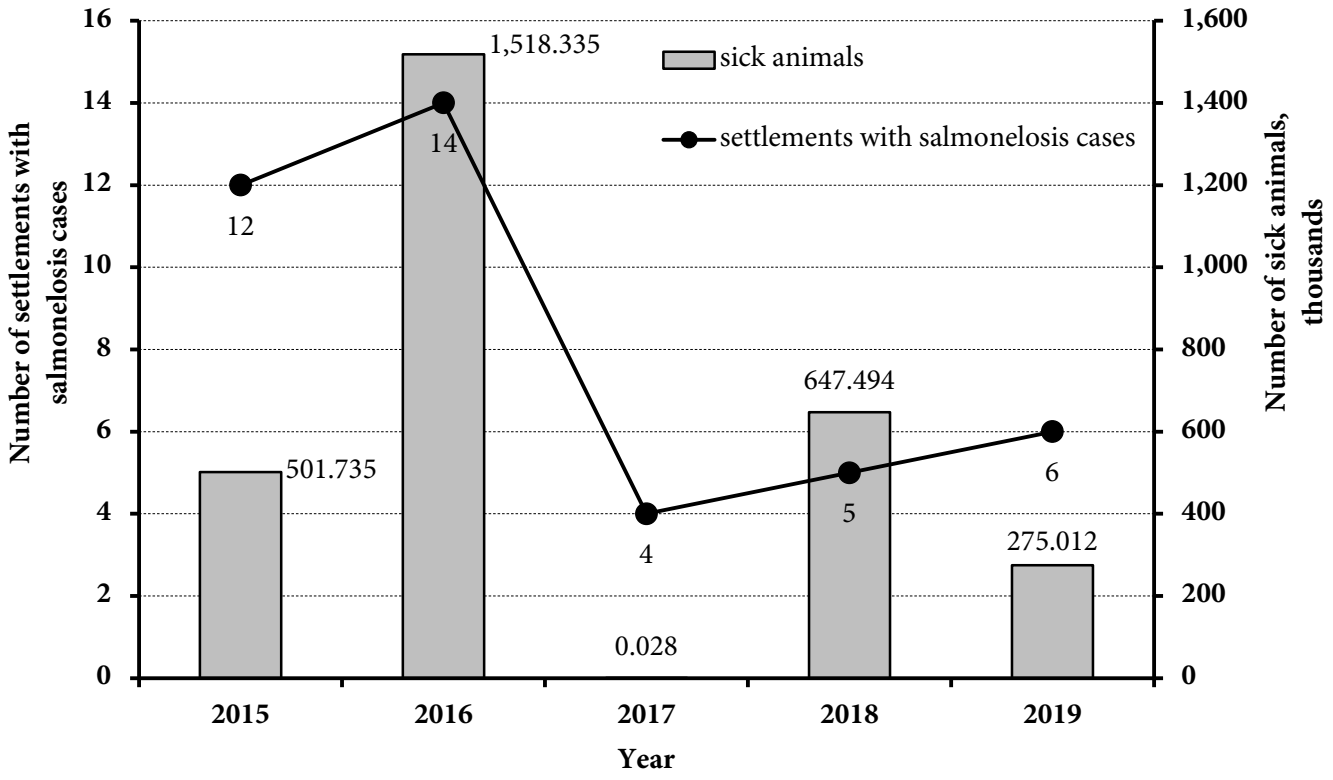
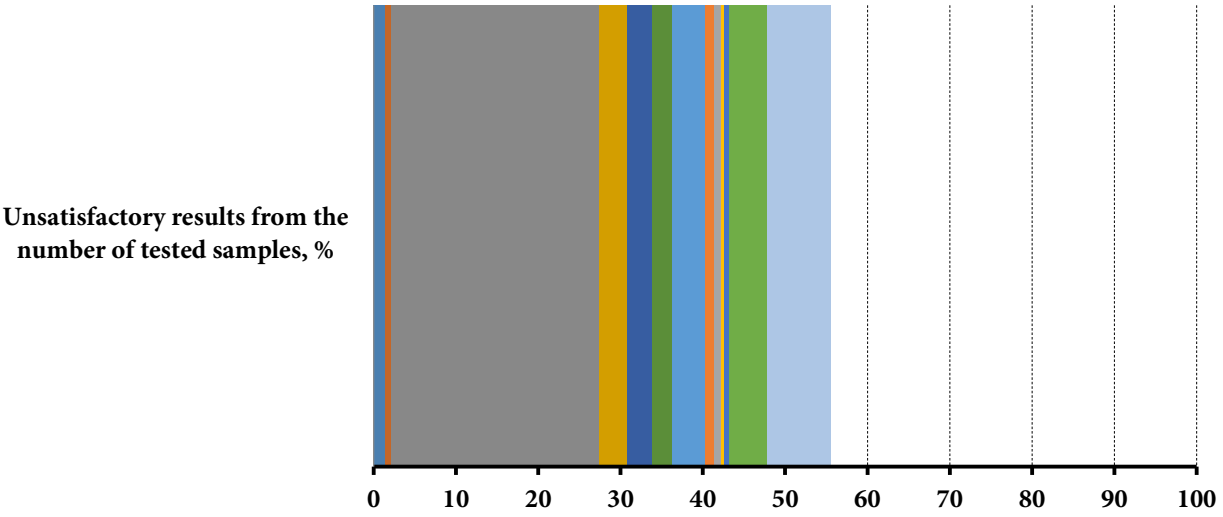


Figure 1. Statistical data on animal salmonellosis in 2015–2019 in Ukraine



	Unsatisfactory results from the number of tested samples, %
pork meat	1.41
beef meat	0.8
poultry minced meat and MDPM	25.21
minced meat and MDM of other types of animal meat	3.4
semi-finished products and culinary products from meat, including poultry	3.05
sausages, including poultry	2.51
semi-finished products and culinary products without meat	3.89
milk and dairy products	1.14
cheese	0.85
eggs	0.35
fresh fisg	0.57
salted, smoked, dried fish, herring	4.68
caviar, mollusks, crustaceans and other seafood	7.69

Figure 2. Standardized histogram of positive results of microbiological studies of food and food raw materials of animal origin in the Dnipropetrovsk Region in 2020

Table 2 — Violation of microbiological indicators in poultry products in the Dnipropetrovsk Region in 2020

Type of poultry products	Groups of dangerous factors, absolute number of samples/percentage of violations					
	MA&OAMO	Bacteria of the *E. coli* group	*Salmonella* spp.	*S. aureus*	Sulfite-reducing clostridia	*L. mono-cytogenes*
Semi-finished and culinary products from poultry meat	961/11 1.14	410/16 3.90	-	39/5 12.82	-	-
Sausages from poultry meat	332/3 0.90	516/11 2.13	-	-	413/2 0.48	-
Minced poultry meat and MDPM	119/13 10.92	-	119/5 4.20	-		96/12 12.50
Chicken eggs	-	-	212/1 0.47	-	-	-

Table 3 — Serological identification of salmonella, which were isolated during microbiological studies of raw materials, food products in the Dnipropetrovsk Region in 2020 (n = 7)

Serological group	Serological variant	Products and their origin		
		Minced meat and MDM from poultry, frozen (domestic), %	Beef meat (domestic), %	Chicken eggs (domestic), %
D	*Salmonella* Enteritidis 0:9, phase (H:g/H:m)	71.4	14.3	14.3

Discussion. Microbiological studies have shown that poultry products in 0.9–10.9% of cases were characterized by high content of QMA&OAMO. The largest number of microorganism-contaminated samples (10.9%) was observed in minced meat and MDM, compared with other types of products, as also reported by other sources (Danylenko et al., 2017). It is known that the nature of microbial contamination is influenced by the physicochemical properties of products. Thus, mechanical processing (making minced meat, etc.) increases the probability of contamination and promotes the homogeneous spread of microorganisms throughout the product (Kovalenko and Zamaziy, 2021).

The analysis of other sanitary-indicative criteria proved the increased number of bacteria of the *E. coli* group in 2.1–3.9% of the studied samples. The presence of an increased amount of *E. coli* bacteria in the finished product indicates unsatisfactory sanitary conditions of processing and storage of the product.

The number of spore-forming sulfite-reducing rods (genus *Clostridium*) is limited because they can multiply intensively in food in the presence of anaerobic conditions and at a concentration of 10^6 or higher in 1 ml/g to cause food poisoning. Their presence in poultry sausages at the level of 0.48% indicates the non-compliance of certain samples with regulatory documentation.

Isolation of *S. aureus* is dangerous due to its ability to produce enterotoxin and cause food poisoning. Increased amounts of coagulase-positive staphylococci in finished products usually indicate secondary contamination of the product: through contact with contaminated equipment, staff hands, or by airborne droplets.

Detection of pathogens *Salmonella* spp. and *L. monocytogenes* in minced meat and MDPM proves that these poultry products are a potential source of human infection. Thus, Zaytseva and Digo (2017) also report the isolation of listeria at the level of 15–80% from poultry meat. Therefore, human infection with listeria in the region is possible through food products at any stage of their receiving, processing, and storage, without the participation of animals in the cycle of transmission. The urgency of the problem of listeriosis is confirmed by reports in 2019. Thus, in Spain, 150 people were injured, and one died; in the Netherlands and Belgium, three people died and one stillborn child as a result of eating meat in which the causative agent of listeriosis was found (European Pravda, 2019a, 2019b).

Regarding the detection of bacteria of the genus *Salmonella*, according to the official data of the RASFF system (Rapid Alert System for Food and Feed) in the EU in the period from 2010 to 2015 in raw materials of animal and plant origin contamination of poultry and poultry meat products dominated (Smajhel and Shadrova, 2018).

As salmonella during mass reproduction in products can cause not only toxicoinfections but also infectious diseases, Commission Regulation (EU) No. 142/2011 requires the absence of the pathogen in 25 g of the product. Council Directive 94/65/EC inclused minced meat to the list of products for which Salmonella control is regulated, which is explained by the scattering of *Salmonella* from the smallest lymph nodes over the entire area and more intensive reproduction of bacteria on a large surface of minced meat (Sevalniev et al., 2020).

The study of the structure of the flagellar H-antigen proved the circulation in the Dnipropetrovsk Region of the serological variant *Salmonella* Enteritidis, which according to the WHO is one of the most important serotypes transmitted from animals to humans in most regions of the world (WHO, 2018). Regarding Ukraine, the analysis of salmonellosis incidence and its etiological structure also shows that the predominant pathogen was *Salmonella* Enteritidis (Zarytsky, Hlushkevych and Bubalo, 2016). Detection of egg samples containing salmonella indicates a risk of eating raw or undercooked eggs, which can cause up to 45% of all known cases of human salmonellosis (EFSA and ECDC, 2021; Whitworth, 2019).

In addition, according to RASFF (Smajhel and Shadrova, 2018), *Salmonella* Enteritidis is the serotype most commonly found in poultry meat. The detection of salmonella in beef indicates the importance of controlling food safety. Thus, 13 cases of disease and one death were recorded in the United States in 2019 due to the use of infected ground beef (CDC, 2019).

Studies of the structure of the somatic O-antigen of *Salmonella* revealed exclusively serological group D in four different types of products, which indicates a geographical serotypic predisposition, caused by the dominance of group D pathogens in the Dnipropetrovsk Region (Martynenko and Rula, 2020). Thus, according to statistical reports of the Sanitary and Epidemiological Service of Ukraine, the dominant serogroup of the pathogen among sick people and carriers remains O-group D (MHU, 2016).

Close attention to poultry products imported to the territory of Ukraine is attracted by the detection of non-compliance of DSTU EN 12824:2004 (DSSU, 2005d) in 2019 (Martynenko and Rula, 2020) and 2020 (MDSFCSIFR, 2020). This situation may be due to the fact that EU legislation does not establish microbiological criteria for the content of bacteria of the genus *Salmonella* in raw materials. Thus, today the number of countries that have international veterinary certificates for the import of minced meat and/or MDPM into the customs territory of Ukraine has increased to seven countries (UK, Denmark, Poland, Hungary, Czech Republic, Lithuania, and Latvia). Thus, imported products are a risk factor, according to a report by EFSA and ECDC (2021), which showed a stable prevalence since 2015 of serovars of salmonella-positive flocks among breeding chickens, laying hens, broilers and turkeys for fattening.

Thus, the analysis of information on animal and poultry diseases from the territories-importers of agricultural products allows to formulate epizootological problems in a timely manner and to ensure the welfare of animals and the health of the population of the importing region.

Infected products were the source of pathogens of food bacterial zoonoses in the Dnipropetrovsk Region in 2020: minced meat and MDM from poultry; chicken eggs and beef, the probability of which is also reported by other authors (Malysh, 2020).

Conclusions. The paper shows problematic in 2020 nosological forms in the structure of pathogens of food bacterial zoonoses and identifies their potential sources.

Microbiological studies have established that the contaminants of poultry products of domestic Ukrainian production in the Dnipropetrovsk Region were sanitary-indicative (Bacteria of the *E. coli* group, MA&OAMO), opportunistic (*S. aureus*, sulfite-reducing clostridia) and pathogenic microflora (bacteria of the genera *Salmonella, Listeria*).

The author observed the geographical serotypic predisposition of *Salmonella*, which is caused by the prevalence of O-group D in the Dnipropetrovsk Region, and the possibility of their passage through the food chain.

It was found that minced meat and MDM from poultry are among the factors in the transmission of a potentially pathogenic for humans and animals species of *Listeria — L. monocytogenes* in the region.

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