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Effect of the Feed Additive Butaselmevit-Plus on the Antioxidant Status of the Rat Body Due to Cadmium and Lead Intoxication

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Abstract. The relevance of the study subject is due to the need to create effective methods for preventing the poisoning of animals with heavy metals because lead and cadmium are among the environmental pollutants that negatively affect agriculture and are potentially dangerous to animal and human health. The purpose of the study was to establish the effect of the feed additive Butaselmevit-Plus on the antioxidant protection of the rat body under the chronic action of cadmium and lead. Experimental studies were performed on 2 groups of male rats, 6 animals each. In the control and experimental groups, rats were given a 16.6% aqueous solution of lead acetate at a dose of 100 mg/kg (0.6 ml/kg) of body weight and 0.029% aqueous solution of cadmium chloride at a dose of 2.0 mg/kg (6.9 ml/kg) of body weight. In the experimental group, the rats were additionally provided with food containing the feed additive Butaselmevit-Plus in the amount of 0.1 g per 100 g of body weight. Experimental lead-cadmium toxicosis in the blood serum of rats demonstrated a substantial decrease in the indicators of the antioxidant protection system (reduced glutathione – by 38.4%, superoxide dismutase – by 27.6%, catalase – by 22.7%). On the fourteenth day of the experiment, the lowest activity of the antioxidant protection system indicators in the blood of control rats was observed with the combined administration of heavy metals. Under the experimental load of lead and cadmium, the feed additive Butaselmevit-Plus demonstrated antioxidant properties, which is due to its chemical composition (milk thistle, selenium, methionine, and vitamins). The introduction of the feed additive Butaselmevit-Plus to the rats of the experimental group contributed to an increase in the activity of superoxide dismutase and catalase in the blood serum by 22.7 and 20.7%, respectively. When providing this feed additive to rats of the experimental group, an increase in the level of reduced glutathione was also identified, which reached its maximum value on the 28th day of the experiment. Thus, the results of the study confirm the effectiveness of using the supplement Butaselmevit-Plus to improve the antioxidant status of animals in conditions of chronic intoxication of the rat body with lead and cadmium. The practical value of the results obtained is to substantiate the feasibility of using the feed additive Butaselmevit-Plus in animal husbandry to prevent the negative impact of heavy metals on the animal body

Keywords: heavy metals, catalase, superoxide dismutase, reduced glutathione, milk thistle, vitamins, selenium

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Introduction

In the conditions of substantial man-made or natural pollution of the environment with heavy metals, the issue of finding and creating modern methods and means to prevent the intoxication of the animal body with them becomes relevant. Compliance with sanitary and hygienic standards and requirements for the content of heavy metals in feed, water, and the air is an important step in the prevention of animal poisoning. However, farming involves the use of machinery, pesticides, and fertilisers of organic and inorganic origin, which creates prerequisites for the accumulation of heavy metals in the body of animals. Therefore, the use of drugs and feed additives that prevent the absorption of heavy metals into the blood from the digestive canal, promote their rapid elimination from the body or limit their pathogenic effect on the body becomes important in the prevention of heavy metal poisoning in animals.

Contamination of the environment with heavy metals as a result of anthropogenic activities has negative consequences for agricultural production [1; 2]. High concentrations of heavy metals in the soil can directly harm crops, limiting photosynthesis, damaging roots, reducing growth, and ultimately causing plant death [3]. The intake of heavy metals in products of plant or animal origin worsens their safety indicators and poses a threat to human health since the consumption of such products can cause diseases. Heavy metals, even in small amounts, negatively affect the body of animals. They do not take part in physiological metabolic processes, but, accumulating in organs and tissues, can cause acute or chronic poisoning. Heavy metals can become an etiological factor in the development of many pathological processes in the animal body. As a result of their toxic effects, the liver, kidneys, bones, and cardiovascular and nervous systems can be affected, blood disorders and various types of cancer occur [4].

Lead and cadmium are substantial environmental pollutants belonging to very toxic heavy metals [5]. Entering the body, these toxicants can accumulate in organs and tissues and cause deterioration of the health of animals and their productivity [6-8]. The reaction of animals to various toxicants and their concentrations is ambiguous and depends on the type and form of heavy metal, the route of its entry into the body, age, gender, and physiological state of the animal, feeding conditions, etc. [9-11]. Heavy metals will enter the body of productive animals mainly with contaminated feed and water, and, to a certain extent, with inhaled air containing metal particles. It is proved that due to environmental pollution with cadmium and lead, the manifestation of toxic effects on the animal's body is determined not only by the toxicity of each metal separately but also by their combined effect [12-14].

To date, it has been established that the occurrence and development of cadmium and lead poisoning causes an imbalance between the processes of free radical lipid peroxide oxidation and the antioxidant defence system of the body, which leads to the development of oxidative stress [15; 16]. As a result of the latter, first of all, changes occur in the membranes of liver cells – the lipid bilayer is delaminated, which substantially disrupts the functioning of membrane enzymes [17; 18]. Subsequently, changes occur in the plasma, mitochondrial, peroxisomal, lysosomal, endoplasmic, and core structural units of cells. There is swelling and lysis of mitochondria, microsomes, organelle dysfunction, polymerisation of proteins and rupture of their polypeptide chain, DNA destructures, cellular enzymes are inactivating, the level of intracellular calcium increases, which leads to cell necrosis [19; 20].

An antioxidant protection system, which consists of enzymatic and non-enzymatic mechanisms functions to maintain a balance between the formation and neutralisation of reactive oxygen species in the body of animals. As you know, reactive oxygen species in the animal body are formed not only as a result of the normal course of cellular metabolism but also as a result of harmful environmental factors (ionising radiation, toxins, heat stress). Therewith, the antioxidant protection system largely determines the adaptive resistance of animals to the influence of negative environmental factors [21; 22].

The functional usefulness and power of the antioxidant protection system largely determines the course of toxicosis and the possibility of full recovery of the body. Therefore, the study of this important biological aspect requires further and in-depth research. Considering the above, *the purpose of the study* consisted in the investigation of the effect of Butaselmevit-Plus on the indicators of the antioxidant protection system during chronic cadmium and lead loading of the rat body.

Literature Review

Various methods and means are used to prevent heavy metal intoxication and treat animals for their occurrence. Enterosorption is one of the safest and most effective methods of removing toxic substances, which is based on the oral use of drugs that can absorb various toxic substances in the lumen of the digestive canal without entering into chemical reactions with them [23]. For the treatment of animals for heavy metal poisoning, enterosorbents of various origins are used: carbon sorbents of natural and synthetic origin; natural polymers: polysaccharides and lignin; aluminosilicates and clays: zeolites, saponites; combined materials: mixtures, composites [24]. The most effective enterosorbents include Enterosgel [25]. Enterosorbents mainly have a non-selective effect and, if used for a long time, will help to remove useful micronutrients from the body. Therefore, their use is limited and appropriate for acute heavy metal poisoning.

Organic and inorganic chelators such as ethylenediaminetetraacetic acid (EDTA), 2,3-dimercaptobursh tic acid, unitiol, and penicillamine are also used to neutralise and remove heavy metals from the body [26]. Although the effectiveness of these drugs is high, quite often their use is accompanied by the appearance of undesirable effects – the elimination of useful macro- and microelements from the body with corresponding consequences, kidney damage, etc.

The considerable attention of researchers is also focused on magnetic biosorbents that are synthesised from various metal nanoparticles. In particular, positive results are shown by magnetic nanoparticles based on iron oxide, which have a high adsorption capacity due to their large surface area and pronounced reaction properties [27]. Therewith, there are still no opportunities for widespread implementation of nanotechnology achievements in practice, and certain aspects of the potential toxic effects of nanoparticles on living organisms are still being actively investigated.

Natural and synthetic antioxidants (quercetin, catechin, taurine, captopril, gallic acid, melatonin, N-acetylcysteine, alpha-lipoic acid, etc.) deserve special attention since heavy metals have pro-oxidant properties. Antioxidants affect biological systems not only by directly neutralising free radicals but also by chelating toxic metals. They can also enhance the mechanism of antioxidant protection of cells through the regeneration of endogenous antioxidants [28].

Materials and Methods

Experimental studies were performed based on the vivarium of the State Research Control Institute of Veterinary Drugs and Feed Additives in Lviv. 12 mongrel white male rats weighing 200-220 g were used in the experiments. Experimental rats were divided into a control and experimental group of 6 animals each. 16.6% aqueous solution of lead acetate at a dose of 100 mg/kg (0.60 ml/kg) of body weight (corresponding to 1/40 DL50) and 0.029% aqueous solution of cadmium chloride at a dose of 2.0 mg/kg (6.9 ml/kg) of body weight (corresponding to 1/40 DL50) were administered intragastrically for 28 days to simulate experimental chronic lead and cadmium intoxication. Additionally, the rats of the experimental group were fed food containing the feed additive Butaselmevit-Plus at a dose of 1000 mg/kg of body weight throughout the experiment. The composition of the feed additive includes such active substances as milk thistle fruits, vitamins (A, D3, E), sodium selenite, methionine [29]. In total, these components are a powerful antioxidant complex that corrects the intensity of lipid peroxide oxidation and free radical formation processes, thereby preventing the development of oxidative stress.

Experiments on rats were performed in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes [30]. During the experiment, the rats had a balanced diet, and drinking water was obtained ad libitum from glass drinkers. On days 1, 7, 14, 21, and 28 of the experiment, blood samples were taken from rats for biochemical studies from the jugular vein. The following indicators were determined in the blood serum: superoxide dismutase activity (EC 1.15.1.1) – according to the method of Dubynina et al. [31]; catalase activity (EC 1.11.1.6) – according to the Koroliuk method [31]; reduced glutathione content – according to the Butler method [31].

The resulting digital data was processed using the Statistica 6.0 software package. The reliability of differences between indicators was determined using the Student's t-test.

Results and Discussion

Endogenous antioxidants include reduced glutathione, which, as a reduced thiol with the highest concentration, has a direct effect on the redox status of cells. It neutralises free radicals, is a cofactor and substrate of the enzyme antioxidant system.

Under conditions of cadmium and lead intoxication, on the 7th day of the experiment, the content of reduced glutathione in the blood serum of rats of the control group decreased by 29.1%, and on the 14th day of the experiment – by 38.4% compared to the initial values (Table 1). On days 21 and 28 of the experiment, the content of reduced glutathione in the blood serum of rats in the control group remained lower, by 24.9% and 21.3%, respectively.

Table 1. Content of reduced glutathione in the blood serum of intoxicated rats when using Butaselmevit-Plus, mmol/l, M ± m, n = 6

Study period	Animal group	
	Control	Experimental
Initial data	2.229 ± 0.044	2.225 ± 0.042
Day 7	1.581 ± 0.041	1.864 ± 0.030*
Day 14	1.372 ± 0.039	1.825 ± 0.036*
Day 21	1.675 ± 0.050	2.067 ± 0.040*
Day 28	1.754 ± 0.043	2.207 ± 0.034*

Note: **P < 0.05, compared with animals in the control group*

For the use of the feed additive Butaselmevit-Plus in rats intoxicated with lead and cadmium, it was identified that in rat blood serum in the experimental group, the content of reduced glutathione substantially increased by 17.9% on the 7th day of the experiment compared with its value in animals of the control group. The level of restored glutathione increased by 33.0% on day 14 of the experiment, while on day 21 – by 23.4% compared with the control group. The highest content of reduced glutathione in the blood serum of rats of this group was noted on the 28th day of the experiment, which is 25.8% higher than the same indicator in animals of the control group.

Superoxide dismutase is a powerful antioxidant due to its high enzyme ability to neutralise free radicals. Under the conditions of lead-cadmium intoxication in rats of the control group, a decrease in the activity of this enzyme in the blood serum on the 7th day of the experiment was established by 13.8% compared with the initial data (Table 2).

Table 2. Superoxide dismutase activity in the blood serum of intoxicated rats when using Butaselmevit-Plus, conditional units, M ± m, n = 6

Study period	Animal group	
	Control	Experimental
Initial data	1.522 ± 0.005	1.540 ± 0.005
Day 7	1.313 ± 0.004	1.471 ± 0.003*
Day 14	1.101 ± 0.004	1.354 ± 0.004*
Day 21	1.153 ± 0.002	1.421 ± 0.005*
Day 28	1.250 ± 0.003	1.492 ± 0.004*

Note: **P < 0.05, compared with animals in the control group*

According to the results obtained, on the 14th day of studies, its activity in the blood serum of these animals was the lowest and corresponded to values 27.6% less than the initial level. Therewith, on days 21 and 28 of the experiment, there was a tendency to increase the activity of superoxide dismutase in the blood serum of rats intoxicated with lead and cadmium, although the indicators differed from the initial ones.

Throughout the experiment, the activity of superoxide dismutase in the blood serum of rats increased due to the introduction of the feed additive Butaselmevit-Plus to the diet. In particular, enzyme activity increased by 12.2% on the 7th day, and by 22.7% on the 14th day of the experiment compared with the values of the control group. The highest activity of superoxide dismutase was established in the blood serum of rats of the experimental group, when it increased by 19.2% compared with the control group on the 28th day of the experiment.

According to Table 3, in control rats, serum catalase activity decreased starting from 7th day after administration of lead and cadmium salt solutions. The lowest catalase activity in the blood serum of animals of this group was established on days 14 and 21 of the experiment, which corresponded to values of 22.7% and 20.0% lower than the initial level, respectively.

Table 3. Catalase activity in the blood serum of intoxicated rats when using Butaselmevit-Plus, μmol/min, M ± M, n = 6

Study period	Animal group	
	Control	Experimental
Initial data	6.751 ± 0.038	6.780 ± 0.040
Day 7	5.824 ± 0.052	6.192 ± 0.062*
Day 14	5.223 ± 0.084	6.303 ± 0.058*
Day 21	5.402 ± 0.054	6.423 ± 0.066*
Day 28	5.981 ± 0.062	6.542 ± 0.070*

Note: **P < 0.05, compared with animals in the control group*

The results of the study indicate that feeding rats the feed additive Butaselmevit-Plus caused a likely increase in the activity of catalase in the blood serum. In particular, the activity of the enzyme was higher by 6.4% and 20.7% in the blood serum of animals of the experimental group, respectively, on days 7 and 14 compared with the corresponding control parameters. On the 21st day of the experiment, an increase in catalase activity by 18.9% was established in the blood serum of rats of the experimental group. The highest activity of the enzyme was established in rats of the experimental group on the 28th day of the experiment. In addition, it was 9.4% higher than the value of this indicator in the animals of the control group. Notably, that catalase is an enzyme of the antioxidant system that effectively cleaves excess hydrogen peroxide to water and molecular oxygen (catalase activity), and is also able to oxidise a number of toxic compounds in the presence of peroxides (peroxidase activity) [32].

Thus, analysing the results of the study in the conditions of chronic experimental poisoning of the rat body with lead and cadmium, it was established that the feed additive Butaselmevit-Plus has a pronounced antioxidant effect. There are two potentially possible mechanisms of action of the feed additive Butaselmevit-Plus. First, this feed additive, due to its active ingredients, suppresses the peroxide oxidation of lipids, and secondly, it activates the processes of antioxidant protection of the body.

We believe that the effectiveness of the feed additive Butaselmevit-Plus is associated with the effect of its active substances on the main pathogenetic links of the process of chronic intoxication of the rat body with lead and cadmium. Since it is the liver that neutralises xenobiotics in the body, hepatocytes are primarily damaged by the negative effects of heavy metals [11]. Previous studies in rats have shown that the elimination of endogenous intoxication syndrome helps to restore the functional state of liver cell membranes and increase the antioxidant status of the rat body [33].

The use of selenium in the feed additive Butaselmevit-Plus prevents the absorption of heavy metals in the digestive canal. Other researchers have also noted that adding selenium to animal diets provides substantial protection against cadmium toxicity by inhibiting its bioaccumulation [34]. In addition, selenium is a part of selenoproteins, which have a wide range of functions, from antioxidant and anti-inflammatory to the production of

thyroid hormones. The main function of selenium in the antioxidant protection system is to take part in the construction and functioning of glutathione peroxidase, an enzyme that neutralises the products of lipid peroxide oxidation [35; 36].

The medicinal plant milk thistle (*Silybum marianum L.*) has a wide application in veterinary medicine. It contains valuable medicinal substances, namely silymarin, which includes silibinin, silidianin, silicristine, and other flavolignans. Thus, the feed additive Butaselmevit-Plus exhibits antioxidant properties due to the interaction of these flavolignans with free radicals in the liver and their conversion into less toxic compounds, which contributes to further interruption of the lipid peroxide oxidation chain and inhibition of these processes, and prevention of destruction of cellular structures [37].

In a number of studies [19; 29; 33], it was identified that silymarin acts as an antioxidant, hepatoprotective, and choleretic agent. Due to this action, the biological membranes of hepatocytes are stabilised. This leads to the restoration of the activity of hepatospecific enzymes in the blood, which ensures high therapeutic effectiveness when feeding animals the feed additive Butaselmevit-Plus.

Methionine, which is one of the components of the drug, combines enzyme and non-enzyme links of anti-radical protection of cell membranes. This amino acid in the body of rats under heavy metal intoxication contributes to the conversion of neutral lipids into phospholipids. This stabilises subcellular membranes, improves the body's antioxidant defences, and increases the resistance of liver cells to the toxic effects of lead and cadmium.

The feed supplement also contains vitamins A, E, and D_3, which during chronic lead-cadmium intoxication in combination with its other components contributed to a decrease in the intensity of free radical formation in the body of rats and had a protective effect, since, as is known [37; 38], these vitamins show the ability to prevent damage to the ultrastructure of cells.

The conducted studies and established patterns confirm the expediency of using the feed additive Butaselmevit-Plus for the prevention of lead-cadmium toxicosis in animals.

Conclusions

Studies have shown that intragastric administration of 16.6% aqueous solution of lead acetate at a dose of 100 mg/kg of body weight and 0.029% aqueous solution of cadmium chloride at a dose of 2.0 mg/kg of body weight to rats for 28 days causes changes in individual links of the antioxidant protection system. During the entire study period, there was a decrease in the content of reduced glutathione in rat blood plasma by 21.3-38.4%. Therewith, the lowest values of this indicator are recorded on the 14th day of the experiment. The activity of catalase and superoxide dismutase in the blood serum of experimental rats also decreases. Therewith, chronic lead and cadmium intoxication in rats is accompanied by a decrease in the activity of the body's antioxidant defence system.

The use of the feed additive Butaselmevit-Plus at a dose of 1000 mg/kg of body weight during chronic lead and cadmium intoxication contributes to an increase in the content of reduced glutathione in rat blood serum by 17.9-33.0%, the activity of superoxide dismutase by 12.2-22.7%, and catalase by 6.4-20.7% compared to similar indicators in animals of the control group. Thus, Butaselmevit-Plus provides stabilisation of the antioxidant defence of the rat body in conditions of chronic heavy metal intoxication. This is due to the selenium, vitamins A, E, and D_3, and milk thistle, present in the supplement.

The feed supplement Butaselmevit-Plus, which contains antioxidants and natural biologically active substances, such as silymarin and a complex of fat-soluble vitamins, can be used to prevent poisoning of animals with heavy metals. It stimulates the potential ability of superoxide dismutase, catalase, and reduced glutathione to neutralise excess reactive oxygen species and lipid peroxidation products that are actively generated due to the action of heavy metals on the animal's body.

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Вплив кормової добавки «Бутаселмевіт-плюс» на антиоксидантний статус організму щурів за інтоксикації кадмієм і свинцем

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Анотація. Актуальність теми досліджень зумовлена необхідністю створення ефективних методів профілактики отруєння тварин важкими металами, адже свинець і кадмій належать до забруднювачів довкілля, що негативно впливають на сільське господарство і є потенційно небезпечними для здоров'я тварин і людей. Мета роботи полягала у встановленні впливу кормової добавки «Бутаселмевіт-плюс» на антиоксидантний захист організму щурів за хронічної дії кадмію та свинцю. Експериментальні дослідження виконували на 2 групах самців щурів, по 6 тварин у кожній. У контрольній і дослідній групах щурам задавали 16,6 % водний розчин свинцю ацетату в дозі 100 мг/кг (0,6 мл/кг) маси тіла і 0,029 % водний розчин кадмію хлориду в дозі 2,0 мг/кг (6,9 мл/кг) маси тіла. У дослідній групі щурам додатково згодовували корм, який містив кормову добавку «Бутаселмевіт-плюс» у кількості 0,1 г на 100 г маси тіла. За експериментального свинцево-кадмієвого токсикозу в сироватці крові щурів спостерігали вірогідне зниження показників системи антиоксидантного захисту (відновленого глутатіону – на 38,4 %, супероксиддисмутази – на 27,6 %, каталази – на 22,7 %). На чотирнадцяту добу досліду за сукупного введення важких металів спостерігали найнижчу активність показників системи антиоксидантного захисту в крові щурів контрольної групи. За експериментального навантаження свинцем та кадмієм, кормова добавка «Бутаселмевіт-плюс» проявляла антиоксидантні властивості, що зумовлено її хімічним складом (розторопша плямиста, селен, метіонін і вітаміни). Додавання до складу раціону кормової добавки «Бутаселмевіт-плюс» щурам дослідної групи сприяло зростанню активності супероксиддисмутази та каталази в сироватці крові, відповідно на 22,7 і 20,7 %. При згодовуванні щурам дослідної групи цієї кормової добавки встановлено також і підвищення рівня відновленого глутатіону, який досягав максимального значення на 28 добу досліду. Отже, результати дослідження підтверджують ефективність застосування добавки «Бутаселмевіт-плюс» для покращення антиоксидантного статусу тварин в умовах хронічної інтоксикації організму щурів свинцем та кадмієм. Практична цінність одержаних результатів полягає у обґрунтуванні доцільності використання у тваринництві кормової добавки «Бутаселмевіт-плюс» для профілактики негативного впливу важких металів на організм тварин

Ключові слова: важкі метали, каталаза, супероксиддисмутаза, відновлений глутатіон, розторопша плямиста, вітаміни, селен

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Morphology of the Digestive Canal Organs and Their Immune Formations in the Mulard Ducks

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Abstract. It is known that in the immune formations of the digestive canal of birds, which belong to the peripheral organs of hematopoiesis and lymphopoiesis, differentiation of T- and B-lymphocytes occurs under the influence of antigens that cause the development of specific (cellular and humoral) immunity. In this regard, the purpose of this study was to identify the features of the morphology of the digestive canal organs and their immune formations in ducks of the hybrid meat breed "Mulard" aged 150 days during puberty. During histological studies, pieces from different areas (oesophagus, parts of the stomach, intestines with Peyer's spots, Meckel's diverticula, and caecum diverticula) were selected, labelled, and fixed in a 10% aqueous solution of neutral formalin and poured into paraffin, according to the generally accepted method. Histological preparations were used to examine the features of the microscopic structure of the digestive canal organs and their immune formations and histotopography, the types of forms of lymphoid tissue were analysed, its area was calculated. It was established that the immune formations of the digestive canal organs of ducks are represented by all levels of structural organisation of lymphoid tissue, which are not equally expressed in certain parts of them. Accumulations of immune formations in the walls of the oesophagus and stomach are located in lamina propria plate of the mucous membrane and submucosal base, and in the intestines – also in the muscle membrane. Lymphoid tissue is best developed in the oesophagal tonsil, caecum diverticula, slightly less in the Meckel diverticula and Peyer's spots of the intestine. In the wall of the oesophagus and stomach of ducks, only minor accumulations of this tissue are observed. The results obtained on the morphofunctional state of peripheral organs of hematopoiesis and lymphopoiesis allow improving technologies for raising and exploiting birds to ensure their high viability and productivity

Keywords: poultry, oesophagus, stomach, intestines, lymphoid tissue, lymphoid nodules

Introduction

It is known that immune (lymphoid) formations are located in the walls of the digestive canal (tract) of birds, which are part of the peripheral organs of hematopoiesis and lymphopoiesis [1-3]. Knowledge of the morphofunctional state of these organs makes it possible to improve technologies for raising and exploiting birds to ensure their high viability and productivity.

Organised immune formations of the digestive canal of birds include tonsils (oesophagal, caecum), Meckel's diverticula, Peyer's spots [4-6]. Most of them are complex structures, the lymphoid tissue of which has a connection with the epithelium and may contain crypts. Immunocompetent structures also include some cellular elements (intraepithelial lymphocytes, plasma cells, macrophages, granulocytes) [7].

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A substantial content of immune formations is concentrated in the mucous membrane and submucosal base of the digestive canal organs [5]. Some of them are located inside it and are macroscopically invisible. In certain areas of the mucous membrane, these formations are very well developed, they can protrude in the form of rollers above the surface [8-10].

The functional basis of peripheral organs of hematopoiesis and lymphopoiesis, including immune formations, is formed by lymphoid tissue, effector cells, which recognise and neutralise antigens and clean the body from them [11; 12]. Thus, it is activated immediately after foreign agents enter the body during the feeding of birds.

The basis of lymphoid tissue is reticular tissue, which is a type of connective tissue. It is formed by reticular cells and intercellular substance. The fibres of the latter form a mesh-like structure. The cells of this grid contain lymphocytes. Reticular cells have a stellate shape, an oval nucleus, and long branched cytoplasmic appendages. The appendages of these cells are in contact and in some cases form dense adhesive contacts or desmosomes [13; 14].

In the structure and development of peripheral organs of hematopoiesis and lymphopoiesis, including immune formations, there are several patterns. The first is the beginning of their formation at the early stages of ontogenesis [15; 16]. Second is that they are always located in areas of possible penetration of foreign agents into the body and in places where they spread in it. The third is the gradual transformation of the structure of their lymphoid tissue, depending on the duration and intensity of exposure to antigens. In connection with the third pattern, there are four stages of differentiation in the development of lymphoid tissue.

At the beginning of the development of lymphoid tissue, a diffuse-scattered (associated) form of this tissue appears in the mucous membrane of internal organs. Its presence is considered as the readiness of the bird body to reproduce an adequate response to oral antigen intake in the intestine. The second stage is characterised by the fact that clusters of lymphoid cells are formed in the diffuse lymphoid tissue – pre-nodules that do not have clear borders. In the third stage, the formation of primary lymphoid nodules occurs, the appearance of which characterises the high morphological maturity of lymphoid tissue and peripheral organs of hematopoiesis and lymphopoiesis, that is, their readiness for the formation of lymphoid cells that provide immunity. The fourth stage is the development of lymphoid tissue. This is the stage of the highest degree of differentiation of the organs of the immune system. It forms lymphoid nodules with light centres (secondary). The formation of the latter is associated with an antigenic effect and indicates a highly protective reaction of the body. Some researchers [2] also distinguish the fifth stage, which is characterised by the disappearance of lymphoid nodules as a result of their reverse development during the age-related transformation of immune formations. Therewith, the area of diffuse lymphoid tissue decreases. In place of nodules, loose fibrous connective tissue grows noticeably, fat cells and lobules of adipose tissue appear. Thus, connective and adipose tissues displace the lymphoid parenchyma [1].

Given the important role of immune formations of the digestive system in the formation of immunity, their topography, microstructure and functional features are quite well investigated in chickens and certain species of wild birds [2; 5; 6]. In turn, in waterfowl, these data are incomplete, few, and contradictory.

The purpose of the study was to identify the structure of the digestive canal organs and morphofunctional features of their immune formations in 150-day-old Mulard Ducks.

Literature Review

Lykova & Kharchenko [17] identified a general pattern of localisation of immune formations in the wall of the digestive canal of birds. According to them, the greatest content of such formations is concentrated in the places of transition from one part of the digestive tube to another. Especially large numbers of them are located on the border between the oesophagus and the glandular part of the stomach, the small intestine, and the rectum. The number of immunocompetent cells increases with the onset of the sexual maturity of birds and then decreases with increasing age. In addition, the authors report that the intensity of the development of immune formations of the digestive organs of birds depends primarily on trophic specialisation and the method of obtaining food.

Khomish *et al*. [5] explain the substantial development of immune formations in the oesophagal wall of birds as compensation for the absence of the Pirogov-Waldeyer pharyngeal lymphoid ring, which is present in mammals. They are represented by scattered lymphoid nodules and the oesophagal tonsil. Lymphoid nodules are located along the entire oesophagus, but their aggregates are located in the thoraco-abdominal part of this organ near the secretory parts of the oesophagal glands. Their lymphoid cells substantially infiltrate the glandular epithelium.

Hanafy *et al*. [18] demonstrated that in ducks, the oesophagal tonsil is ring-shaped and grey-pink in colour. In the folds of the mucous membrane, this area, there is lymphoid tissue, which is represented by all levels of structural organisation (diffuse and pre-nodular forms, nodules without light and with light centres), which indicates its structural and functional maturity.

In the stomach of birds, immune formations are unevenly located in different parts of it. Their greatest content is located in the area of the sphincters of the intermediate zone and the pyloric part of the stomach, which regulate the flow of food entering the muscular part of the stomach and the duodenum, respectively [19]. To date, the immune formations of the stomach of chickens, especially its glandular part, are quite well investigated. They are located in the mucous membrane between the superficial tubular glands, in the lobules of the deep glands, and between them [20].

The intestinal wall with its immune formations of broiler Ducks of Blagovarsky cross in the age aspect is most fully investigated by Mazurkevych [6; 21]. According to the author, the duodenum and ileum contain one Peyer's spot, in the jejunum of ducks aged from one to 240 days there are three of them, in 330-day-olds – two, in 420-day-olds – one, in each caecum – 60-80 spots arranged in a chain. Feather spots are macroscopically detected from the age of 5 days of ducks and come in various shapes and sizes. Their size increases to 120-150 days of age. In the older bird, these indicators decrease. The Meckel diverticulum of ducks has the appearance of a tube with a narrowed tip, on which there may be a yellow sac residue by the age of

20 days of the bird. The length of the Meckel diverticulum increases to 150 days, and the width – to 120 days of age. By the 420-day age of ducks, these indicators decrease. Caecum diverticulae, as immune formations, are established up to 330 days of age in ducks. In the immune formations of the ducks' intestines, the author located early and mature B-lymphocytes, naive T-cells, T-helper cells, T-suppressors and natural killers, and in certain areas of it, ducks aged 180 days also have blood stem cells.

Materials and Methods

Classical methods of morphological research were used in the course of the study. All interventions and slaughter of ducks were conducted in compliance with the requirements of the "European Convention for the Protection of Vertebrates Used for Experimental and Scientific Purposes" (Strasbourg, 1986) [22] and the Law of Ukraine No. 692 "On the Protection of Animals from Cruelty" (3447-IV) of 02/21/2006 [23].

The material was obtained from six ducks of the hybrid meat breed "Mulard" aged 150 days. The birds were clinically healthy and demonstrated no signs of disease. The research was conducted based on the laboratory of the Department of Animal Anatomy, Histology, and Pathomorphology named after academician V.G. Kasyanenko of the National University of Life and Environmental Sciences of Ukraine during 2021-2022.

During histological studies, samples were cut out from various areas of organs (oesophagus, parts of the stomach, intestines with Peyer's spots located in them, Meckel's diverticulum, and Caecum diverticula were separated from the intestines where they are located). The samples were fixed in a 10% neutral formalin solution for 48 hours. Then they were washed with tap water for 24 hours and dehydrated with ethyl alcohol of increasing concentration (60%, 70%, 80%, 96%, and absolute alcohol). Before making the sections, the samples were sealed with paraffin. The resulting blocks (samples with paraffin) were glued to wooden blocks made of hard trees, from which sections with a thickness of 5-10 microns were cut on a special device – a sledge (skid) microtome. The obtained histological sections were stained with hematoxylin-eosin – to establish the general microstructure of the oesophagus, stomach and intestines and their immune formations, according to Mallory – to register collagen fibres of fibrous connective tissue and impregnated with a 1-2% solution of silver nitrate according to the Kelemen method – to detect reticular fibres. A drop of balsam was applied to the painted and impregnated sections and covered with a covering glass.

Histological preparations were investigated using Olympus and MBS-2 light microscopes. Features of the microscopic structure of the membranes of the wall of the digestive canal organs with their immune formations in ducks were determined, varieties of forms of lymphoid tissue and histotopography of immune formations were investigated. The area of lymphoid tissue was calculated by the "point counting" method using a binocular microscope and a measuring grid.

The results of the conducted studies were recorded in protocols, and their digital indicators were processed statistically using a computer using Excel. Individual histological preparations were photographed using an Olympus microscope with a Nikon Coolpix S3100 camera.

Results and Discussion

Morphology of the oesophagus and its immune formations

It is known that in the oesophagus of birds, two parts are distinguished by length: cervical (cranial) and thoraco-abdominal (caudal). True crop unlike chickens, is absent in waterfowl, instead there is a fusiform expansion [24; 25].

The mucous membrane of the oesophagus of ducks forms deep longitudinal folds (5-9), which facilitate the passage of food from the oral cavity to the stomach and has the ability to stretch. It is lined with a multi-layered flat partially keratinised epithelium, which directly near the glandular part of the stomach becomes thin and changes to a simple cylindrical glandular one. The lamina propria layer of the mucous membrane forms deep connective tissue papillae and is represented by loose connective and reticular tissues, where a large number of secretory parts of the oesophagal glands are located, the excretory ducts of which open on the surface of this membrane. The glands secrete mucus, which promotes the movement of food through the oesophagus. Bundles of smooth myocytes form a muscle plate of the mucous membrane, which is poorly developed and sometimes intermittent. Near the glandular stomach, the oesophagal glands disappear, and lobules of deep glands appear in the submucosal layer, which are characteristic of this stomach. The muscle membrane of the oesophagal wall is formed by separate layers of smooth muscle tissue. The inner (longitudinal) layers are attached to the submucosal base, and the outer (circular) layers are attached to the serous membrane. In some places, a longitudinal layer is also located outside the circular layer in this membrane. The inner layer takes part in the formation of high folds of the mucous membrane, going deeper into them. The adventitious membrane of the cervical oesophagus is formed by loose connective tissue. In the thoraco-abdominal part of this organ, it is replaced by a serous membrane, which has a similar structure but is covered with mesothelium.

The inner lining of the oesophagus of ducks contains minor accumulations of immune formations. In the lamina propria layer of this membrane, lymphoid elements are represented by small clusters of scattered lymphoid tissue, which are located under the epithelium, around blood vessels, in the wall of secretory parts of the oesophagal glands and their lumen, and less often it is located in the submucosal layer on the border with the muscle membrane. This tissue has no clear borders, it is uniform, without noticeable seals or rarefaction. It is based on reticular tissue, which has a mesh structure. Its loops contain lymphoid cells and macrophages. Lymphoid cells locally infiltrate the surface epithelium, epithelium of the secretory parts of the oesophagal glands, and their ducts. In addition to diffusely located lymphocytes, single lymphoid nodules are also located under the epithelium and near the oesophagal glands (Fig. 1). Some of them have light centres (reactive centres).

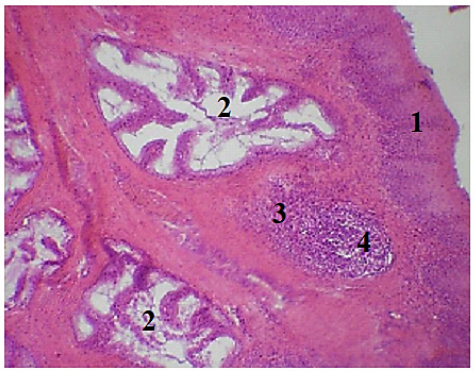


Figure 1. Duck oesophagus aged 150 days: 1 – epithelium; 2 – oesophagal glands; 3 – diffuse lymphoid tissue; 4 – secondary lymphoid nodule. Hematoxylin and eosin staining, ×63

Lymphoid nodules are mainly spherical and oval in shape with a well-defined membrane, in the formation of which reticular and collagen fibres take part. Primary lymphoid nodules are evenly filled with lymphoid cells. Reticular fibres in their central areas form grids with large cells. In lymphoid nodules with reactive centres, most lymphocytes are concentrated on the periphery and form a mantle zone. In the central areas of these nodules, reticular fibres disappear and are located only in the membrane and under it. The fibres are thicker, not tightly arranged, and do not form a grid.

According to the authors [26], activation, blasttransformation, proliferation, and antigen-dependent differentiation of B-lymphocytes into plasmocytes that are producers of specific antibodies of the IDA class takes place in the reactive centres of lymphoid nodules. When passing through epithelial cells, a secretory component is attached to the IgA and a secretory IgA (SIgA) molecule is formed. The latter provides protection (antiviral and antibacterial) on the surface of the mucous membrane.

In the area of the caudal part of the oesophagus, there are more immune formations. The surface epithelium of the mucous membrane in their places is infiltrated by lymphocytes, and perivascular immune formations are also recorded in the submucosal base. In the caudal part of the duck's oesophagus, during its transition to the glandular part of the stomach (oesophagal-gastric communication), lymphoid tissue is well developed and forms the oesophagal tonsil, which is consistent with the data of other authors [8; 10]. This immune formation is macroscopically visible and has the appearance of a ring-shaped strip with uneven edges, a folded and bumpy surface with a yellowish tinge.

According to [5], in the oesophagal tonsil of birds, which is macroscopically visible, the lymphoid tissue is located compactly, and in those species in which it is not detected – diffusely. In connection with this feature, they suggest classifying the oesophagal tonsils into compact and diffuse.

In the oesophagal tonsil of ducks, lymphoid tissue occupies almost the entire area of the lamina propria of the mucosal plate and submucosal base (Fig. 2).

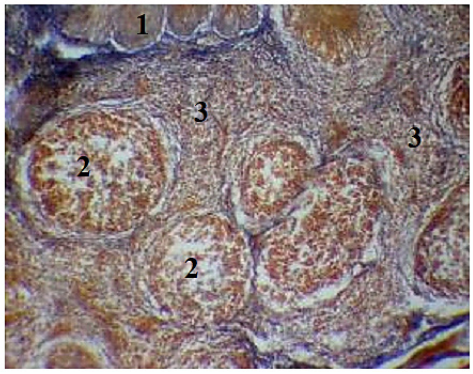


Figure 2. Oesophagal tonsil of a duck aged 150 days: 1 – superficial epithelium; 2 – secondary lymphoid nodules; 3 – diffuse lymphoid tissue. Mallory staining, ×90

It contains large lymphoid nodules and their precursors – pre-nodules, which are limited to accumulations of diffuse lymphoid tissue. Lymphoid nodules in the oesophagal tonsil are located mainly at the base of the folds of the mucous membrane, and substantial accumulations of diffuse lymphoid tissue are located at the tops of these folds. Nodules are mostly rounded and oval, less often ovate, pear-shaped, and irregular in shape. Crypt-like formations, which are well expressed on histopreparations, are limited to the layer of lymphoepithelium and open into the lumen of the oesophagal tonsil area. They are recorded on the side surfaces and at the base of the folds.

Morphology of the stomach and its immune formations

The stomach of birds is located between the oesophagus and intestines [21; 27; 28]. According to modern international anatomical nomenclature, it has three parts: glandular with its intermediate zone (Isthmus), muscular, and pyloric, which are not equally expressed in certain bird species [29].

The glandular part of the stomach of ducks has the appearance of a short fusiform (thick-walled) tube. Its anterior and posterior ends are narrowed, and the middle part is expanded. The muscular part of the stomach is located caudoventrally and is covered with accumulations of adipose tissue – a fat capsule. It has a disc-shaped shape and thick walls, on the lateral surfaces of which tendon mirrors are located and in the cranial and caudal parts blind sacs of the same name. The Isthmus of the glandular part of the stomach opens into the cranial blind sac. Next to it is the pyloric part of the stomach – the exit to the duodenum.

The mucous membrane of the glandular part of the stomach of ducks is the thickest. It is covered with a simple cylindrical glandular epithelium. Numerous surface glands permeate the lamina propria layer of this membrane. According to the authors [19; 28], these glands produce a viscous secret that has bactericidal properties. In the mucous membrane, the muscle plate is represented by bundles of longitudinally oriented smooth myocytes. In some places, it is intermittent. In the submucosal layer of the glandular part of the stomach of ducks, deep complex glands are located, a characteristic feature of which is a complex system of excretory ducts. The glands are grouped into lobules, which are arranged in one or two rows and have a predominantly polygonal shape.

According to some authors, the secretory parts of deep complex glands are formed by glandulocytes of the same type and combine the secretion of pepsinogen and hydrochloric acid, which gave reason to call them oxinto-peptic [21]. Their excretory ducts open with papillae on the surface of the mucous membrane. In the area of the intermediate zone, there are no deep glands.

The muscular membrane of this part of the stomach of ducks is three-layered and is represented by two longitudinal (inner and outer) and circular (middle) layers. In the muscle membrane, between its layers, layers of loose connective tissue are noticeable, in which blood vessels and nerve plexuses pass.

The mucous membrane of the muscular part of the stomach is covered with a simple cubic epithelium. The lamina propria layer of this membrane contains gastric glands, the secret of which covers the entire surface of the mucous membrane with a dense pellicle – the cuticle. The latter protects it from mechanical damage. It also contains a lot of collagen fibres that do not have a specific orientation. The muscle plate is absent. The submucosal base is well defined. It is formed by dense fibrous connective tissue. The muscle membrane of this part of the stomach is best developed and formed by muscle tissue, which forms two groups of powerful smooth muscles: intermediate and lateral. The intermediate muscles are located in the area of the blind sacs, and the lateral muscles lie on the body of the stomach and form its edges.

The pyloric part of the stomach of ducks is similar in structure to the muscular one, but the cuticle on the mucous membrane is weakly expressed, and the muscle membrane is thinner and consists of two layers of smooth myosites: internal-circular and external-longitudinal. However, a muscle plate appears in the mucous membrane of this part of the stomach. It is formed by fragmented bundles of smooth muscle cells.

Immune formations are unevenly located in different parts of the stomach of ducks. They are best expressed in the glandular part of the stomach (Fig. 3).

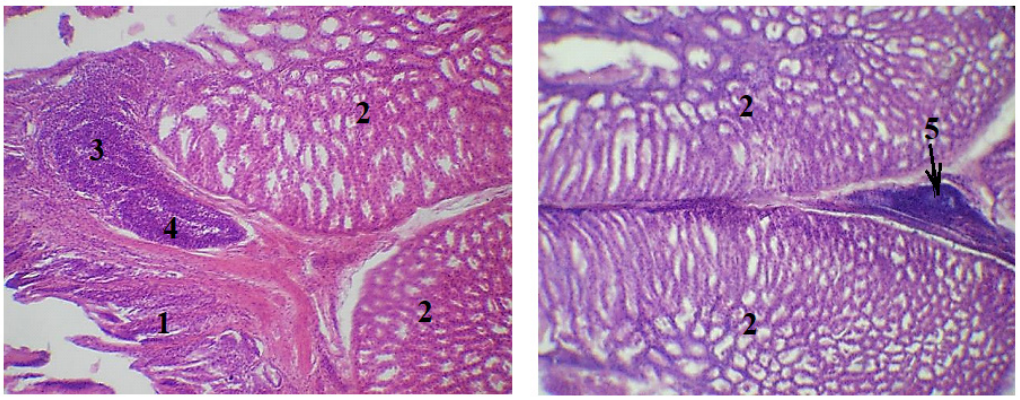


Figure 3. Glandular part of the stomach of a duck aged 150 days: 1 – superficial tubular glands; 2 – lobules of deep glands; 3 – diffuse lymphoid tissue; 4 – secondary lymphoid nodule; 5 – primary lymphoid nodule. Hematoxylin and eosin staining, ×63

Immune formations are mainly located at the base of the folds of the mucous membrane between and under the surface tubular glands. In places of these accumulations, the structure of the mucous membrane's lamina propria changes. It registers collagen and reticular fibres. The latter intertwine in different areas and form a small-cell grid, the cells of which contain lymphoid cells and macrophages. The nature of the structure of the surface and glandular epithelium of the mucous membrane also changes. Locally, due to infiltration by lymphoid cells, it becomes spongy. Diffuse lymphoid tissue and lymphoid nodules are also recorded in and between the lobules of the deep glands. Their lymphoid cells enter the glandular epithelium of the lobules. Single lymphoid nodules are located in the fibrous connective tissue of the submucosal base, which is located near the muscle plate of the mucous membrane. Some accumulations of diffuse lymphoid tissue of the submucosal base can connect to those of the mucous lamina propria plate. Single rounded lymphoid nodules are also located in the subserous base of the serous membrane.

In the intermediate zone, immune formations are localised mainly in areas close to the glandular part of the

stomach. They are located in the lamina propria layer of the mucous membrane and less often in the submucosal base. In lamina propria, accumulations of lymphoid tissue are located between the tubular glands and at their base. Nodules are placed mainly singly. Some of them bend the muscle plate, violating its integrity.

In the mucous membrane of the muscular and pyloric parts of the stomach, immune formations are poorly developed and are represented by a diffuse form of lymphoid tissue and single secondary round lymphoid nodules. They are located between the tubular glands in the lamina propria of this membrane. The epithelium of the mucous membrane at the sites of immune formations is infiltrated by lymphoid cells. These same cells also infiltrate the tubular glands.

Morphology of the intestine and its immune formations

The small intestine of birds consists of the duodenum, jejunum, and iliac, and the colon – two blind and rectum, which ends in the cloaca. Among the components of the small intestine, the jejunum is the longest, the duodenum is much shorter, and the ileum is the shortest [6; 30].

The intestinal mucosa of ducks is well developed. It forms villi, crypts, and folds. In the small intestine, the villi are high and thin, and in the large intestine – low and wide. The epithelium of the villi is simple cylindrical bordered. Between its cells are bordered and goblet-shaped. Border cells have a cylindrical shape, on their apical pole there are microvilli. It is characterised by the appearance of a thin line on the surface of these cells. The border increases the absorption surface of the intestine.

Goblet cells are known to be single-celled glands that produce mucus. The latter covers the epithelial layer of the mucous membrane [28]. They have a goblet shape. Their apical pole is expanded and filled with large granules of light (mucosal) secretions. The number of such cells increases in the distal parts of the intestine.

The surface epithelium goes deeper into the lamina propria layer of the intestinal mucosa and forms numerous intestinal glands of various depths. It is known that undifferentiated epithelial cells are located at their bottom, which are stem cells for the epithelium. The lamina propria layer of the mucous membrane is formed by loose connective tissue, which contains reticular and collagen fibres. It also has many vascular plexuses. The muscle plate of the mucous membrane is located between the lamina propria layer and the submucosal base. It is weakly expressed and is represented by bundles of smooth myocytes. The submucosal base has the appearance of a thin layer of loose connective fibrous tissue, contains numerous blood vessels and nerve plexuses.

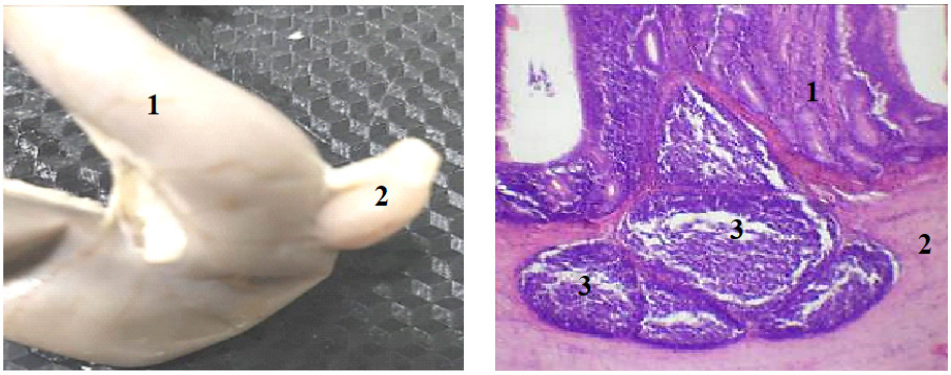
The muscular lining of ducks' intestines is formed by two layers of smooth muscle. The inner (circular) layer is better developed than the outer longitudinal one. In the duodenum, this membrane is three-layered: the outer and inner layers are longitudinal and the middle one is well developed – circular. Loose connective tissue with blood vessels and nerve nodes is contained between the layers of the muscle membrane and bundles of smooth myocytes.

In the intestinal wall of ducks, Meckel's diverticulum, Peyer's spots, and two glaucous diverticulae are registered among the immune formations. Unlike other birds, ducks do not have pronounced caecum tonsils, which is consistent with the data of other authors [6].

Peyer's spots are located in the wall of the small intestine and caecum. They are not detected in the rectum. The spots are visible visually without the use of special methods and have a lighter colour, a thinned bumpy wall and a spongy-porous appearance due to the numerous holes of the crypts. One such spot was located in the duodenum and ileum, and 3-4 in the jejunum. The largest number of spots is located in the caecum – 27-46. They are round, oval, and conical in shape.

Meckel's diverticulum is a remnant of the yolk sac duct. It is visible in ducks in the form of a pear-shaped protrusion on the antimesenteric surface of the jejunum, and its tip is directed cranially (Fig. 4A). The caecum of ducks on its cone-shaped tips contains lymphoid tissue that forms apical caecum diverticula. The latter have a lighter colour compared to other areas of the caecum.

Some authors explain the presence of well-developed lymphoid tissue in Peyer's spots and caecum diverticula by colonising them with urea-processing bacteria [9]. In all immune formations of the duck intestine, the lymphoid tissue is morphofunctional mature and is represented by a diffuse form and secondary lymphoid nodules (Fig. 4B, 5, 6). Pre-nodules and primary lymphoid nodules occur only on certain histoses of these structures. It is located mainly in the villi, between the crypts and in the submucosal base. In the areas where the lymphoid tissue is located, thickening of the mucous membrane is observed. In some places, it deapens between the surface bundles of smooth myocytes of the circular layer of the muscle membrane. That is, lymphoid tissue is located not only in the mucous membrane, but also in the muscular lining of the intestines of ducks. Lymphoid cells of this tissue locally infiltrate the villi epithelium and crypts. Such infiltration leads to the formation of lymphoepithelium. Primary and secondary lymphoid nodules are mostly elongated-oval, triangular (with a wide base), and rounded in shape. Reticular fibres in nodules have the greatest thickness in the membrane, giving them shape. Usually, secondary lymphoid nodules are larger than primary ones.



A B

Figure 4. Meckel diverticulum of the duck's empty intestine at the age of 150 days: A. Macropreparation: 1 – Jejunum; 2 – Meckel's diverticulum. B. Histopreparation:1 – mucous membrane; 2 – muscle membrane; 3 – secondary lymphoid nodules. Hematoxylin and eosin staining, ×90

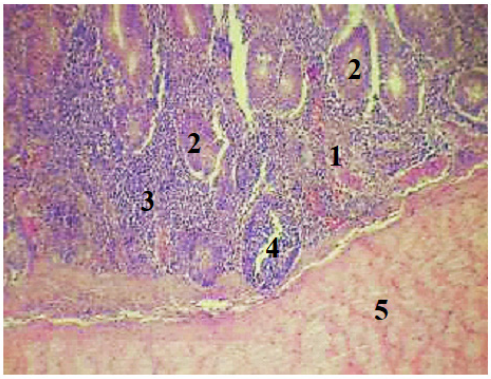


Figure 5. Peyer's spot of 12-duodenum of a duck aged 150 days: 1 – mucous membrane; 2 – crypts; 3 – diffuse lymphoid tissue; 4 – secondary lymphoid nodule; 5 – muscle membrane. Hematoxylin and eosin staining, ×63

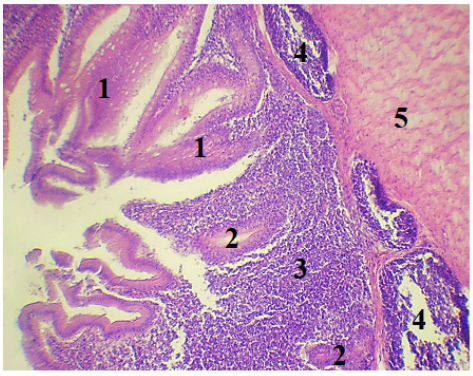


Figure 6. Apical caecum diverticulum of a duck aged 150 days:1 – villi; 2 – crypts; 3 – diffuse lymphoid tissue; 4 – secondary lymphoid nodules; 5 – muscle membrane. Hematoxylin and eosin staining, ×63

Lymphoid nodules are not equally located in the mucous and muscular membranes. In the mucosa – they are localised mostly perpendicular to the surface, and in the muscle-parallel to the bundles of gdad myocytes. Nodules are arranged singly, in pairs, or in larger groups. In the diverticulum of Meckel ducks, secondary lymphoid nodules are located behind the entire plane of lamina propria of the mucous membrane and submucosal base, and in the muscle membrane, they are deepened in a circular layer and placed in groups. In Peyer's spots and caecum diverticula, lymphoid nodules are arranged in a chain in the muscle membrane at the border with the submucosal base.

Content of lymphoid tissue in the digestive system

As noted above, lymphoid tissue forms the basis of immune formations. In the walls of the digestive canal organs, it occupies a different area (Table 1).

Table 1. Area of lymphoid tissue in the walls of the digestive canal of ducks aged 150 days, %, m ± m, n = 6

Organs and their parts	Area of lymphoid tissue
Cervical oesophagus	5.45 ± 0.32
Thoraco-abdominal part of the oesophagus	6.39 ± 0.72
Oesophagal tonsil	61.22 ± 0.65
Glandular part of the stomach	19.18 ± 0.21**
Intermediate zone of the glandular part of the stomach	22.34 ± 0.27**
Muscular part of the stomach	3.14 ± 0.56
Pyloric part of the stomach	5.63 ± 0.23*
Peyer's spots 12-duodenum	38.32 ± 0.87
Peyer's empty bowel spots	44.28 ± 0.77

Table 1, Continued

Organs and their parts	Area of lymphoid tissue
Peyer's spots of the ileum	55.12 ± 0.89
Peyer's caecum spots	36.55 ± 0.58
Meckel's Diverticulum	49.45 ± 0.92
Coccygeal diverticula	62.21 ± 0.39

Note: * P < 0.01, compared to the muscular part of the stomach; **P < 0.001, compared to the muscular and pyloric parts of the stomach

As can be seen from the data in Table 1, the smallest area of lymphoid tissue is occupied in the cervical and thoraco-abdominal parts of the oesophagus, the muscular and pyloric parts of the stomach. Therewith, the content of this tissue in the thoraco-abdominal part of the oesophagus is 0.94% higher than in the cervical part of this organ, and in the pyloric part of the stomach – by 2.49% (p<0.01), from its muscular part. A large area of lymphoid tissue is recorded in the glandular part of the stomach and its intermediate zone, respectively, by 16.04% (p<0.001) and 19.2% (p<0.001), compared with the muscular part of the stomach, and by 13.55% (p<0.001) and 16.71% (p<0.001) – with the pyloric part of the stomach. It is located locally in the mucous membrane and submucosal base of these organs and their parts. A substantial content of lymphoid tissue is observed in immunocompetent structures – tonsils, diverticula and Peyer's spots in the intestines of ducks. The largest area of lymphoid tissue was recorded in the diverticula and oesophagal tonsil (over 60%) and slightly smaller in Peyer's bowel spots and Meckel's diverticula (35-55%). Therewith, as noted above, the lymphoid tissue in the oesophagal tonsil is located in the mucous membrane and submucosal base of this area of the oesophagus, and in the immune formations of the intestine, it is also well expressed in the muscle membrane.

Conclusions

Immune formations of the digestive organs of ducks are associated with the mucous membrane and submucosal base, in the intestines, they are also located in the muscle membrane. Some of them are macroscopically invisible. In certain areas, they are well developed and protrude above the surface of the mucous membrane and have specific names (tonsils, Peyer's spots). The basis of immune formations is formed by lymphoid tissue, which in the organs of the digestive canal of ducks is morphofunctional mature and is represented by a diffuse form, primary and secondary lymphoid nodules, which are not equally expressed in certain parts of them.

Diffuse lymphoid tissue has no clear borders, and the membranes in the lymphoid nodules are well defined. Most nodules have light centres that are formed as a result of antigenic stimulation. Lymphoid tissue is best developed in substantial immunocompetent structures (oesophagal tonsil, caecum diverticula, Meckel diverticula, and Peyer's intestinal spots). In the wall of the oesophagus and stomach of ducks, only minor local accumulations of it are located.

It is important to consider the results obtained when raising and operating this poultry, which will ensure the formation of an appropriate level of specific immunity.

Further studies should be aimed at investigating the morphological features of the digestive canal organs and their immune formations in Mulard ducks in the age aspect.

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Морфологія органів травного каналу та їхніх імунних утворень в качок породи «Мулард»

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Анотація. Відомо, що в імунних утвореннях травного каналу птахів, які відносять до периферичних органів гемопоезу та лімфопоезу, відбувається диференціація Т- та В-лімфоцитів під впливом антигенів, які зумовлюють розвиток специфічного (клітинного і гуморального) імунітету. У зв'язку з цим, метою цього дослідження було з'ясувати особливості морфології органів травного каналу та їх імунних утворень у качок гібридної м'ясної породи «Мулард» віком 150 діб на період статевої зрілості. Під час проведення гістологічних досліджень шматочки з різних ділянок (стравоходу, частин шлунка, кишок із розташованими в них плямками Пейера, дивертикул Меккеля та сліпокишкові дивертикули) відбирали, етикетували та фіксували у 10 % водному розчині нейтрального формаліну і заливали в парафін, відповідно до загальноприйнятої методики. На гістологічних препаратах вивчали особливості мікроскопічної будови органів травного каналу та їхніх імунних утворень і гістотопографію, досліджували різновиди форм лімфоїдної тканини та підраховували площу цієї тканини. Встановлено, що імунні утворення органів травного каналу качок представлені всіма рівнями структурної організації лімфоїдної тканини, які неоднаково виражені в певних їхніх частинах. Скупчення імунних утворень в стінках стравоходу і шлунка розташовані у власній пластинці слизової оболонки та підслизовій основі, а в кишечнику – ще й у м'язовій оболонці. Найкраще лімфоїдна тканина розвинена у стравохідному мигдалику, сліпокишкових дивертикулах, дещо менше у дивертикулі Меккеля та плямках Пейєра кишечника. У стінці стравоходу та шлунка качок спостерігаються лише незначні скупчення цієї тканини. Отримані результати щодо морфофункціонального стану периферичних органів гемопоезу та лімфопоезу дають можливість удосконалювати технології вирощування і експлуатації птахів з метою забезпечення їхньої високої життєздатності та продуктивності

Ключові слова: свійська птиця, стравохід, шлунок, кишечник, лімфоїдна тканина, лімфоїдні вузлики

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Cellular Composition of the Lymphoid Tissue of the Cecal Immune Formations in Ducks

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Abstract. Observing the quantitative and qualitative composition of immunocompetent cell populations of the lymphoid tissue of the immunogenic organs allows to determine the immune status of the organism in a certain age period. The object of research is determing the cellular composition of the lymphoid tissue of the cecal Peyer's patches and cecal (apical) diverticula in ducks in age-concerned aspect. Material simples for research were selected from broiler ducks of the Blagovarsky cross. Cytological testss were performed on imprint specimens. Immunoblasts, lymphocytes, proplasmocytes, and plasmocytes, monocytes, and macrophages are distinguished among the cells of the lymphoid tissue of the cecal Peyer's patches and the cecal diverticula. The content of these cells is not the same. Population of lymphocytes in imprint specimens is the biggest. It consists of subpopulations of small, medium, and large lymphocytes, the ratio is uneven. The largest is a subpopulation of small lymphocytes, and the smallest is a subpopulation of large ones. The total content of lymphocyte in cecal Peyer's patches and cecal diverticula decreases with age of the subject ducks. The content of small and medium-sized lymphocytes in the cecal diverticula and small lymphocytes in the cecal Peyer's patches as well decreases. Simultaneously, the content of large lymphocytes in the cecal diverticula, large and medium lymphocytes in the cecal Peyer's patches increases. The immunoblasts content in the lymphoid tissue of the studied immune formations decreases with age of ducks, while the quantity of macrophages and monocytes conversely increases. Proplasmocytes and plasmocytes are detected in the lymphoid tissue of cecal Peyer's patches and cecal diverticula from the age of 10 days in ducks. Their content increases significantly with the poultry age. Reticular cells observation if complicated due to their location under a dense layer of lymphoid cells. Fibroblasts, M-cells, erythrocytes, and granulocytes in imprint specimens are detected in trace amounts. The established changes in the cellular composition of the lymphoid tissue of the cecal Peyer's patches and the cecal diverticula in ducks in the age-related aspect confirm the occurring immune reactions within them. Consideration of these changes will improve the effectiveness of anti-epizootic measures

Keywords: Peyer's patches, cecal diverticula, lymphocytes, plasmocytes, macrophages

Introduction

The intensive development of poultry farming in Ukraine and the rapid growth of production volumes require significant attention from morphologists in conducting the necessary comprehensive studies of the morphogenesis of all body systems, especially the poultry digestive system, to prevent diseases, effectively treat them, and obtain high-quality food products [1].

The main function of immunocompetent organs and structures of the body is the formation of immunity. They can produce cellular and humoral factors that free the body of foreign proteins (antigens). Therefore, the morphofunctional state of the immunogenesis organs substantially affects the viability and productivity of poultry. Improvement of poultry breeding and exploitation technologies in order to ensure their viability and Table increase in productivity is possible only in the presence of deepened knowledge about the peculiarities of the morphofunctional formation of the immunogenous organs in the postnatal

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period of ontogenesis, including their cellular composition. Study on quantitative and qualitative characteristics of immunocompetent cells populations of lymphoid tissue of the immune formations in mammals and birds digestive tract make it possible to assess the immune status of the organism in a certain age period and, accordingly, to form a diet and organize anti-epizootic measures.

The immune system includes the hematopoietic and lymphopoietic organs, which also include clusters of different lymphocyte populations are localized in all tissues of the organism. The birds' immune system ensures homeostasis of their organism by destroying genetically foreign agents. It produces substances and cells that neutralize antigens through immune recognition responses. These processes are characteristic of peripheral hematopoietic and lymphopoietic organs, including immune formations of the digestive system [2; 3].

In birds, lymphoid (immune) formations of the digestive canal is associated with mucosa, consist of separately located lymphoid nodules, their clusters (Peyer's patches), Meckel's diverticulum and cecal (apical) diverticula. Among the peripheral hematopoietic and lymphopoietic organs, they are the first to encounter antigens that enter the bird's organism through feed and water, and are constantly exposed to them [4]. Due to the significant antigenic load, the lymphoid tissue of the digestive canal is extremely well developed and makes up about 70% of the lymphoid tissue of all immunocompetent structures of the animal organism. In addition, it constantly differentiates harmless antigens present in feed or commensal bacteria from pathogenic bacteria. As a result, the lymphocytes population of immune formations of the digestive organs is substantially larger than in all secondary lymphoid organs combined [5].

Among the organs of the bird's digestive canal, immune formations are extremely well developed in the ceca, which is explained by the peculiarities of their purpose, namely: they provide the absorption of feed nutrients and water, as well as conversion of uric acid and the production of volatile fatty acids. The ceca are inhabited by microbiota involved in the utilization of feed with significant fibre content. They are peculiar antigens that affect the cecal mucosa, inducing the formation of immune formations in it [6-8].

Immune formations of the birds ceca are represented by cecal diverticula, cecal tonsils (one in each intestine), and numerous Peyer's patches. The latter are mainly localized in the body and apex of the intestines. As mentioned above, these immune formations, which are characterised by lymphocyte-epithelial symbiosis, belong to the peripheral organs of immunogenesis. The transformation of lymphocytes into effector cells occurs under the action of antigens within them. Effector cells, together with the secreted substances, determine the formation of cellular and humoral immunity [9-11]. It is the optimal balance of immunocompetent cells from pluripotent stem cells to effector cells (lymphocytes, plasmocytes, and macrophages) that ensures the formation of immunity. These cells are constantly in the processes of proliferation, differentiation, migration, cooperation, and apoptosis [12; 13].

In ducks, immune formations are located at the base of the ceca and are represented by numerous Peyer's patches and cecal (apical) diverticula. The literature contains information on the morphogenesis of the mentioned immune formations [14-16], but data on the cellular composition of their lymphoid tissue is insufficient.

The purpose of the study was to identify the dynamics of the quantitative and qualitative composition of immunocompetent cells populations of the lymphoid tissue of Peyer's patches and the cecal diverticula in ducks with age.

Materials and Methods

Cytological studies were performed in the scientific laboratory of immunomorphology of the Department of Anatomy, Histology and Pathomorphology of Animals named after academician Volodymyr Kasyanenko of National University of Life and Environmental Sciences of Ukraine during 2011-2021.

All manipulations and euthanasia of subject poultry were performed by acute exsanguination under ether anesthesia, for which chloroethyl was used in the form of inhalations. All procedures were conducted in accordance with the "General Ethical Principles of Animal Experiments" (Ukraine, 2001) [17], which is consistent with the Law of Ukraine "On the Protection of Animals from Cruelty" of 02/21/2006 No. 3447-IV [18] and the provisions of the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" [19].

Material for the study was selected from 80 broiler ducks of the Blagovarsky cross of 1, 5, 10, 15, 20, 25, 30, 60, 90, 120, 150, 180, 210, 240, 330, and 420 days old (5 ducks of each age).

Cytological studies were performed on imprint specimens. A section of the immune formation (Peyer's patch, cecal diverticulum) was cut with a sharp blade to prepare specimen samples. Futher, using filter paper, excess moisture was removed from it and applied to a defatted slide at the appropriate incision site. The obtained imprint specimens were air-dried. For cytological studies, they were stained according to Wright with commercial dyes Leucodif 200 (Erba Lachema, Czech Republic) and according to Papenheim with Hemocolor dyes (Merck, Germany) [20]. Stained imprint samples were examined using microscope Olympus (Japan) (× 1000). Cells were differentiated and their number determined. Cells were counted using microscope in 5 fields of view in one specimen. 50-70 cells were counted in one observed area [21]. The specimens were examined using light microscopes MBS-2, "Biolam" and "Olympus".

The results of the studies were entered into the protocols, and their digital indicators were subjected to statistical processing using a personal computer using the StatSoft Statistica 13.1 (2015) program, considering the specific features of statistical methods in biomedical studies [22].

Results and Discussion

As a result of the examination of imprint-samples of cecal Peyer's patches and cecal diverticula of ducks in the age-related aspect, populations of cells were identified in their lymphoid tissue: lymphoid lineage – immunoblasts, lymphocytes, proplasmocytes, and plasmocytes; blood cells – erythrocytes, granulocytes, monocytes, including macrophages, as well as tunica mucosa tissues – reticular, fibroblasts, epithelial, and M-cells (Figs. 1, 2).

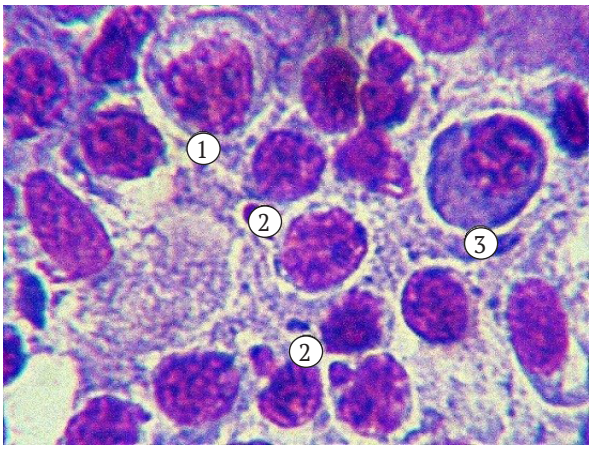

Figure 1. Cells of the cecal Peyer's patches in 240 day-old duck
Note: 1 – immunoblast; 2 – lymphocytes; 3 – plasmocyte. Imprint specimen. Wright staining, ×1000

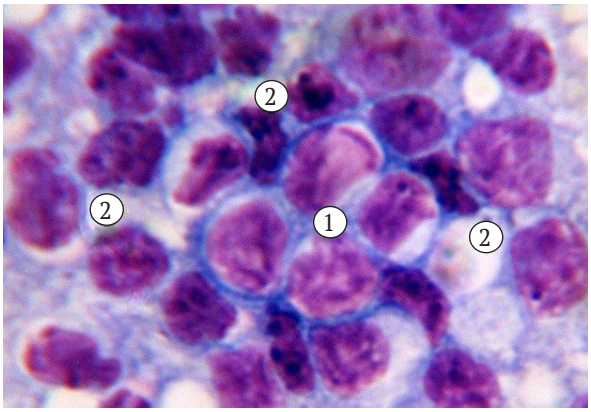

Figure 2. Cells of the cecal diverticula in 90 day-old duck
Note: 1 – immunoblasts; 2 – lymphocytes. Imprint specimen. Wright staining, ×1000

These cells in the immune formations of the digestive canal of birds were also identified by other researchers [23-25]. According to the results of the conducted studies, it was discovered that the content of populations of these cells is variable. Determining the content of reticular cells is complicated by their placement under a dense layer of lymphoid cells. Fibroblasts, M-cells, erythrocytes, and granulocytes in imprint specimens are detected in trace amounts.

Among the cells in the cecal Peyer's patches and the cecal diverticula in ducks, the lymphocytes are prevailing (Table 1, 2). This outcome corresponds consistent with data from other researchers who have studied the cellular contents of the esophageal tonsil [23], Peyer's patches [24] in chickens, and Peyer's patches in ducks [25]. Their structure is similar to that in mammals and other bird species. Lymphocytes on imprint specimens are predominantly rounded. Their nuclei are very large and occupy almost the entire volume of the cell and are basophilic, have different shapes and uneven contours (see Figs. 1, 2). The shape of the nuclei can vary from round to slightly elongate. Heterochromatin is attached in substantial amounts to the inner membrane of the nuclear envelope, and also in the form of grains and lumps is freely located throughout the nucleoplasm. Due to the high content of heterochromatin, it is difficult to differentiate the nucleolus in the nucleus. The cytoplasm of lymphocytes is poorly distincted. It is coloured slightly basophilic and surrounds the nucleolus in the form of a thin blue stripe.

Table 1. The cell content in cecal Peyer's patches in duck, %, M ± m, n = 5

Age, days	Immunoblasts	Lymphocytes	Proplasmocytes and plasmocytes	Macrophages and monocytes
1	33.57 ± 0.56	64.41 ± 0.53	–	2.02 ± 0.16
5	33.02 ± 0.39	64.91 ± 0.41	–	2.07 ± 0.09
10	32.17 ± 0.39	64.34 ± 0.28	0.87 ± 0.10	2.61 ± 0.08
15	31.79 ± 0.57	61.53 ± 0.27	3.34 ± 0.28*	3.34 ± 0.25
20	31.12 ± 0.54	60.58 ± 0.16	4.15 ± 0.4	4.14 ± 0.24
25	30.06 ± 0.35	59.66 ± 0.09	5.85 ± 0.22	4.43 ± 0.23
30	29.42 ± 0.53	59.49 ± 0.44	6.71 ± 0.17	4.37 ± 0.13

Table 1, Continued

Age, days	Immunoblasts	Lymphocytes	Proplasmocytes and plasmocytes	Macrophages and monocytes
60	29.71 ± 1.29	57.88 ± 1.34	7.69 ± 0.51	4.72 ± 0.41
90	28.34 ± 0.34	59.08 ± 0.39	7.10 ± 0.16	5.47 ± 0.14
120	29.12 ± 0.29	59.89 ± 0.41	6.88 ± 0.44	4.11 ± 0.33
150	29.27 ± 0.36	59.69 ± 0.41	6.29 ± 0.44	4.74 ± 0.04
180	28.91 ± 0.34	59.53 ± 0.37	6.42 ± 0.19	5.14 ± 0.12
210	26.94 ± 0.11	58.92 ± 0.23	8.18 ± 0.13*	5.96 ± 0.08
240	26.25 ± 0.07	59.39 ± 0.13	8.44 ± 0.17	5.92 ± 0.14
330	25.95 ± 0.19	59.21 ± 0.25	9.06 ± 0.32	5.78 ± 0.07
420	25.14 ± 0.15	59.25 ± 0.28	9.15 ± 0.46	6.46 ± 0.26

Note: * P < 0.05, compared to the indicator in the previous age group

Table 2. The cell content in the cecal diverticula in duck, %, M ± m, n = 5

Age, days	Immunoblasts	Lymphocytes	Proplasmocytes and plasmocytes	Macrophages and monocytes
1	28.84 ± 0.23	69.38 ± 0.26	–	1.78 ± 0.15
5	32.25 ± 0.30*	66.03 ± 0.39	–	1.72 ± 0.15
10	33.66 ± 0.47	63.76 ± 0.55	0.39 ± 0.11	2.18 ± 0.20
15	30.38 ± 0.46	62.97 ± 0.19	3.35 ± 0.29*	3.30 ± 0.30
20	30.41 ± 0.43	61.18 ± 0.21	3.86 ± 0.36	4.56 ± 0.25
25	29.86 ± 0.36	59.83 ± 0.17	5.80 ± 0.34	4.51 ± 0.20
30	29.09 ± 0.48	59.09 ± 0.42	7.03 ± 0.25	4.77 ± 0.16
60	30.01 ± 1.19	56.96 ±1.09	7.61 ± 0.39	5.42 ± 0.50
90	27.48 ± 0.40	57.80 ± 0.45	8.31 ± 0.19	6.40 ± 0.16
120	24.63 ± 1.87	62.66 ± 1.65	7.58 ± 0.33	5.12 ± 0.47
150	27.13 ± 0.44	60.59 ± 0.72	7.04 ± 0.54	5.25 ± 0.06
180	28.07 ± 0.47	58.46 ± 0.55	7.49 ± 0.24	5.99 ± 0.14
210	25.79 ± 0.12	57.81 ± 0.23	9.49 ± 0.14*	6.91 ± 0.09
240	25.08 ± 0.09	58.21 ± 0.14	9.82 ± 0.20	6.88 ± 0.16
330	24.69 ± 0.22	58.18 ± 0.24	10.45 ± 0.35	6.68 ± 0.08

Note: * P < 0.05, compared to the indicator in the previous age group

The content of lymphocytes in the lymphoid tissue of the cecal Peyer's patches and the cecal diverticula decreases with the increasing of the ducks age (see Table 1, 2). This is due to their differentiation into effector cells. Changes in the content of lymphocytes in the lymphoid tissue of the esophageal tonsil in chickens were also recorded by N.V. Dyshliuk [23]. According to the data, up to the 60-day age of chickens, there is an increase in this indicator, and in older birds – a decrease. In cecal Peyer's patches from 1 day to 420 days of the ducks age, the content of lymphocytes decreases by 1.09 times. In the cecal diverticula, this indicator decreases more rapidly (by 1.19 times) in a shorter period – from 1-day to 330-day age of ducks (the age when the cecal diverticula are detected for the last time). The decrease in the number of lymphocytes occurs unevenly and in waves. By the age of 60 days, the content of these cells in the cecal Peyer's patches decreases by 1.11 times, and in the cecal diverticula – by 1.22 times. Moreover, in the first five days of the duck's life, this indicator increases (by 1.01 times) in Peyer's patches of ducks, and the most intense (by 1.05 times) decrease is recorded in the cecal diverticula. The content of lymphocytes in the cecal Peyer's patches decreases most intensively (by 1.05 times) from 10 to 15 days. Over the period from 60 to 120 days, the content of lymphocytes in the cecal immune formations increases. Thus, in the cecal Peyer's patches, this indicator increases by 1.04 times (from 60 to 90 days – by 1.02 times, from 90 to 120 days – by 1.01 times). In cecal diverticula, during this period, the increase in the content of lymphocytes occurs more intensively – by 1.10 times (from 60 to 90 days – by 1.01 times, from 90 to 120 days – by 1.08 times). In older ducks, this indicator changes in waves, decreasing in the cecal Peyer's patches by 1.01 times to 420-day age, and in the cecal diverticula – by 1.08 times to 330-day age.

In the cecal Peyer's patches and the cecal diverticula, we found small (d up to 7 μm), medium (d 7-10 μm), and large (d more than 10 μm) lymphocyte among the total content of lymphocytes. The content of populations of these cells is not the same (see Table 3, 4).

Table 3. The content of different groups of lymphocytes in the cecal Peyer's patches in ducks, %, M ± m, n = 5

Age, days	Lymphocytes		
	Small	Medium	Large
1	56.44 ± 0.45	35.93 ± 0.71	7.63 ± 0.67
5	56.09 ± 0.93	35.83 ± 0.72	8.08 ± 0.71
10	55.38 ± 0.47	36.09 ± 0.66	8.52 ± 0.4
15	54.64 ± 0.36	35.93 ± 0.36	9.42 ± 0.57
20	54.86 ± 0.29	35.94 ± 0.49	9.20 ± 0.54
25	54.93 ± 0.94	36.23 ± 0.47	8.84 ± 0.64
30	53.83 ± 0.47	37.93 ± 0.76	8.24 ± 0.47
60	53.94 ± 0.22	35.92 ± 1.72	10.15 ± 1.23
90	52.43 ± 0.72	38.26 ± 0.1	9.31 ± 0.67
120	51.53 ± 0.27	38.06 ± 0.28	10.41 ± 0.38
150	52.46 ± 0.92	36.47 ± 0.81	11.07 ± 0.35
180	52.99 ± 0.33	36.19 ± 0.35	10.81 ± 0.35
210	53.28 ± 0.36	35.60 ± 0.39	11.12 ± 0.23
240	53.67 ± 0.13	35.25 ± 0.23	11.08 ± 0.17
330	53.12 ± 0.15	35.56 ± 0.18	11.32 ± 0.14
420	51.45 ± 0.42	36.69 ± 0.62	11.86 ± 0.54

Table 4. The content of different groups of lymphocytes in the cecal diverticula in ducks, %, M ± m, n = 5

Age, days	Lymphocytes		
	Small	Medium	Large
1	58.39 ± 0.47	35.88 ± 0.89	5.74 ± 0.62
5	58.56 ± 0.99	35.18 ± 0.88	6.26 ± 0.59
10	58.25 ± 0.65	34.92 ± 0.84	6.83 ± 0.36
15	57.68 ± 0.47	35.43 ± 0.44	6.89 ± 0.56
20	57.72 ± 0.49	34.33 ± 0.60	7.94 ± 0.63
25	58.65 ± 0.85	34.43 ± 0.40	6.92 ± 0.61
30	57.41 ± 0.38	35.13 ± 0.65	7.46 ± 0.50
60	56.42 ± 0.47	35.57 ± 1.29	8.00 ± 0.91
90	56.53 ± 0.73	36.58 ± 0.41	7.89 ± 0.81
120	55.27 ± 0.38	35.42 ± 0.29	9.31 ± 0.45
150	54.51 ± 1.21	35.21 ± 1.02	10.28 ± 0.32
180	57.73 ± 0.41	32.66 ± 0.30	9.61 ± 0.40
210	57.35 ± 0.53	32.60 ± 0.44	10.04 ± 0.11
240	58.12 ± 0.15	31.23 ± 0.17	10.66 ± 0.24
330	56.41 ± 0.19	33.32 ± 0.13*	10.27 ± 0.15

Note: * P < 0.05, compared to the indicator in the previous age group

In the studied immune formations in ducks of all age groups, small lymphocytes detected most of all. Their nucleus is very large, contains a lot of heterochromatin, and is intensely coloured. The cytoplasm volume of small lymphocytes is insubstantial. It has the appearance of a narrow blue stripe, which in the form of a sickle does not completely surround the nucleus. The content of small lymphocytes in the cecal Peyer's patches is less than in the cecal diverticula (see Table 3, 4). This indicator decreases unevenly by almost 1.10 times from 1-day to 420-day of age. The most intense decrease in this indicator is observed from 330 to 420 days (by 1.03 times) (see Table 3). The content of small lymphocytes in the cecal diverticula decreases unevenly by almost 1.04 times from 1-day to 330-day of age. Simultaneously, the minimum value of this indicator is recorded in 150-day-old poultry (54.51 ± 1.21%), and the maximum value is recorded in 25-day-old poultry (58.65 ± ± 0.85%) (see Table 4).

The results obtained are consistent with the data of K.O. Medvid [26] on the cellular composition of the cecal

tonsil in chickens. According to this information, the cecal tonsil in chickens aged from 1 to 90 days is mainly represented by diffuse lymphoid tissue, which is formed mainly by small lymphocytes, among which plasmoblasts and macrophages are found in small amounts.

Medium lymphocytes have a larger volume of the nucleus and cytoplasm. The heterochromatin saturation of the nuclei is lower, as a result of which they are less intensely stained (see Fig. 1). The content of medium lymphocytes in the lymphoid tissue of the cecal Peyer's patches and cecal diverticula is less than that of small lymphocytes (see Table 3, 4). In 1-day-old ducks in the immune formations, this indicator is almost the same (see Table 3, 4). In the cecal Peyer's patches and the cecal diverticula, the content of medium lymphocytes from 1-day to 90-day of age changes unevenly in waves, generally increasing almost by 1.07 and 1.02 times, respectively. In older poultry, up to 240 days of age, the content of medium lymphocytes in both immune formations decreases unevenly, reaching minimal values. From 90 to 240 days, the content of medium lymphocytes in the cecal Peyer's patches decreases by almost 1.09 times, and in the cecal diverticula – by 1.17 times. The most intense decrease in this indicator in the cecal Peyer's patches is recorded from 120 to 150 days (by over 1.04 times), in the cecal diverticula – from 150 to 180 days (by 1.08 times). In older poultry, the content of medium lymphocytes in the cecal Peyer's patches increases by 1.04 times, and from 330 to 420 days over by 1.03 times (see Table 3). In cecal diverticula from 240 to 330 days, the most intense increase (by 1.07 times) of this indicator is noted (see Table 4).

The content of large lymphocytes in the lymphoid tissue of the immune formations is the smallest (see Table 3, 4). Among lymphocytes, these cells have the largest volume of the nucleus and cytoplasm. The nucleus contains small lumps of heterochromatin and is less intensely stained. The cytoplasm appears as a thin, slightly basophilic streak. In the cecal Peyer's patches in ducks, the content of large lymphocytes is higher than in the cecal diverticula (see Table 3, 4). In the immune formations, this indicator increases unevenly. Thus, in the cecal Peyer's patches from 1-day to 420-day of age in ducks, the content of large lymphocytes increases by over 1.55 times (see Table 3), and in the cecal diverticula from 1-day to 330-day of age in ducks – by 1.79 times (see Table 4). The most intense growth of this indicator in Peyer's patches is observed in ducks aged from 30 to 60 days (by 1.23 times), and in the cecal diverticula – from 90 to 120 days (by 1.18 times) (see Table 3, 4).

Immunoblasts are cells of the fifth class of lymphopoiesis [27]. They are larger than lymphocytes. The shape of immunoblasts varies from round to slightly elongate. The cytoplasm of these cells has a much larger volume compared to lymphocytes (see Figs. 1; 2). It surrounds the nucleus in the form of a slightly basophilic streak of unequal thickness. The nucleus is round. Most immunoblasts contain two nucleoli. There is less heterochromatin in the nucleus than in lymphocytes. It is evenly sprayed in the nucleoplasm, and part of it is fixed near the inner membrane of the nucleolemma. The content of immunoblasts in the immune formations in ducks of all age groups is less than that of lymphocytes (see Table 1, 2). In most age groups of ducks, this indicator is higher in the cecal Peyer's patches than in the cecal diverticula. In general, the content of immunoblasts in the cecal Peyer's patches in ducks unevenly decreases from 1-day to 420-day of age by over 1.34 times (see Table 1), and in the cecal diverticula – by 1.17 times (see Table 2). This indicator decreases most intensively in Peyer's patches in ducks aged from 180 to 210 days (by 1.09 times). In the cecal diverticula in ducks from 1 to 10 days, the content of immunoblasts increases almost by 1.17 times, reaching its maximum value (see Table 2). A more pronounced increase in this indicator (by 1.12 times) is recorded from the first to 5 days. In older poultry, the content of immunoblasts decreases unevenly by over 1.36 times by the age of 330 days. With a maximum intensity (by 1.12 times), this process occurs in ducks aged from 90 to 120 days (see Table 2).

In the intestines of poultry, the content of antigens of various origins increases with age, in the neutralisation of which antibodies play a decisive role [28-30]. With the help of antigen-presenting cells, such as M cells, dendritic cells and macrophages, antigens from the intestinal lumen enter the lymphoid tissue of intestinal immune formations [25; 31; 32]. As a result of the action of the antigen, an increase in the number of plasma cells capable of producing antibodies is observed [23; 33; 34], which is confirmed by the results of our study.

Plasmocytes and proplasmocytes are found in small amounts in imprint specimens. Plasmocytes are effector cells of B-lymphocytes, proplasmocytes, are precursors of plasmocytes. Proplasmocytes are mostly small in size. Their shape varies from slightly elongated to irregular oval. The nucleus of proplasmocytes has uneven contours and shape. It is intensively coloured due to the high content of heterochromatin. The latter is partially sprayed in the nucleoplasm, but most of it is fixed to the inner membrane of the nucleolemma in the form of triangles and trapezoids. One or two nucleoli are found in the nucleus. The cytoplasm is stained basophilic and has a larger volume than that of lymphocytes. Plasmocytes are also small cells with an eccentrically arranged nucleus. They are characterised by a rounded or oval shape (see Fig. 1). There are many lumps of highly condensed heterochromatin in the nucleus, which is fixed to the inner membrane of the nucleolemma mainly in the form of triangles, forming a characteristic pattern of a wheel or watch face. The nucleolus cannot be detected in the nucleus. In the cytoplasm near the nucleus, a zone of brightening is found – this is the place of the Golgi complex localisation. The volume of the cytoplasm exceeds the volume of the nucleus. Proplasmocytes and plasmocytes in the lymphoid tissue of the cecal Peyer's patches and the cecal diverticula in ducks are detected in small amounts from the age of 10 days (see Table 1, 2). The content of these cells in the cecal Peyer's patches increases over tenfold with the age (by 10.52 times), in 420-day-old birds is 9.15 ± 0.46%, in the cecal diverticula – by 26.8 times, and in 330-day-old ducks, it is 10.45 ± 0.35 %. The maximum apparent increase in this indicator is recorded in ducks aged from 10 to 15 days: in the cecal Peyer's patches – by 3.84 times and in the cecal diverticula – by 8.59 times. Moreover, it is during this period that the cecal Peyer's patches show the most intense (by 1.05 times) increase in the content of lymphocytes.

Monocytes are known to be progenitors of macrophages. These are large cells that have a horseshoe-shaped or bean-shaped nucleus. Heterochromatin in the nucleus

condenses into lumps that are evenly distributed throughout the nucleoplasm. All common-functions organelles are well developed in the cytoplasm. Macrophages are big in size, have a round or elongated oval shape with uneven edges. The macrophage nucleus is small comparing to the cell volume. Heterochromatin is located in large amounts on the inner membrane of the nucleolemma and in the nucleoplasm. The cytoplasm of macrophages has a substantial volume and forms protrusions (cytopodia) of various sizes and shapes. It contains many large round shape lysosomes, many phagosomes of different sizes and shapes, fewer mitochondria, tubules of the endoplasmic reticulum, and elements of the Golgi complex. The content of monocytes and macrophages in the lymphoid tissue of the cecal Peyer's patches and the cecal diverticula in ducks of almost all studied age groups is the lowest (see Table 1, 2). This indicator in the cecal Peyer's patches increases unevenly from 1-day to 420-day of age by 3.20 times. A rapid increase in the content of monocytes and macrophages in the lymphoid tissue of the cecal Peyer's patches is recorded in the period from 5 to 20 days. Thus, from 5 to 10 days it increases by 1.26 times, from 10 to 15 days – by 1.28 times and from 15 to 20 days – by 1.20 times. In the cecal diverticula lymphoid tissue in ducks, this indicator increases unevenly from 1-day to 330-day age by 3.75 times. The maximum increase in monocytes and macrophages' content in the cecal diverticula's lymphoid tissue is recorded in ducks aged from 10 to 15 days (by 1.51 times).

Conclusions

Immunoblasts, lymphocytes, proplasmocytes, plasmocytes, monocytes, and macrophages are found among the cells of the lymphoid tissue of the cecal Peyer's patches and the cecal diverticula of all studied age groups in ducks. The quantitative characteristics of these cells populations are not the same. The most numerous population of the cecal Peyer's patches and the cecal diverticula in the imprint specimens are the lymphocytes. Their content decreases by 1.09 and 1.19 times, respectively, with the age of ducks. Among the lymphocytes, small, medium, and large forms are distinguished. Most of all, small lymphocytes are detected, and least of all – large ones. With the age of ducks, the content of small lymphocytes in the cecal Peyer's patches and the cecal diverticula decreases by 1.04 and 1.10 times, respectively, and medium lymphocytes in the cecal diverticula – by 1.08 times. The content of large lymphocytes increases in the cecal diverticula by 1.79 times, in the cecal Peyer's patches – by 1.55 times, and medium lymphocytes – by 1.02 times.

The content of immunoblasts in the lymphoid tissue of the cecal Peyer's patches and the cecal diverticula decreases by 1.34 and 1.17 times with the age in ducks, respectively.

Proplasmocytes and plasmocytes in the lymphoid tissue of the cecal Peyer's patches and the cecal diverticula are detected from the age of 10 days of ducks. Their content in the immune formations increases substantially with the age of the bird – by 10.52 and 26.80 times, respectively.

The content of macrophages and monocytes in the lymphoid tissue of the cecal Peyer's patches and the cecal diverticula increases by 3.20 and 3.75 times with the age in ducks, respectively.

In the future, it is planned to examine certain immunohistochemical characteristics of lymphocytes subpopulations and some cells of lymphoid tissue in the intestinal immune formations of Blagovarsky cross ducks.

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Клітинний склад лімфоїдної тканини імунних утворень сліпих кишок качок

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Анотація. Встановлення закономірностей щодо змін кількісного і якісного складу популяцій імунокомпетентних клітин лімфоїдної тканини органів імуногенезу дозволяє визначати імунний статус організму в певний віковий період. Метою дослідження було вивчити клітинний склад лімфоїдної тканини плямок Пейєра сліпих кишок і сліпокишкових (апікальних) дивертикулів качок у віковому аспекті. Матеріал для дослідження відібрали від

бройлерних качок Благоварського кросу. Цитологічні дослідження проводили на препаратах-відбитках. Серед клітин лімфоїдної тканини плямок Пейєра сліпих кишок та сліпокишкових дивертикулів виявляються імунобласти, лімфоцити, проплазмоцити і плазмоцити, моноцити і макрофаги. Вміст цих клітин неоднаковий. Популяція лімфоцитів у препаратах-відбитках є найбільшою. Вона складається із субпопуляцій малих, середніх і великих лімфоцитів, які є нерівнозначними. Найбільшою є субпопуляція малих лімфоцитів, а найменшою – великих. Загальний вміст лімфоцитів у плямках Пейєра сліпих кишок та сліпокишкових дивертикулах зменшується з віком качок. Водночас уміст малих і середніх лімфоцитів в сліпокишкових дивертикулах та малих лімфоцитів у плямках Пейєра сліпих кишок зменшується. Вміст великих лімфоцитів у сліпокишкових дивертикулах та великих і середніх лімфоцитів у плямках Пейєра сліпих кишок збільшується. Кількість імунобластів у лімфоїдній тканині досліджених імунних утворень з віком качок зменшується, а макрофагів і моноцитів – зростає. Проплазмоцити і плазмоцити у лімфоїдній тканині плямок Пейєра сліпих кишок та сліпокишкових дивертикулів виявляються з 10-добового віку качок. Уміст їх із віком птиці значно збільшується. Ретикулярні клітини розташовані під щільним шаром лімфоїдних клітин, тому їх визначення ускладнюється. Фібробласти, М-клітини, еритроцити і гранулоцити у препаратах-відбитках виявляються в слідових кількостях. Встановлені зміни клітинного складу лімфоїдної тканини плямок Пейєра сліпих кишок і сліпокишкових дивертикулів качок у віковому аспекті підтверджують перебіг в них імунних реакцій. Врахування цих змін дозволить підвищити ефективність проведення протиепізотичних заходів

Ключові слова: плямки Пейєра, сліпокишкові дивертикули, лімфоцити, плазмоцити, макрофаги

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Comparative Morphology of Tubular Bones of Blagovarsky Cross Ducks in the Postnatal Period of Ontogenesis

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Abstract. The microstructure of limb skeletal bones is closely related to ontogenetic age, localised skeletal growth dynamics, biomechanical modes of bone load, and possible taxonomic differences. This is important for the investigation of the problematic issues of ontogenetic changes in the compact bone tissue of domestic ducks. The purpose of the study was to compare the microstructure of the mid-diaphysis of the humerus and femur bones of Blagovarsky cross ducks, depending on age and gender. The material for research was the tubular bones of the thoracic (humerus) (n = 72) and pelvic (femur) (n = 72) limbs of Blagovarsky cross ducks aged 1 day, 10, 20, 30, 90, 196, 268, 341 and 483 days of postnatal ontogenesis of both sexual groups (females and males of 36 ducks each), a total of 72 ducks. The timing of the selection of ducks coincided with the technological cycle of their cultivation. Histological sections with a thickness of 5-10 microns were obtained, which were dyed with hematoxylin Karatsi and eosin, and according to Van Gieson for connective tissue differentiation. Morphometry determined the quantitative indicators of compact bone tissue in the middle of the diaphysis of tubular bones: the diameter of the diaphysis, the thickness of the periosteum, compact bone tissue, the diameter of osteons and central channels of osteons (Havers channels). It was discovered that the growth of the humerus and femur bones in length and thickness is completed on the 196th day of the postnatal period of ontogenesis in both female and male ducks. It was established that medullary (cerebral) bone tissue (1.01 ± 0.10 mm) appears from the femoral endostus of females, which is formed on the 196th day of the postnatal period of ontogenesis (the beginning of sexual maturity of females) and is further observed in intensive periods of their egg production on 268 and 341 days (2.43 ± 0.56 and 2.55 ± 0.62 mm, respectively), and disappears on the 483rd day (0.20 ± 0.03 mm) when the duck is not laying eggs. This study for the first time determined the age dynamics of morphometric parameters of microstructures of compact bone tissue of the humerus and femur and established their sex differences in ducks of the Blagovarsky cross. The results of comparative morphology studies are necessary for determining the age, sex, and species of birds based on the microstructure of compact bone tissue in the middle of the diaphysis of tubular bones, and for the ability to differentiate changes in the occurrence of limb pathologies in poultry

Keywords: humerus and femur, age groups, female, male, microstructure, diaphysis

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Introduction

Ontogenetic adaptations regarding the shape of limb bones in birds during the postnatal period of ontogenesis are associated with the distribution of static resistance and dynamic load on the musculoskeletal system. Constant load on the skeleton of body weight is an important factor in the formation of the structure, development and strength of tubular bones [1-3].

The microstructure of the limb skeleton bones is closely related to the age of the bird, the dynamics of localised growth of skeletal elements, biomechanical modes of bone load, and possible taxonomic differences. The variability of the microstructure of bones from different parts of the skeleton indicates differences in the rate of its growth in response to functional loads and the possible development of pathology [4-6].

Microstructural adaptations are most pronounced in the lamellar bone. However, compact bone tissue consists of units known as the Havers system, but some bird species may not have the typical Havers system described in mammals.

Some studies have shown that long bones, namely their structure and location of the Havers system, differ depending on the type of animal and the position of the bone in the skeleton of the limbs. Therewith, histological variations observed in the compact bone tissue of the mammalian and avian species under study can be associated with both the mechanical load acting on long bones and their different lifestyle [7; 8].

Medullary bone is an ephemeral type of bone tissue that occurs only in sexually mature female birds and is associated with reproductive function. This tissue is a rapidly mobilised source of calcium and phosphorus for egg shell formation [9]. The systemic distribution of medullary bone in the avian skeleton is used to identify reproductive tissues in tubular bones [10; 11].

Therefore, all of the above is of great importance for a better understanding of the micro - and macrostructure, and functional purpose of tubular bones in the postnatal period of ontogenesis of various bird species, depending on age and gender characteristics.

The purpose of the study was to determine age and sex differences in the microscopic structure in the middle of the bone diaphysis of the stylopodia of ducks of the Blagovarsky cross.

Literature Review

The dynamics of morphological, geometric, densitometric, and mechanical parameters of tubular bones vary depending on the type of animal with different growth rates, and the position of the bone in the skeleton of the limbs [12-14].

Thus, different microscopic structures and the absence of the typical Havers system observed in mammals were discovered in the compact bone tissue of tubular bones in different bird species [7]. The compact femoral bone tissue of the sparrow has one side vascular, and the other – avascular. Vascularisation is represented by primary vascular channels surrounded by several misplaced osteocytes inside their lacunae, which form primary osteons. Reduced vascular supply and the appearance of secondary osteons are associated with bone rearrangement in birds such as sparrows. On the other hand, in ducks and geese, compact bone tissue was similar and consisted mainly of dense Havers tissue, while in some parts of it Primary osteons were discovered. Morphological variations in the tubular bone observed in sparrows compared to ducks and geese in terms of microstructure and its different density can be explained by the fact that the biomechanical load on the tubular bone occurs differently in each of the investigated bird species [7].

In the postnatal period of ontogenesis, the tubular bones of chickens are characterised by a rough surface with numerous grooves (depressions) that contain small holes. On the other hand, the bones of chicks, which are as large as those of adults, but more slender, are characterised by the presence of single depressions and holes. According to the histological examination, the author confirmed that these rough surface textures belong to fibrolamellar bone tissue. Instead, the smooth textured surface of the tubular limb bones in adult birds is formed after the growth of bones in width stops and coincides with their plumage and reaching sexual maturity [15].

On the example of the growth and development of Avialans – *Cretaceous enantiornithine,* those who lived in the Mesozoic Era can trace the presence of intra-skeletal osteohistovariability, which identifies complex pre-structures in tubular bones. Their osteohistological data indicate a unique strategy for the growth of the middle diaphysis of tubular bones, characterised by a high rate in *Mirarce eatoni* (terrestrial Late Cretaceous bird). The radius consists of parallel fibrous bone, similar to histological descriptions of other enantiornithines. Other elements of bones, on the contrary, are noticeably different from other representatives of these bird species. The humerus consists of a parallel fibrous bone with a middle layer of the original fibrolamellar bone and several growth lines in the outer surrounding layer and at the border with the endosteum. The structure of the endosteal and periosteal layers of bone indicates cyclical changes in the rate of its growth. The femur is represented by sections of coarse compact spongy and parallel-fibrous bone with numerous secondary osteons, and only one growth line. Therewith, tarsometatarsus is predominantly fibrolamellar in texture with several asymmetric growth lines located behind the entire compact bone tissue, which forms a strong cortical drift. Growth lines in both the endosteal and periosteal parts of compact bone indicate that, as in the humerus, the growth rate of this bone changed cyclically. Therewith, the two phalanges of the fingers investigated consist of parallel fibrous bone and remodelling in areas of rough compact spongy bone [8].

In addition, variability in the evolution of *Avialans* is confirmed by changes in the evolution of the three main bone layers behind the entire compact bone, both between species and within the same species of extinct Avialans. However, the most noTable is the adaptive change from the general lamellar (parallel-fibrous) bone tissue to the fibrolamellar complex in the middle part of the compact bone, both in representatives of the *Pygostylia,* and *Jeholornithiformes*. This change is usually associated with an increase in the density of compact bone tissue and complexity in the structure of the neurovascular network [13].

Changes in the condition of the skeleton of the limbs of poultry depend on the speed of its growth. More body weight, and in particular, the pectoral muscles, play

an important role in the development of pathologies of the pelvic limb skeleton. Such pathologies can be detected by the method of geometric morphometry, which is a tool for analysing the dynamics of bone shape and its correlation with changes in the skeletal movements of broiler chickens. Weight gain is positively correlated with the curvature of the anterior-posterior tibial axis; tibial shape and active behaviour during movement have a positive correlation by which the genetic lines of broiler chickens can be distinguished; the predominance of static behaviour also correlated with the curvature of the anterior-posterior tibial axis [16].

The movements of poultry (laying hens) that are raised in enclosures that differ in structure depend on the type of locomotion (the duration of standing, sitting, walking, running, flying, and the indicators of jumping, flying, group running, and walking), the occurrence of genetic stress associated with the musculoskeletal system and on breed characteristics. Thus, white-feathered young birds had proportionally stronger shins and femurs compared to brown ones. Therewith, the type of locomotion of poultry did not affect the strength of tubular bones and their deformation indicators. However, differences in the design of enclosures for raising chickens can affect the types of movement of birds and, in turn, the development of their skeleton, which is confirmed by the appearing deformities [17].

In addition, the condition of the limb skeleton can be assessed by comparative analysis of bird bone tissue, depending on age, breed, and calcium content in it. Such an assessment is possible by applying the coefficient of uniformity of bone hardness and its correlation with the age of birds and the strength of bone material, which allowed reliable assessment of the qualitative condition of tubular bones, depending on the influence of the internal and external environment. Based on the developed approach, it is easy to trace the features of the growth and development of tubular bones in the skeleton of any animal [4].

In the tubular bones, during egg production of female birds, a medullary bone is formed, which is estrogen-dependent and its deposition is dictated by skeletal distribution patterns, both pneumaticity and bone marrow; two environmental factors (body size, food production). Thus, the presence of medullary bone in the skeleton can be widely represented in small and diving birds, but more limited – in large-bodied bird species and those that fly intensively [10; 18].

The microscopic structure of tubular bones, namely compact bone tissue, is used to differentiate bone fragments from different animal species. Currently, a large number of histological studies devoted to the dynamics of compact mammalian bone tissue are covered in scientific Ukrainian and foreign literature, but there are insufficient papers on the microstructure of tubular bones in poultry.

Materials and Methods

The object of the study in 2018 was ducks of the Blagovarsky cross in the postnatal period of ontogenesis. Ducks were raised in private production (village Tsebrykove, Velykomykhailivskyi district, Odesa region). According to the technology of breeding, the duck population is divided into the following age groups: rearing young birds aged 1 day, 10, 20, 30 and 90 days, and breeding stock aged 196 days (the beginning of egg production). Therewith, 268 and 341 days are intensive periods of egg production, and on 483 days egg production stops.

Keeping ducks is outdoor. The ducks were fed balanced feeds according to their age: wheat, corn, sunflower, and soy meal, fish meal, vegeTable oil, melusa (shell rock), monocalcium phosphate, Table salt, baking soda, premix (vitamins: retinol (a), cholecalciferol (D3), tocopherol (E), menadiol (K3), thiamine (B1), riboflavin (B2), niacin (B3), pyridoxine (B6), folic acid (B9), cyancobalamin (B12), magnesium, iron, copper, cobalt, iodine, selenium, methionine, lysine) [19]. Up to 32 days of the postnatal period of ontogenesis, a complete diet with the addition of premix was used (the duck is intensively gaining body weight); after 32 days, a diet without the addition of premix was used.

On the farm, ducks were vaccinated against pasteurellosis at the age of 40 days and hepatitis at the age of 60 days.

When working with experimental poultry, the "General Ethical Principles of Animal Experiments" adopted at the first National Congress on Bioethics (Kyiv, 2001) were adhered, which is consistent with the provisions "On the protection of animals from cruelty" [20] and "European Convention for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) [21].

Before slaughter, the ducks were weighed on VLKT-500 g-M scales (at the age of 1, 10 days) and electronic scales of the company "Nova" (at the age of 20, 30, 90, 196, 268, 341, and 483 days) and removed the humerus and femur.

The material for research was the tubular bones of the thoracic (humerus) (n = 72) and pelvic (femur) (n = 72) limbs of Blagovarsky cross ducks. Female and male ducks (36 heads each) were selected for the study at the age of 1 day, 10, 20, 30, 90, 196, 268, 341, and 483 days of postnatal ontogenesis.

Later, after slaughter, duck carcasses were dissected and the tubular bones of the thoracic and pelvic limbs were removed. The left tubular bones were fixed in a 10% neutral aqueous formalin solution. Then, columns were cut from the middle of the diaphysis (0.5 cm) and decalcified in an 8% nitric acid solution, and degreased and dehydrated in 96% and 100% alcohol. After that, the alcohol-ether was poured into celloidin. Using a Microtome (Easily ERM3000), histological sections with a thickness of 5-10 microns were obtained, which were dyed with Karatsi hematoxylin and Van Gieson eosin for connective tissue differentiation [22].

Light microscopy and photographing of histological preparations were performed using a MICROmed XS 5520 microscope and a MICROmed – 5.0 Mega CMOS camera. Subsequently, using the method of histomorphometry, quantitative indicators of bone tissue were obtained by optical-visual method. When describing the structure of tissues, histological nomenclature was observed [23].

The obtained digital results of the study were subjected to statistical processing using the "Microsoft Excel" software package. Therewith, the arithmetic mean and its error (M ± m), the probability level (P) were calculated according to the Student's Table ($P < 0.05$; $P < 0.01$; $P < 0.001$) [24].

Results and Discussion

As a result of the study of tubular bones of ducks of the Blagovarsky cross in the postnatal period of ontogenesis, the microscopic structure of the middle of the diaphysis was established and morphometric indicators of microstructures of compact bone tissue were obtained (Table 1).

Table 1. Dynamics of morphometric parameters of compact bone tissue in the middle of the humerus diaphysis, mm, M ± m, n = 8

Age, days	Sex ♀♂	Diaphysis diameter	Periosteum thickness	Thickness of compact bone tissue	Osteones diameter	Central osteones canals (Havers channels) diameter
1	♀	1.10 ± 0.22	0.04 ± 0.01	0.15 ± 0.06	0.04 ± 0.01	0.03 ± 0.01
	♂	1.19 ± 0.15	0.05 ± 0.01	0.16 ± 0.04	0.05 ± 0.01	0.04 ± 0.01
10	♀	1.71 ± 0.15	0.06 ± 0.01°	0.21 ± 0.03	0.07 ± 0.01°°	0.05 ± 0.01°
	♂	1.65 ± 0.04°	0.06 ± 0.01	0.21 ± 0.02	0.08 ± 0.01°	0.06 ± 0.01°
20	♀	3.12 ± 0.20°°	0.08 ± 0.02°°°	0.42 ± 0.06°	0.14 ± 0.06	0.05 ± 0.00*
	♂	3.22 ± 0.21°°°	0.06 ± 0.01	0.59 ± 0.10°	0.07 ± 0.01	0.04 ± 0.00°
30	♀	4.61 ±0.34°°	0.11 ± 0.023	0.54 ± 0.10	0.08 ± 0.01	0.06 ± 0.00°
	♂	4.82 ± 0.34°°	0.08 ± 0.01°°	0.55 ± 0.10	0.07 ± 0.01	0.05 ± 0.01
90	♀	7.76 ± 0.23°°°	0.04 ± 0.01°	0.79 ± 0.03	0.17 ± 0.05	0.09 ± 0.07
	♂	8.10 ± 0.44°°	0.04 ± 0.01°°	0.75 ± 0.03	0.09 ± 0.01	0.80 ± 0.26
196	♀	7.69 ± 0.33	0.03 ± 0.01	0.60 ± 0.05°	1.96 ± 0.41°°°	0.61 ± 0.21
	♂	7.25 ± 0.30	0.03 ± 0.01	0.68 ± 0.07	2.33 ± 0.27°°°	1.23 ± 0.23°
268	♀	6.44 ± 0.28°	0.03 ± 0.02	0.62 ± 0.05	2.05 ± 0.17	0.88 ± 0.20*
	♂	7.07 ± 0.19	0.01 ± 0.01	0.59 ± 0.02	1.23 ± 0.39	0.19 ± 0.17°
341	♀	6.95 ± 0.44	0.04 ± 0.01	0.49 ± 0.02°	0.07 ± 0.01°°°	0.01 ± 0.00°°
	♂	6.69 ± 0.66	0.03 ± 0.01°°°	0.59 ± 0.06	0.07 ± 0.01°	0.02 ± 0.00*
483	♀	6.95 ± 0.51	0.02 ± 0.01°	0.58 ± 0.02	0.07 ± 0.01	0.01 ± 0.00
	♂	7.05 ± 0.46	0.03 ± 0.01	0.65 ± 0.04	0.06 ± 0.01	0.01 ± 0.00°°

Note: °P < 0.05; °°P < 0.01; °°°P < 0.001, compared to the previous age;
* P < 0.05, compared to the female and male of the previous age

Based on the results obtained, presented in Table 1 it follows that on day 1 of the postnatal period of duck ontogenesis, there was no statistically substantial difference in the dynamics of morphometric parameters of compact bone tissue in the middle of the humerus diaphysis. On the 10th day, compact bone tissue continues to rebuild and its size increases. Thus, the diameter of the humerus diaphysis of females substantially increases by 1.6 times (P < 0.05), and males – by 1.4 times (P < 0.05), compared to the previous age. Therewith, the diameter of the femoral diaphysis of females increases by 1.7 times (P < 0.05) (Fig. 1, c), and males – 1.6 times (P < 0.05) compared to the previous age (see Table 2).

Table 2. Dynamics of morphometric parameters of compact bone tissue of the middle of the femoral diaphysis, mm, M ± m n = 8

Age, days	Sex ♀♂	Diaphysis diameter	Periosteum thickness	Thickness of compact bone tissue	Diaphysis diameter	Central osteones canals (Havers channels) diameter
1	♀	1.64 ± 0.04	0.04 ± 0.01	0.23 ± 0.01	0.04 ± 0.01	0.03 ± 0.00
	♂	2.04 ± 0.34	0.03 ± 0.01	0.36 ± 0.12	0.05 ± 0.01	0.03 ± 0.01
10	♀	2.73 ± 0.37°	0.05 ± 0.01	0.30 ± 0.05	0.06 ± 0.01°	0.04 ± 0.00°
	♂	3.19 ± 0.17°	0.06 ± 0.01°°	0.43 ± 0.04	0.08 ± 0.01	0.05 ± 0.01
20	♀	4.84 ± 0.07°°	0.05 ± 0.01	0.53 ± 0.03°°	0.07 ± 0.01	0.03 ± 0.00
	♂	5.26 ± 0.08°°°**	0.07 ± 0.01	0.69 ± 0.04°°*	0.07 ± 0.01	0.03 ± 0.00
30	♀	5.87 ± 0.24°°	0.07 ± 0.01	0.70 ± 0.06°	0.07 ± 0.01	0.02 ± 0.00
	♂	6,11 ± 0.19°°	0.07 ± 0.01	0.60 ± 0.02	0.08 ± 0.01	0.03 ± 0.01
90	♀	6.00 ± 0.19	0.02 ± 0.01°°	0.70 ± 0.04	0.15 ± 0.03°	0.39 ± 0.37
	♂	6.86 ± 0.29	0.06 ± 0.01°*	0.59 ± 0.05	0.14 ± 0.03	0.27 ± 0.22
196	♀	6.29 ± 0.31	0.04 ± 0.01°°	0.59 ± 0.03	2.44 ± 0.11°°°*	0.54 ± 0.32
	♂	6.91 ± 0.33	0.02 ± 0.01°	0.65 ± 0.06	1.30 ± 0.45°	0.47 ± 0.29
268	♀	5.95 ± 0.13	0.03 ± 0.02	0.56 ± 0.02	1.72 ± 0.09°°°	0.46 ± 0.27
	♂	5.93 ± 0.34	0.02 ± 0.01	0.63 ± 0.05	2.02 ± 0.10	0.44 ± 0.24

Table 2, Continued

Age, days	Sex ♀♂	Diaphysis diameter	Periosteum thickness	Thickness of compact bone tissue	Diaphysis diameter	Central osteones canals (Havers channels) diameter
341	♀	6.36 ± 0.28	0.06 ± 0.03	0.54 ± 0.03	0.08 ± 0.01°°	0.02 ± 0.01
	♂	6.59 ± 0.21	0.03 ± 0.01	0.66 ± 0.02*	0.07 ± 0.01°°°	0.02 ± 0.01
483	♀	5.98 ± 0.42	0.02 ± 0.01	0.65 ± 0.05	0.08 ± 0.01	0.02 ± 0.01
	♂	6.64 ± 0.13	0.02 ± 0.01	0.74 ± 0.08	0.23 ± 0.17	0.01 ± 0.00°

Note: °P < 0.05; °°P < 0.01; °°°P < 0.001, compared to the previous age;
* P < 0.05; * * P < 0.01, compared to the female and male of the previous age

In the humerus of females, osteons of irregular shape with radially directed central channels were discovered, which increase by 1.8 times (P < 0.01), and in males – by 1.6 times (P < 0.05); in the femur of females, osteons increase by 1.5 times (P < 0.05), and in males – by 1.6 times compared to the previous age (see Table 1; 2).

A study of the humerus of ducks discovered that on day 1, the bone tissue of the middle of the diaphysis was formed, but uneven in thickness. There is an osteonic type of structure of compact bone tissue. Osteons of irregular elongated circular shape with a large number of longitudinal expanded central channels, the concentric bone plates of which are in the stage of formation (Fig. 1). However, the density of osteons is uneven. The outer and inner surrounding plates are not formed. The outer and inner surfaces of the osteon layer of the diaphysis are uneven, and the proliferation of cells of the osteogenic layer of the periosteum is present in its cavities. The bone marrow in the humerus of females is predominantly red and red-yellow in males, while in the femur it is red-yellown both females and males.

On the 10th day of the postnatal period of duck ontogenesis, proliferation of cells of the osteogenic layer of the periosteum was detected (Fig. 1, a-d, 1), the formation of deep lacunae and wide cavities (Fig. 1, a-d, 2).

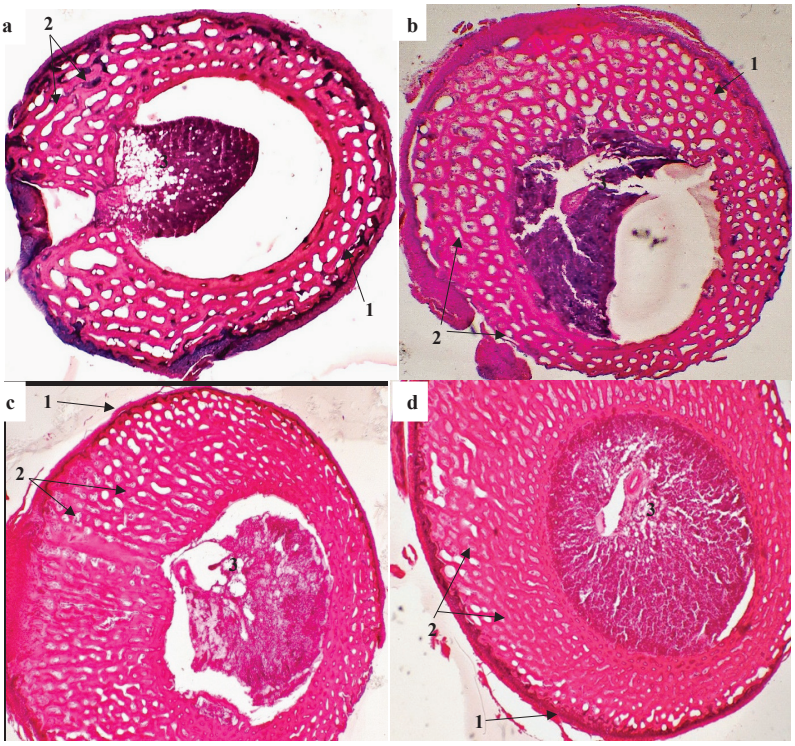

Figure 1. Microstructure of the cross-section of the middle of the diaphysis of the humerus (a, b) and femoral (c, d) bones of ducks aged 10 days (female – a, c; male – b, d): 1 – proliferation of cells of the osteogenic layer of the periosteum; 2 – central channels of osteons of various shapes; 3 – bone marrow. Staining with hematoxylin Karatsi and eosin. Magnification ×32 (a-d)

The formation of the outer and inner surrounding plates begins. The bone marrow in the humerus of females is red-yellow and red in males, and in the femur of females and males – red (Fig. 1, a-d, 3).

On the 20th day of the postnatal period of duck ontogenesis, the diameter of the humerus diaphysis of females increases 1.8 times (P < 0.01), males – 2.0 times (P < 0.001); femur of females – 1.8 times (P < 0.01), males – 1.7 times (P < 0.001) compared to the previous age. Therewith, the density of osteons increased compared to that of birds of the previous age, and the diameter of the central osteon channels (Havers channels) decreased (see Table 1, 2).

On the 30th day of the postnatal period of duck ontogenesis, the compact bone tissue of the humerus and femur

is represented by densely arranged osteons of various diameters and shapes (see Table 1). The diameter of the humerus diaphysis of females increases 1.5 times ($P < 0.01$), and males – 1.5 times ($P < 0.01$); femur of females – 1.2 times ($P < 0.01$) and males – 1.2 times ($P < 0.01$), compared to the previous age (see Table 1, 2).

In ducks aged 90 days (see Table 1) the cross-sectional diameter of the humerus diaphysis of females increased by 1.7 times ($P < 0.001$), and males – by 1.7 times ($P < 0.01$) compared to the previous age. Therewith, there is no statistically substantial difference in the diameter of the cross-section of the femoral diaphysis of females and males with such indicators of the previous age (see Table 2), (Fig. 2, a-d). Osteons are densely arranged, have a longitudinal and transverse course in the periosteal, mesostal and endostal zones (Fig. 2, a-d 1), and the spaces between osteons are filled with insertion (interstitial) bone plates (Fig. 2, a-d, 2).

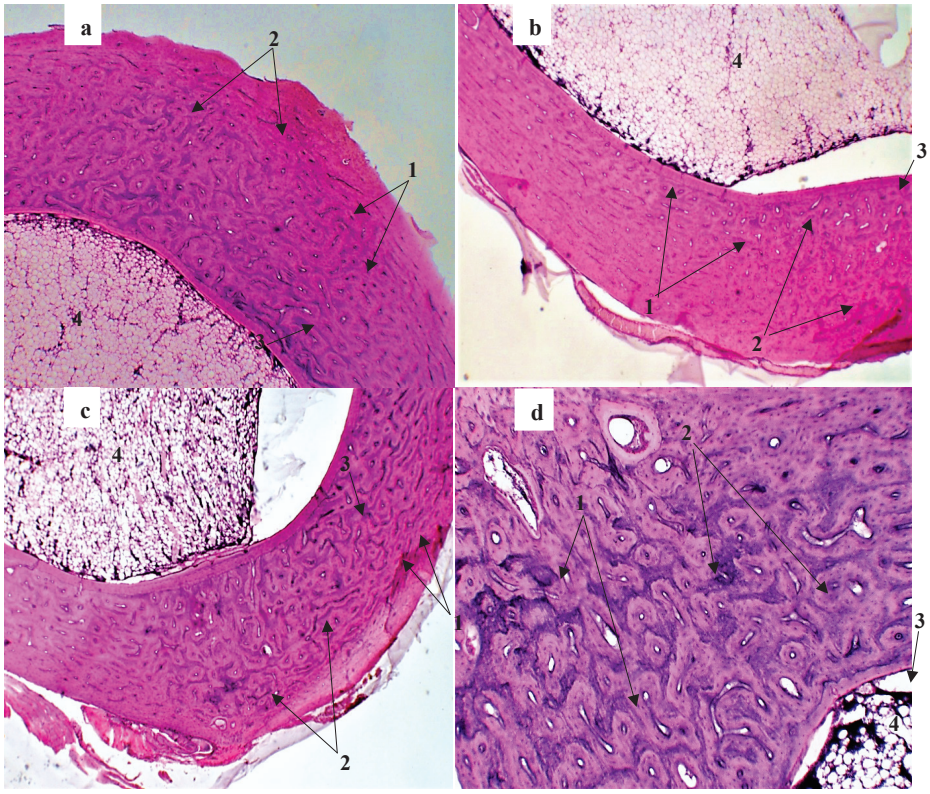

Figure 2. Microstructure of compact bone tissue of cross-section of the middle of the diaphysis of the humerus (a, b) and femoral (c, d) bones of ducks aged 90 days (female – a,c; male – b, d):
1 – osteons with longitudinally and transversely directed central channels; 2 – insertion (interstitial) bone plates; 3 – formed internal surrounding system of bone plates with Cementation lines; 4 – bone marrow.
Karatsi hematoxylin and eosin staining, ×30 (a-b), ×75 (g)

The diameter of the central osteon channels (Havers channels) increased slightly during this age period (see Table 1, 2). External surrounding system of bone plates in the process of formation. The internal surrounding system of bone plates is formed and clearly separated by Cementation lines, it borders the endoost (Fig. 2, a-d, 3). Bone marrow in the humerus and femur of females and males, mostly yellow (Fig. 2, a-d, 4). The compact bone tissue of the middle part of the humerus diaphysis on the 196th day of the postnatal period of duck ontogenesis is formed due to densely located osteons in the peri-, meso-, and endostal zones, which have mainly longitudinal and transverse directions (Fig. 3, a-d, 1).

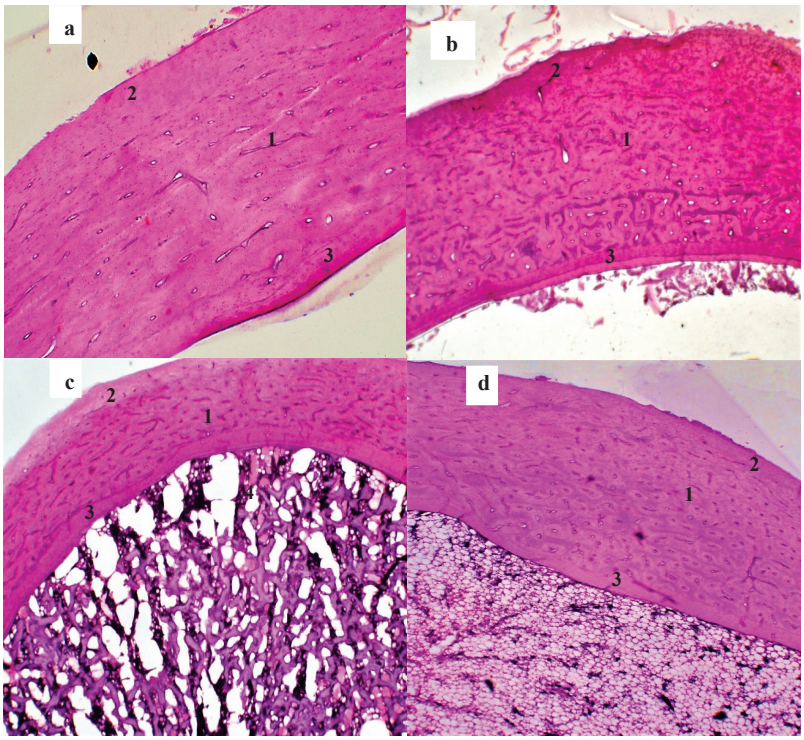

Figure 3. Definitive microstructure of the cross-section of the middle of the diaphysis of the humerus (a, b) and femoral (c, d) bones of ducks aged 196 days (female – a, c; male – b, d): 1 – osteons of compact bone tissue; 2 – formed external and 3 – Internal surrounding systems of bone plates; 4 – medullary (brain) bone tissue. Karatsi hematoxylin and eosin staining, ×30 (b-d), ×75 (a)

On day 196 of the postnatal period of duck ontogenesis, there was no statistically substantial difference in the diameter of the humerus and femoral diaphysis, both with those of the previous age and between the female and male. Diameter of osteons in the humerus of females (see Table 1) increases by 1.5 times ($P < 0.001$) and males – by 25.9 times compared to the previous age. Therewith, the diameter of osteons in the femur of females increases by 16.3 times ($P < 0.001$) and males – by 9.3 times ($P < 0.05$) compared to the previous age (see Table 2). Therewith, the diameter of osteons in the femur of females increased by 1.9 times ($P < 0.05$), compared with this indicator in males of this age.

During this age period, external (Fig. 3, a-d, 2) and internal (Fig. 3, a-d, 3) the surrounding systems of bone plates are formed, their surface is mostly smooth. Medullary bone tissue is absent in the humerus. The bone marrow in the humerus of female and male ducks is yellow. This structure corresponds to the definitive stage of tubular bone development.

In females of this age period, medullary (cerebral) bone tissue appears in the bone marrow cavity of the femur, its thickness is 1.01 ± 0.10 mm, which displaced and replaced part of the bone marrow. Medullary tissue is represented by bone junctions, and the cavities between them are filled with oxyphilic protein fluid (Figs. 3, b, 4). This is due to the onset of sexual maturity and preparation for the egg production period of the breeding stock of ducks. The bone marrow in the tubular bones of both females and males is predominantly yellow.

The microstructure of the cross-section of the middle of the duck humerus diaphysis on day 268 of the postnatal period of ontogenesis is characterised by the presence of an external surrounding system of bone plates, an osteon layer that includes osteons and insertion systems of bone plates (Fig. 4, a-d, 1) and internal surrounding systems of bone plates.

The diameter of the humerus diaphysis of females decreases by 1.2 times ($P < 0.05$) compared to the previous age. Therewith, the diameter of osteons in the compact femoral bone tissue of females decreases by 1.4 times ($P < 0.001$) compared to the previous age.

External (Fig. 4, a-d, 2) and internal (Fig. 4, a-d, 3) surrounding systems of bone plates on the periosteal and endosteal surfaces of the osteon layer of compact bone are formed. Medullary bone tissue is absent in the humerus of females.

In the femur of females, a layer of medullary bone tissue of uneven thickness in most of the compact bone tissue of the middle of the diaphysis. Therewith, the intertrabecular spaces of medullary bone tissue are filled with protein masses and blood cells (Fig. 4, c, 4). This indicates an intensive period of egg production of the breeding stock of ducks, which corresponds to the technology of raising poultry. The bone marrow in the humerus and femur of females and males is yellow (Fig. 4, c, d, 5).In ducks on 341 days of postnatal ontogenesis, compact bone tissue is represented by densely located osteons in the peri-, meso- and endostal zones (Fig. 5, a-d).

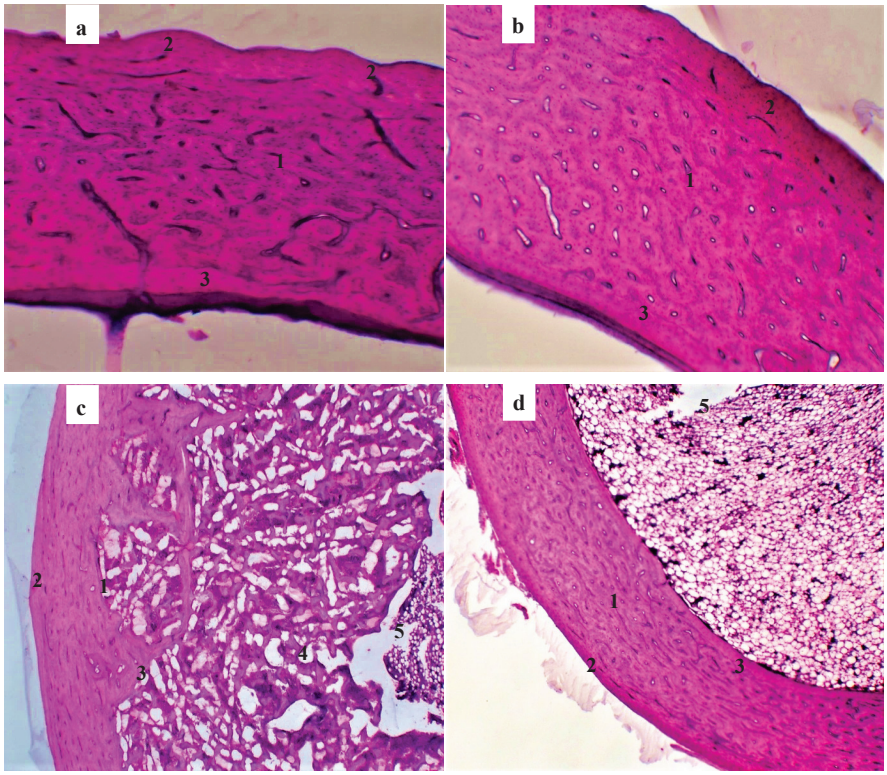

Figure 4. Microstructure of the cross-section of the middle of the diaphysis of the humerus (a, b) bone of ducks aged 268 days (female – a; male – b): 1 – dense osteon Layer; 2 – external, and 3 – iternal surrounding systems of bone plates. Karatsi hematoxylin and eosin staining, ×30 (c, d); ×75 (a, b)
Microstructure of the cross-section of the middle of the femoral diaphysis (b, d) of ducks aged 268 days (female – c; male – d): 1 – dense osteon Layer; 2 – external, and 3 – internal surrounding systems of bone plates; 4 – layer of medullary bone tissue; 5 – bone marrow.
Karatsi hematoxylin and eosin staining, ×30 (c, d); ×75 (a, b)

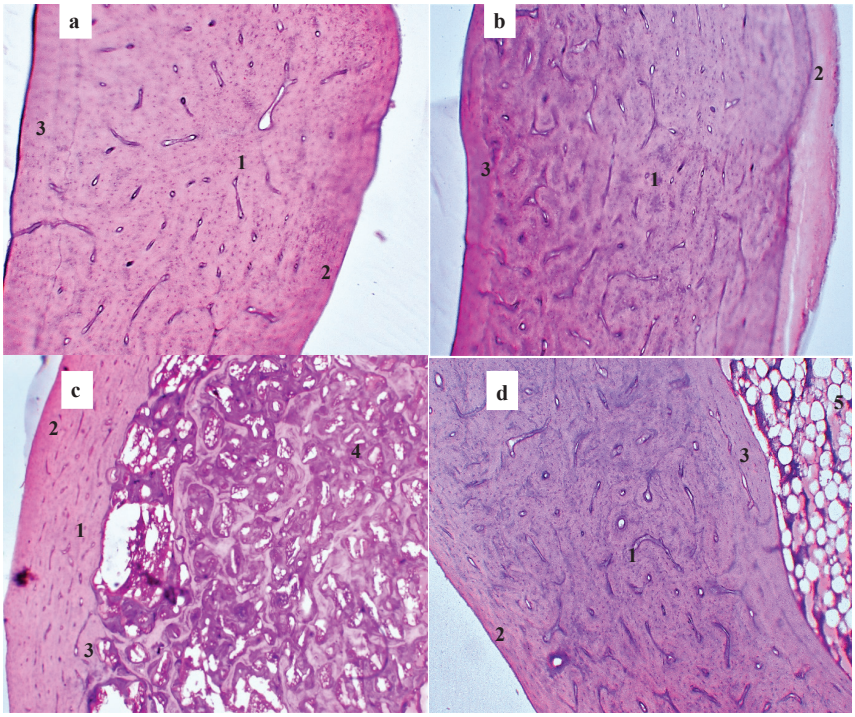

Figure 5. Microstructure of the cross-section of the middle of the diaphysis of the humerus (a, b) and femoral (c, d) bones of ducks aged 341 days (female – a, c; male – b, d): 1 – dense osteon Layer; 2 – external, and 3 – internal surrounding systems of bone plates; 4 – layer of medullary bone tissue; 5 – bone marrow. Staining with hematoxylin Karatsi and eosin, ×30 (a, b); ×75 (c, d)

Insertion (interstitial) systems of bone plates were discovered, which are parts of previously formed osteons that were preserved during bone reconstruction. The thickness of the compact bone tissue of the humerus of females decreases by 1.3 times ($P < 0.05$) compared to the previous age. Therewith, in the femur of males, the thickness of compact bone tissue is 1.2 times greater ($P < 0.05$) than in females (see Table 1, 2).

The diameter of osteons in the compact bone tissue of the humerus of females decreases by 29.3 times ($P < 0.001$), and in males – by 17.6 times ($P < 0.05$) compared to the previous age. The diameter of osteons in the compact femoral bone tissue of females decreases by 21.5 times ($P < 0.01$), and males – by 28.9 times ($P < 0.001$) compared to the previous age.

The diameter of the central osteon channels (Havers channels) in the compact humerus bone tissue of females decreases by 88.0 times ($P < 0.01$) compared to the previous age. Instead, the diameter of the central osteon channels (Havers channels) in the compact humerus bone tissue of males is 2.0 times larger ($P < 0.05$) than that of females (see Table 1).

External (see Fig. 5, a-d, 2) and internal (Fig. 5, a-d, 3) surrounding systems of bone plates are formed. Medullary bone tissue is absent in the humerus of female ducks. During this period, the medullary bone layer of the femur of females has the greatest thickness (2.55 ± 0.62 mm), the cavities between its bone layers are filled with oxyphilic protein fluid (see Fig. 5, c, 4). The bone marrow in the humerus of females and males is yellow, and in the femur – red-yellow (see Fig. 5, d, 5).

The middle of the duck humerus diaphysis is represented by compact bone tissue on day 483 of the postnatal period of ontogenesis. It is formed by the outer surrounding system of bone plates, the osteon layer, insertion (interstitial) bone plates, and the inner surrounding system of bone plates. In terms of diaphysis diameter, compact bone thickness, and osteon diameter, there was no statistically substantial difference with the previous age of ducks (see Table 1, 2). Instead, the thickness of the periosteum in the humerus of females decreases by 2.0 times ($P < 0.05$) compared to previous ages. Therewith, the diameter of the central channels of osteons (Havers channels) in the compact bone tissue of the humerus ($P < 0.01$) and femur ($P < 0.05$) of males decreases by 2.0 times compared to previous ages (see Table 1, 2).

Medullary bone tissue is absent in the humerus of females. The medullary bone layer of the femur of females almost disappears, its thickness is 0.20 ± 0.03 mm. The bone marrow is yellow, both in the tubular bones of females and males.

Thus, according to the conducted studies, it was established that the compact bone tissue of ducks of the Blagovarsky cross was represented by a formed, but uneven in thickness layer of bone tissue with a large number of expanded central channels of osteons. With age, the osteon layer of compact bone tissue increases, the osteons are denser, and the Havers channels decrease in diameter. Another study reported that compact bone tissue *Muscovy ducks* and *Egyptian geese*was similar and consisted of dense Havers tissue with a large number of rounded clusters of osteons, separated from each other by thick dark lines [7].

Other osteohistological studies have established a unique strategy for the growth of the middle of the diaphysis of tubular bones in *Mirarce eatoni* (terrestrial Late Cretaceous bird). The humerus consists of parallel-fibrous bone (with extremely high vascular porosity for a mature skeleton of *Enantiornithes*) with the middle layer of the initial fibrol-amellar bone and several growth lines in the outer surrounding layer and near the endoost. The endosteal and periosteal layers in slow-growing bone can be followed by cyclical changes in the growth rate of the limb skeleton. In the femur, there are areas of rough compact, spongy, and parallel-fibrous bone with numerous secondary osteons, and only one growth line. Therewith, to achieve the appropriate size and structure characteristic of an adult, various remodeling processes occurred in the tubular bone, including cycles of slow and rapid growth, asymmetric growth within a single bone element [5; 8; 12].

Morphological variations and dynamics of osteon density observed in the tubular bones of the above-mentioned bird species can be explained by different biomechanical loads on these bones, which depend on body weight, habitat conditions of wild and domestic birds [16-18].

Therewith, the presence of medullary bone tissue in the middle of the femoral diaphysis of female ducks of the "Blagovarsky" cross, which appears during egg production of the breeding stock, is confirmed by data from other studies on the formation of the brain bone in the medullary cavity of long bones of domestic and wild birds, considering their body weight, ecological (terrestrial, semi-aquatic, aquatic, migration) and reproductive status (stage in the egg cycle) [9; 11; 18].

Conclusions

A study of the tubular bones of Blagovarsky cross ducks using morphological methods demonstrated that the results obtained vary depending on their age and gender.

From 1 to 10 days of the postnatal period of ontogenesis, microscopic structure the middle of the diaphysis of tubular bones of ducks is represented by formed, uneven in thickness bone tissue, which consists of osteons, whose vascular channels are directed longitudinally, irregular elongated circular shape, do not have formed concentric bone plates.

By 20-30 days of the postnatal period of duck ontogenesis, due to the proliferation of cells of the osteogenic layer of the periosteum, resorption of osteon endoost cells and bone tissue from the brain cavity, tubular bones grow in thickness. This process slows down from the 30th day of the postnatal period of duck ontogenesis, the osteon layer of bone tissue is compacted and from the 90th day the internal surrounding system of bone plates with cementation lines is formed. Therewith, the external surrounding system of bone plates is also formed by the 196th day of the postnatal period of ontogenesis.

The compact bone tissue of the investigated tubular bones acquires a definitive state on the 196th day of the postnatal period of ontogenesis of female and male ducks of the Blagovarsky cross.

On day 196, female ducks form medullary bone tissue from the femoral endoost. The process of formation of medullary bone tissue coincides with the beginning of sexual maturity and the period of egg production of the breeding stock of ducks (196 days) and intensive periods of egg production on 268 and 341 days, and on 483 days when the duck does not lay eggs – completely disappears. In turn, medullary bone tissue is absent in the humerus of females.

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Порівняльна морфологія трубчастих кісток качок кросу «Благоварський» у постнатальному періоді онтогенезу

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Анотація. Мікроструктура кісток скелета кінцівок тісно пов'язана з онтогенетичним віком, локалізованою динамікою росту скелета, біомеханічними режимами навантаження на кістку та можливими таксономічними відмінностями. Це має важливе значення для вивчення проблемних питань онтогенетичних змін компактної кісткової тканини свійської качки. Мета дослідження полягала в проведенні порівняння мікроструктури середини діафіза плечової та стегнової кісток качок кросу «Благоварський» залежно від віку та статі. Матеріалом для досліджень слугували трубчасті кістки грудної (плечові кістки) (n = 72) та тазової (стегнова кістка) (n = 72) кінцівок качок кросу «Благоварський» у віці 1 доби, 10, 20, 30, 90, 196, 268, 341 і 483 доби постнатального періоду онтогенезу обох статевих груп (самок і самців по 36 качок), всього 72 качки. Терміни відбору качок збігалися із технологічним циклом їх вирощування. Виготовляли гістологічні зрізи товщиною 5-10 мкм, які фарбували гематоксиліном Караці та еозином, і за Ван Гізон для диференціювання сполучних тканин. Морфометрією визначено кількісні показники компактної кісткової тканини середини діафіза трубчастих кісток: діаметр діафіза, товщину окістя та компактної кісткової тканини, діаметр остеонів і центральних каналів остеонів (Гаверсові канали). Встановлено, що ріст плечової і стегнової кісток в довжину та товщину завершується на 196 добу постнатального періоду онтогенезу як у самок, так і в самців качок. Встановлено, що з боку ендоосту стегнової кістки самок з'являється медулярна (мозкова) кісткова тканина (1,01 ± 0,10 мм), що формується на 196 добу постнатального періоду онтогенезу (початок статевої зрілості самок) і у подальшому спостерігається в інтенсивні періоди їх несучості на 268 і 341 добу (відповідно, 2,43 ± 0,56 і 2,55 ± 0,62 мм), і зникає на 483 добу (0,20 ± 0,03 мм), коли качка не несеться. Цим дослідженням вперше визначено вікову динаміку морфометричних показників мікроструктур компактної кісткової тканини плечової та стегнової кісток і встановлено їх статеві відмінності у качок кросу «Благоварський». Результати досліджень порівняльної морфології необхідні для визначення віку, статі та виду птахів за мікроструктурою компактної кісткової тканини середини діафіза трубчастих кісток, а також для вміння диференціювати зміни за виникнення патологій кінцівок у свійської птиці

Ключові слова: плечова та стегнова кістки, вікові групи, самка, самець, мікроструктура, діафіз

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Embryo Flushing in Cows under Various Superovulation Schemes

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Abstract. The use of biotechnological reproduction methods is a relevant issue since the embryo transfer, obtained after stimulating superovulation, can accelerate reproduction and improve the number of cattle. The purpose of the study was to evaluate the effectiveness of various schemes for stimulating superovulation in cows of the Ukrainian black-pock dairy breed. Therewith, the study analysed the ovarian response to the drug "FSH-Super" under different introduction schemes: Step-up (gradual increase in the dose) and Step-down (gradual dose reduction) and recorded the number of embryos suitable for transplantation. Donor cows were administered the drug "Estrofan" to synchronise the sexual cycle. After 7 days, the drug "Ovarelin" was injected, and after another 7 days, the injection of the drug "Estrofan" was repeated in the same dose. Stimulation of superovulation began on the 10th day of the sexual cycle with the drug "FSH-super" in the form of eight gradually increasing (Step-up) and gradually decreasing (Step-down) doses within 4 days. Artificial insemination was performed 12 and 24 hours after the start of oestrus. During the study, it was discovered that in the group of cows with gradual dose reduction of the drug "FSH-Super", 83.3% reacted with superovulation, and in the group with the gradual increase – only 71.4%. Therewith, the number of yellow bodies on two ovaries in a donor cow averages 15.6 and 9.2, respectively. An average of 12.4 and 7.8 embryos (Step-down and Step-up) were obtained from the donor, of which 7.8 and 4.2 are suitable for transplantation, respectively. However, in the group of cows with gradually decreasing doses, a higher number of embryos unsuitable for transplantation was obtained – 4.6 and unfertilised oocytes – 2.6, compared with the group of donors with gradually increasing doses, where these indicators are 3.6 and 1.0, respectively. Thus, the use of the drug "FSH-super" to donor cows according to the step-down introduction scheme allows getting more embryos suitable for transplantation. This will allow managing the biotechnological aspects of cattle reproduction and effectively and in a controlled manner accelerate the breeding process in farms of various forms of ownership, fixing the desired genotype in the herd

Keywords: FSH-Super, cattle, follicle, Step-up, Step-down

Introduction

According to the forecasts of researchers, by the middle of the 21st century, the world's population will grow to 9.5 billion people, which will require an increase in the global production of food products, including animal origin, and as a result, will cause a substantial number of problems associated with their production [1; 2].

The use of modern biotechnological methods of reproduction of cattle, and careful selection and culling of animals, considering their genetic potential, can contribute to solving the problem of increasing the production of meat and dairy products [2], because the reproductive ability of cows determines the economic efficiency of cattle breeding. A substantial obstacle to the rapid increase in the number of highly productive cows is the long period of pregnancy and the ability to bear only one embryo. Despite the fact that only a small number of oocytes are sold during economic use,

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cows have a generative potential of hundreds of thousands of oocytes. That is why the use of biotechnological reproduction methods is quite important and always relevant. Transplant embryos obtained after stimulation of superovulation, can accelerate reproduction and improve the number of cattle [3].

After the first successful mammalian embryo transfer in 1890, approximately 60 years passed before progress in basic bovine embryo transfer technology was reported [4]. The commercialisation of bovine embryo transfer began in North America in the early 1970s [5]. However, the specialists of that time used surgical methods of both flushing and embryo transfer. It wasn't until 1987 that Craig Smith introduced the concepts of multiple ovulation (superovulation) and embryo transplantation (MOET) at the University of Guelph (Canada). It demonstrated how well-designed MOET programmes can lead to increased selection intensity and shorter generation intervals, leading to improved genetic stock.

Currently, there are dozens, if not hundreds, of commercial enterprises worldwide that use biotechnological methods, which include superovulation, embryo extraction from the donor's uterine horns, embryo transfer, cryopreservation, and in vitro fertilisation [7; 8]. Bovine embryo transfer is a big international business. Every year, over five hundred thousand bovine embryos are produced worldwide using superovulation stimulation techniques [7]. According to the International Society for Embryo Transplantation (IETS), the number of embryos that were transplanted and obtained by the method *in vivo* for one donor cow in the world is 6.7 [9]. For its part, the American Embryo Transplantation Association [10] states that the number of transplanted embryos obtained by the method *in vivo* per donor cow is 6.6 – for meat and 5.7 – for dairy breeds of cattle.

The procedures used in cattle breeding biotechnology have already been defined, but there are opportunities to improve them. For example, superovulation plays an important role in embryo transplant programmes. Its purpose is to ovulate a large number of follicles and a high yield of good quality embryos suitable for transplantation. One of the most problematic aspects of this stage is the variable response of the donor animal to superovulation stimulation and, as a result, unstable superovulation results [11]. Despite much attention paid to this issue, little progress has been made over the past 40 years. Therefore, researchers around the world continue to search for optimisation of existing schemes for stimulating the superovulation of cattle.

Given the above, optimisation of superovulation stimulation schemes in cattle is quite relevant, so the purpose of this study was to compare the effectiveness of two approaches to superovulation stimulation Step-up (gradual increase) and Step-down (gradual decrease) in the dose of exogenous follicle-stimulating hormone (FSH).

The purpose of the study was to evaluate the effectiveness of various schemes for stimulating superovulation in cows of the Ukrainian black-pock dairy breed.

Literature Review

The basic principle of superovulation and transfer of embryos are the production of embryos from a donor animal with a higher genetic value, after which the embryos are transferred to recipient animals with a lower genetic value. The method is aimed at increasing the number of offspring from the donor, while slowing the spread of recipient genes among the herd [5].

International embryo trade is a growing business, and embryos are also considered a safe way to exchange genetic material between countries. Since the risk of disease transmission is negligible for most pathogens, provided that the recommendations of the International Society for Embryo Transplantation are followed [12]. The main stages of superovulation and embryo transfer are: stimulation of ovarian follicle growth with gonadotropins, induction of oestrus with luteolytic drugs, insemination and flushing of embryos on the 7th day after the first insemination, and transfer of fresh embryos to synchronised recipients or freezing of embryos [5].

Stimulation of superovulation usually begins in the middle of the luteal phase of the oestrous cycle – from 8 to 12 days after oestrus. The physiological principle of this approach is based on follicular waves, of which there are usually two or three during the oestrous cycle, the second occurs on about 10 days, in most cycles. The goal is to target exogenous follicle-stimulating hormone (FSH) to new follicles that respond to gonadotropin, avoiding the effect of the dominant follicle on them [5].

It is possible to identify donor cows with as many new follicles as possible by determining the level of antimulerer hormone (AMH) [13]. This allows assessing the ovarian reserve and selecting cows with a potentially better response to stimulation of superovulations.

As for the groups of drugs for superovulation, the most widely used drugs are: pituitary FSH and pregnant mare serum gonadotropin (PMSG) [14; 15].

Pregnant mare serum gonadotropin (PMSG) is a serum, complex glycoprotein, that contains luteinising hormone (LH) and FSH [16]. Substantial variability in the results of stimulation of superovulation with PMSG drugs is due to the not optimal ratio of FSH and LH fractions, and their insufficient concentration [17]. In addition, PMSG drugs cause cystic changes in the ovaries and stimulate the production of antibodies, which reduces the possibility of repeated use of donor cows [18].

Therefore, now purified preparations of pituitary origin are increasingly used to induce superovulation, with a ratio of FSH to LH, as one to one or one to two, respectively [19], with the presence of sialic acid in the preparation, which ensures its active effect. Notably, that pituitary drugs, depending on the degree of their purification, have a different pharmacologic half-life. Thus, this indicator is for "FOLLTROPIN" (the main active substance – FSH extracted from the pituitary gland of pigs, 700 IU in a bottle) and "Pluset" (the main active substance – FSH and LH, extracted from the pituitary glands of pigs, 500 IU in a bottle) is up to 6 hours [11], while for "FSH-Super" (the main active substance is FSH extracted from the pituitary glands of pigs, 1000 IU in a bottle) – up to two days. Due to the relatively short pharmacologic half-life of available FSH preparations, two daily injections (with an interval of 12 hours) are necessary to maintain an equilibrium level of FSH during ovarian stimulation [20].

Currently, there are two approaches to stimulating superovulation using follicle-stimulating hormone in the world. Thus, in North America, a high initial dose of FSH (Step-down) is usually used with a gradual decrease

in it, while in Europe – a low (Step-up) with a subsequent increase in the level of the hormone. [21].

Materials and Methods

Experiments on cows were conducted based on the Faculty of Veterinary Medicine of the National University of Bioresources and nature management of Ukraine and LLC "Golden Meadows" in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals [22] used for Experimental and Other Scientific Purposes of 1986, and the Law of Ukraine "On the Protection of Animals from Cruel Treatment" of 02/21/2006 No. 3447-IV as amended on 04.08.2017 [23].

The study was conducted in the period from December 2019 to March 2020 in LLC "Zoloti Luky" village of Pariivka, Illinetskyi district, Vinnytsia region, on 13 clinically healthy cows of the Ukrainian black-pock dairy breed, aged from 4 to 8 years with a body weight of 600-650 kg, on the 45th-120th day of lactation, with a pre-lactation yield of 7-8 thousand kg of milk, with a fat content of over 3.6%.

Animals with a plasma concentration of antimulerer hormone in the range of 0.1-0.2 ng/cm^3 were selected for the experiment. Blood for this study was taken before the start of superovulation stimulation. Blood was taken from the tail vein into vacuum tubes with a coagulation activator. Then the blood tubes were settled at room temperature (18-20^{o}C) to separate the serum from the clot. Undiluted blood serum (20 µL) was used for analysis. The concentration of AMH was determined by enzyme-linked immunosorbent analysis (ELIA) using the AccuBind® AMH test kit (Monobind – Los Angeles, USA).

All experimental animals were divided into two groups according to the analogue principle (n = 13). In the first group, the ovarian response (number of yellow bodies) to the drug "FSH-Super" (the main active substance – FSH extracted from the pituitary glands of pigs, 1000 IU in a bottle, manufacturer "Agrobiomed", RF) was investigated and the number of embryos suitable for transfer was determined for using the superovulation stimulation scheme Step-up (gradual increase in the dose of the drug under study), in the second group – for using the superovulation stimulation scheme Step-down (gradual decrease in the dose of the drug under study). All experimental animals were subjected to synchronisation of the sexual cycle, regardless of the initial stage.

Donor cows were given 2 cm^3 of the "Estrofan" drug intramuscularly (Bioveta Czech Republic) to synchronise the sexual cycle. After 7 Days, 2 cm^3 of the drug "Ovarelin" (Ceva Sante Animale, France) were injected intramuscularly, and after another 7 days repeated the injection of the drug "Estrofan" in the same dose.

Cows were subjected to two-time artificial insemination by the recto-cervical method 12 and 24 hours after the start of oestrus. Using frozen-thawed sperm in straws with a volume of 0.25 cm^3 with a sperm concentration of 50-60 million. FSH-Super (Agrobiomed, RF) was used to compare different superovulation schemes (Table 1).

Table 1. Schemes for stimulating superovulation of donor cows with FSH-Super

Sexual cycle, day	Input time							
	Experimental group of donor cows no. 1				Experimental group of donor cows no. 2			
	8.00 h		20.00 h		8.00 h		20.00 h	
	FSH-Super, IU	Estrofan, cm^3	FSH-Super, IU	Estrofan, cm^3	FSH-Super, IU	Estrofan, cm^3	FSH-Super, IU	Estrofan, cm^3
0	*Oestrus*							
10	100	–	100	–	250	–	250	–
11	150	–	150	–	200	–	200	–
12	200	4	200	–	150	4	150	–
13	250	–	250	–	100	–	100	-
14 (1)	–		Oestrus		–		Oestrus	
	Insemination							
7	Embryo flushing							

Superovulation stimulation was performed for four days and started after synchronisation on the 10th day of the sexual cycle. Notably, that in the investigated schemes, the total dose of the hormone was identical and amounted to 1400 IU.

Before embryo flushing, donor cows underwent ovarian sonography to record the number of yellow bodies using the KX5200 ultrasound device (Kaixin, China).

Embryo flushing from each horn was performed alternately through a 2-channel silicone catheter CH20 with a Foley-type connection (Minitube, Germany) and the EmSafe filter system for flushing cattle embryos (Minitube, Germany) using a phosphate buffer solution (Sigma, USA) with the addition of 0.05 g/dm^3 of bovine serum albumin (Sigma, USA), and 12 µg/cm^3 of gentamicin (Arterium, Ukraine). The medium was used at the rate of 500 cm^3/uterine horn. Flushing was conducted by gravity method. At the end of flushing, the filter was cleaned using 50 cm^3 of Eagle environment (modified Dulbecco environment) using a sterile plastic syringe with a capacity of 50 cm^3 (Minitube, Germany). After the procedure, each animal was injected with 3 cm^3 of the drug "Estrofan" ("Bioveta", Czech Republic) for lysis of yellow bodies.

Using the SZM-45B stereomicroscope (Spectro Lab, Ukraine), embryos were searched and evaluated by quality and stage of development in accordance with the recommendations of the International Society for Embryo Transplantation [24].

Results and Discussion

In this experiment, 7 cows were selected for experimental group No. 1, and 6 cows with an average AMH level of 0.15 ng/cm^3 were selected for experimental group No. 2 (Table 2).

Table 2. AMH level and number of yellow bodies in cows of experimental groups

Experimental group of donor cows No. 1				Experimental group of donor cows No. 2			
No.	**No. of cow**	**AMH, ng/cm^3**	**Number of yellow bodies**	**No.**	**No. of cow**	**AMH, ng/cm^3**	**Number of yellow bodies**
1	6768	0.14	8	1	8611	0.18	17
2	8452	0.19	12	2	8447	0.19	15
3	8527	0.18	9	3	8492	0.15	14
4	7264	0.16	8	4	6893	0.12	13
5	8531	0.17	9	5	8508	0.20	19
6	7114	0.13	-	6	7140	0.10	-
7	6979	0.12	-	-	-	-	-
M ± m	-	0.15 ± 0.01	9.20 ± 0.73	-	-	0.15 ± 0.01	15.60 ± 1.07

Considering the same average level of AMH in the experimental groups, it can be argued that they are balanced and have the same ovarian reserve. The level of AMH among cows in the study groups ranged from 0.10 to 0.20 ng/cm^3.

In experimental group No. 1, out of seven cows exposed to superovulation stimulation, only five responded. The two cows that did not respond had the lowest AMH levels among the group – 0.12 ng/cm^3 and 0.13 ng/cm^3, respectively. A similar result was observed in experimental group No. 2, where only one of the six cows with the lowest level of AMH – 0.10 ng/cm^3, did not react. Depending on the scheme of FSH introduction, different results were obtained, despite the fact that both experimental groups responded to superovulation stimulation of 5 cows, and the average group level of AMH is identical.

The effectiveness of the effect of "FSH-super" on the manifestation of superovulation under different introduction schemes was determined and it was established that in experimental group No. 2 cows that reacted with superovulation were 5 out of 6 heads, that is, 83.30%, and in experimental group No. 1 – 5 out of 7 heads, 71.40%, respectively.

Before the embryo flushing procedure, an ultrasound examination of the ovaries of donor cows was performed to determine the number of yellow bodies (Fig. 1). Obtained and evaluated embryos (Figure 2) in accordance with the recommendations of the International Society for Embryo Transplantation. [24]. The obtained data were processed, and the results are presented in Table 3.

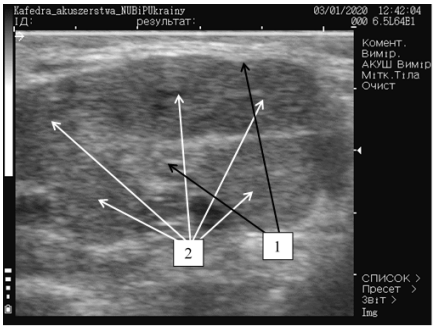

Figure 1. Ultrasound examination of the ovary of a donor cow

Note: 1) ovarian parenchyma, 2) corpus luteum

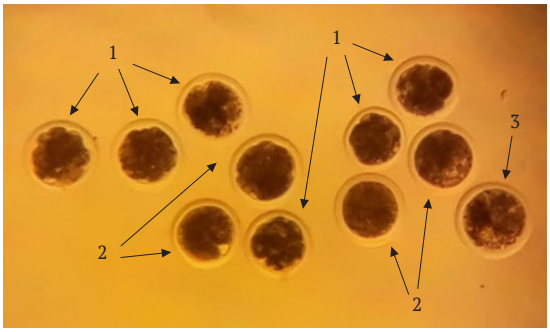

Figure 2. Cattle embryos at different stages of development obtained by flushing after stimulation of superovulation with FSH-super

Note: 1) early morula, 2) late morula, 3) early blastocyst. Native drug, ×100

Table 3. Impact effectiveness of "FSH-super" on the manifestation of superovulation in donor cows under different introduction schemes, M ± m

Indicator	Experimental group of donor cows No. 1	Experimental group of donor cows No. 2
Number of animals, n	7	6
Donor cows that responded with superovulation, n (%)	5 (71.40)	5 (83.30)
Number of yellow bodies in 1 donor	9.20 ± 0.73	15.60 ± 1.07*
Number of embryos received from 1 donor	7.80 ± 0.82	12.40 ± 1.42*
Number of embryos suitable for transplantation, %	4.20 ± 0.58 (53.84)	7.80 ± 0.73 (62.90)*
Number of degenerated embryos	3.60 ± 0.24	4.60 ± 0.24
Number of oocytes	1.00 ± 0.32	2.60 ± 0.40
Fertilisation rate, %	84.78	79.40

Note: *P < 0.05, probably compared to the experimental group of donor cows No. 1

Based on the results obtained, presented in Table 3, it follows that the average number of yellow bodies on the ovaries of donor cows in experimental group No. 2 is 1.69 times (P < 0.05) higher than in donor cows in experimental group No. 1. Therewith, the number of embryos obtained from 1 donor of cows of experimental group No. 2 is 1.59 times (P < 0.05) higher than that of donor cows of experimental group No. 1. Thus, the quantity of suitable for transplantation embryos in donor cows of experimental group No. 2 is 1.86 times larger than in donor cows of experimental group No. 1.

However, there was no statistically substantial difference in the number of degenerated embryos in donor cows of experimental group No. 2 compared to experimental group No. 1, although this number was higher. Therewith, the number of oocytes in donor cows of experimental group No. 1 is less compared to donor cows of experimental group No. 2, and fertilisation is higher by 5.38%.

There is a direct pattern between low plasma AMH levels and low reproductive performance of cows [25], which was confirmed by this study because cows with the lowest AMH values did not respond to superovulation stimulation. According to data of B. M. Guerreiro *et al*. [13], cows with high AMH levels have more visible aspirated follicles, respectively, more oocyte-cumulus complexes and subsequent embryos.

Thus, in this study, the number of embryos obtained in one flushing was 10.1, similar to the data obtained by Chebel *et al*. – 10.9 [26]. However, the number of yellow bodies was twice as low – 12.4 compared to the data of Garcia Guerra *et al*. – 24.0 [19].

According to V. Chankitisakul *et al*. [17], the number of yellow bodies corresponded to a value of 11.4, and the suitability for embryo transplantation was 61.24%. For comparison, in experimental group No. 2 (Step-down) of the conducted study, the results obtained were 15.60 and 62.90%, respectively. Therewith, in experimental group No. 1 (Step-up), the number of yellow bodies was 9.20, and the suitability for embryo transplantation was 53.84%.

According to M. Drillich *et al*. [27], the number of embryos suitable for transplantation was 7.8, and according to this study – 6.0. However, according to the data of L. Vieira *et al*., the number of embryos suitable for transplantation corresponded to a value of 2.4 [28]. This is due to the difference in superovulation stimulation patterns. Notably, the level of gonadotropic hormone in the blood of a donor cow, its individual characteristics, and the feeding factor can affect the number of embryos suitable for transplantation obtained during one flushing. [29].

The results obtained indicate an active superovulatory response, which is confirmed by data of H. Kohram and M. Poorhamdollah [30], who claim to have obtained 4 to 6 transplant-eligible embryos after superovulation in lactating cows. However, Peippo *et al*. [31] obtained 2.2 degenerated embryos in one flushing, and in this study, the number is 4.1.

Conclusions

Determination of serum antimulerer hormone levels can serve as a marker for selecting donor cows. Ultimately, animals with a deficient level of it show a low result or its absence. With an identical dose of the drug "FSH-super" (1400 IU) to stimulate superovulation, the use of the Step-down scheme allows getting an average of 7.80 (62.90%) embryos suitable for transplantation from donor cows, which is 3.60 (9.10%) more compared to the Step-up scheme, for the use of which to donor animals, this Figure is 4.20 (53.80%). The potential of a sufficient or substantial ovarian reserve may not be identified due to an unsuccessful selection of the superovulation stimulation scheme.

Promising areas are the investigation of the correlation between the level of antimulerer hormone in the blood serum of cows and the number of follicles that respond to superovulation stimulation. It is also necessary to determine the correlation between the level of antimulerer hormone in the blood serum of cows, ovarian reserve, and reproductive longevity. In addition, it remains a priority to examine the effect of the dose and method of introduction of exogenous follicle-stimulating and luteinising hormones on the superovulatory response and the yield of embryos suitable for transplantation.

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Вимивання ембріонів у корів за різних схем суперовуляції

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Анотація. Використання біотехнологічних методів відтворення є актуальним питанням, оскільки трансплантація ембріонів, отриманих після стимулювання суперовуляції, здатна прискорити відтворення та поліпшити поголів'я великої рогатої худоби. Метою роботи було оцінити ефективність різних схем стимуляції суперовуляції в корів української чорно-рябої молочної породи. При цьому, в роботі вивчали реакцію яєчників на препарат «ФСГ-супер» за різних схем введення Step-up (поступове підвищення дози) та Step-down (поступове зменшення дози) і реєстрували кількість ембріонів придатних до трансплантації. Для синхронізації статевого циклу коровам-донорам вводили препарат «Естрофан». Через 7 діб ін'єктували препарат «Оварелін», а ще через 7 діб повторювали ін'єкцію препарату «Естрофан» у тій же дозі. Стимуляцію суперовуляції розпочинали на 10 добу статевого циклу за допомогою препарату «ФСГ-супер» у вигляді восьми поступово зростаючих (Step-up) і поступово спадаючих (Step-down) доз упродовж 4 діб. Штучне осіменіння проводили через 12 і 24 год від початку охоти. Під час дослідження було встановлено, що в групі корів з поступовим зменшенням доз препарату «ФСГ-супер» зреагували суперовуляцією 83,3 %, а в групі з поступовим їх підвищенням лише 71,4 %. Водночас кількість жовтих тіл на двох яєчниках у корови-донора в середньому становить 15,6 і 9,2, відповідно. Від донора у середньому отримано 12,4 і 7,8 ембріонів (Step-down та Step-up), із них придатних до трансплантації 7,8 і 4,2, відповідно. Проте, в групі корів з поступово спадаючими дозами отримано більшу кількість непридатних до трансплантації ембріонів – 4,6 і незапліднених яйцеклітин – 2,6, порівняно з групою донорів, яким застосовували поступове підвищення доз, де ці показники становлять 3,6 і 1,0, відповідно. Таким чином, використання коровам-донорам препарату «ФСГ-супер» за схемою введення Step-down дає можливість отримати більшу кількість придатних до трансплантації ембріонів. Це дозволить управляти біотехнологічними аспектами відтворення великої рогатої худоби та ефективно й контрольовано пришвидшити селекційний процес у господарствах різних форм власності, закріплюючи у стаді бажаний генотип

Ключові слова: ФСГ-супер, велика рогата худоба, фолікул, Step-up, Step-down

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Correction of Triacylglycerols and Free Fatty Acids in Rat Bile in Experimental Hepatic Steatosis

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Abstract. The relevance of the scientific study is associated with a substantial spread of hepatic steatosis in domestic animals (up to 40%) and the development of health-threatening complications in the form of cirrhosis of the liver, liver failure, and cancer. The purpose of this study was to determine the corrective effectiveness of the "FLP-MD" dietary supplement based on milk phospholipids in relation to the content of triacylglycerols and free fatty acids in the bile of rats with tetracycline-induced hepatic steatosis. Modelling of the drug form of hepatic steatosis was conducted by intragastric administration of a 4% solution of tetracycline hydrochloride at the rate of 0.5 g/kg of animal body weight for seven days. As a corrective therapy, for nine days the animals were intragastrically administered a dietary supplement "FLP-MD" based on milk phospholipids at a dose of 13.5 mg/kg of body weight. At the end of the experiment, bile samples were taken from rats for three hours every 30 minutes, in which the content of triacylglycerols and free fatty acids was determined by thin-layer chromatography. It was determined that the concentration of triacylglycerols in the bile of sick rats at the third hour of its selection is 63.0% lower than the control indicators. In laboratory rats that received a phospholipid-containing supplement against the background of modelling drug-induced hepatosis, this indicator in bile corresponded to the values of the control group. Therewith, the concentration of free fatty acids in bile samples at the third hour of its selection in sick rats was marked by a decrease of 47.2% compared to the control. The use of the dietary supplement under study in sick animals caused an increase in the concentration of free fatty acids in bile by 2.85 times compared to the control, which reduces the intensity of their use for the synthesis of triacylglycerols and prevents the development of fatty liver infiltration. Therefore, the phospholipid-containing dietary supplement is a highly effective corrective agent for impaired metabolism of triacylglycerols and free fatty acids in rats with drug-induced hepatic steatosis. This gives grounds to recommend it as a corrective therapy and for the prevention of the development of hepatic steatosis, especially in the case of the use of tetracycline antibiotics in animals

Keywords: milk phospholipids, biologically active supplement, tetracycline hydrochloride, corrective therapy

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Introduction

Fatty liver disease or Hepatic steatosis is a hepatopathology, common in domestic animals (up to 40%), which is characterised by a disruption of the balance between the synthesis and utilisation of triacylglycerols, their excessive accumulation in hepatocytes, dyslipidemia, activation of fatty acid peroxide oxidation, and disorders of the bile secretory function of the liver [1; 2]. A particular threat to their health is the consequences of this pathology in the form of cirrhosis of the liver (in 25% of sick animals), further development of liver failure and hepatocellular carcinoma [1; 3].

The liver plays a leading role in lipid metabolism in the body of mammals. The development of hepatic steatosis primarily consists in the intrahepatic accumulation of triacylglycerols [1]. Their excessive deposition in hepatocytes can be reversed or vice versa. Therewith, this disease can occur secondary to various pathological conditions and be a consequence of viral infection, alcohol consumption, incorrect use of medications, etc. [3; 4]. In particular, excessive accumulation of lipids in the liver parenchyma is observed in obesity, metabolic syndrome, diabetes mellitus with insulin resistance [5; 6]. The most common type of fatty liver degeneration is non-alcoholic steatohepatitis (NASH). In addition, there is a tendency to increase cases of hepatic steatosis caused by drugs [5; 7]. Both non-alcoholic hepatosis and hepatic steatosis are caused by various groups of drugs are characterised by damage to hepatocytes, inflammatory processes in the liver parenchyma, and fibrosis, which substantially increases the risk of other complications [1; 3].

The molecular mechanisms of hepatic steatosis development are still poorly understood. It is believed [1] that the accumulation of triacylglycerols in hepatocytes in hepatic steatosis is not the leading factor of lipidotoxicity. Along with this, abnormal accumulation of triacylglycerols in liver cells is accompanied by an increase in the intrahepatocytic content of other lipids, which in turn, are able to trigger numerous intracellular mechanisms of lipidotoxicity, namely: activation of death receptors, endoplasmic reticulum stress, modification of mitochondrial function, and oxidative stress. In particular, with fatty degeneration of the liver, there is an accumulation of cholesterol in its cells, which, with its excessive intracellular amount, exhibits a number of toxic effects leading to liver damage. Maintenance of intrahepatic cholesterol homeostasis depends on the smooth flow of a number of complex processes: the synthesis of cholesterol in hepatocytes, the transport of cholesterol through the sinusoidal (basal) and, especially, canalicular (apical) domains of the plasma membrane of hepatocytes (this mechanism allows removing cholesterol in the bile), the conversion of cholesterol into primary bile acids involving specific intrahepatocytic enzyme, and the absorption of cholesterol in the intestine [6]. Along with this, it was determined that in laboratory rats with an experimental drug form of hepatic steatosis, the excretion of cholesterol and, especially, cholesterol esters to the bile tubules is suppressed and their ratio in bile is substantially disrupted, which is likely due to their accumulation in liver cells [8].

Notably, such a common disease in dogs as chronic hepatitis is accompanied by hypercholesterolemia and hypertriacylglycerolemia [9]. Therewith, dyslipidemia in dogs with chronic hepatitis, which is considered a disease similar to non-alcoholic fatty degeneration of the liver in humans, has several causes: increased synthesis of cholesterol and triacylglycerols in the liver, inhibition of triacylglycerol catabolism, and cholestatic phenomena with an increase in the concentration of bile acids in the blood of sick animals. In this case, a violation of the excretion of both excess cholesterol and excess triacylglycerols in the bile is one of the possible causes of an increase in the level of these lipid compounds in the blood.

However, it is still an open question which of the mechanisms – increased synthesis of cholesterol and triacylglycerols in hepatocytes, inhibition of their catabolism, or disruption of the transport of these lipids from hepatocytes is leading in the pathogenesis of hepatic steatosis [9]. In addition, it was also determined that when triacylglycerols are stimulated to enter the primary bile tubules in case of disruption of the further outflow of bile by the biliary system, for example, as noted in pancreatobiliary reflux, their excessive accumulation in the bile ducts and gallbladder is observed. This, in turn, leads to the fact that triacylglycerols become one of the main factors in the formation of gallstones in the gallbladder [10].

It should be noted the important role of free fatty acids in liver damage due to its fatty degeneration, which occurs due to increased oxidative stress and the subsequent development of inflammatory processes in the parenchyma. There is a hepatoprotective effect of measures that help reduce the content of free fatty acids in hepatocytes, which prevents the progression of fatty degeneration of this organ [11]. Drugs that can correct lipid metabolism in the liver include bile acids (in particular, ursodeoxycholic acid preparations), omega-3 of unsaturated fatty acids [12; 13]. Phospholipids play an important role in the treatment of patients with fatty liver degeneration [14; 15]. Largely the content of various fractions of lipid components in bile reflects the course of lipid metabolism in liver cells. Considering the close relationship between the metabolism of free fatty acids and triacylglycerols and their role in the course of pathologies with fatty liver degeneration, the purpose of the study was to examine the corrective effect of phospholipid-containing biologically active supplement (BAS) "FLP-MD" on the dynamics of the content of free fatty acids and triacylglycerols in rat bile under the conditions of modelling the drug form of hepatic steatosis.

Literature Review

The liver is the central organ of the metabolism of fatty acids, which both enter liver cells from the blood and are synthesised in hepatocytes *de novo*. Free fatty acids are either oxidised in cells as an energy resource or used for the synthesis of triacylglycerols, which, as part of low-density lipoprotein complexes, enter the blood plasma and then enter all organs and tissues of the body with its flow. According to the physiological state, both free fatty acids and triacylglycerols (up to 5% of the organ mass) do not accumulate in hepatocytes in substantial amounts [16]. It is noted that the intracellular accumulation of triacylglycerols, which is characteristic of hepatic steatosis, is primarily associated with a violation of their excretion from hepatocytes, in particular, as part of lipoprotein complexes, and not with an increase in the intake of fatty acids to liver cells from the blood [17].

Steatosis of the liver due to its fatty degeneration caused by metabolic disorders is associated with the excessive synthesis of triacylglycerols in the liver using fatty acids obtained from various sources: from white adipose tissue, as a product of lipogenesis *de novo*, and from endocytic residues rich in triacylglycerol lipoproteins [17]. Due to the high content of lipids in the liver, the elimination of very low-density lipoproteins into the bloodstream should be expected to increase, which becomes the main cause of complex dyslipidemia characteristic of patients with hepatic steatosis. Measures that reduce the secretion of very low-density lipoproteins into the blood can prevent the development of dyslipidemia, but simultaneously cause exacerbation of hepatic steatosis [17; 18]. This means that the balance between intracellular accumulation of lipids and their secretion from hepatocytes into the blood is a critical parameter that determines the course of diseases associated with lipid metabolism disorders and fatty degeneration of the liver.

To date, it has already been confirmed [18] that increasing energy processes using fatty acids as an energy resource reduces the lipid load on the liver. In addition, hepatocellular synthesis of triacylglycerols from fatty acid derivatives can positively change the course of fatty liver damage. However, more studies are needed to determine the effect of individual transporter proteins, enzymes, and their isoforms on the development of steatosis and dyslipidemia *in vivo*. Attention should also be paid to the factor of insufficient enzymatic transformations of cholesterol lipoproteins, which leads to their substantial accumulation in the cytosol of hepatocytes. Although it appears to be less important compared to other reasons. However, "cholesterol load" affects the course of inflammatory processes and leads to the progression of fatty liver degeneration associated with metabolic disorders [18].

Transport systems of the plasma membrane of liver cells play a substantial role in maintaining a certain intracellular content of free fatty acids and triacylglycerols: translocase of fatty acids ((FAT)/CD36), a plasma membrane protein that binds fatty acids (FABPpm) and Caveolin-1. It is noted that the activity of these transport systems and their significance in the development of fatty liver degeneration require in-depth investigation [16]. Notably, hypertriacylglycerolemia is defined as one of the risk factors for developing cholelithiasis [10; 19; 20]. Both metabolic and genetic factors affect the content of triacylglycerols in liver cells, identifying their effects by various mechanisms. Therefore, as noted in the review papers [21-23], further investigation is needed to determine the effects of metabolic and genetic interaction, which will allow the development of personalised and potentially effective therapeutic measures for correcting lipid metabolism disorders in fatty liver degeneration.

Materials and Methods

Experimental animal studies and biochemical analysis of bile were conducted based on the scientific laboratory of the educational and Scientific Centre "Institute of High Technologies" of Taras Shevchenko National University of Kyiv in the second half of 2021. In the study of the effect of BAS "FLP-MD" based on milk phospholipids on the content of triacylglycerols and free fatty acids in bile, male laboratory rats of the line were used Wistar, whose body weight was 200 ± 50 g (n = 13). ORION OS-0k22 electronic scales (ORION ELECTRONICS LTD, Europe) were used for weighing animals. Laboratory rats were kept in standard vivarium conditions at a temperature of 22-24 C with a 14-hour light period of the day, on a standardised full-fledged diet and with free access to water. During the experiment, they adhered to the "European Convention for the Protection of Vertebrates Used for Experimental and Scientific Purposes" (Strasbourg, 1986) [24], and the Law of Ukraine No. 692 "On the Protection of Animals from Cruelty" (3447-IV) of 02/21/2006 [25]. All surgical interventions were performed with the use of painkillers (thiopental sodium at a dose of 7 μg/100 g of body weight) and compliance with the rules of humane treatment of laboratory animals.

Reproduction of hepatic steatosis was conducted according to the author's own technique [26]. Using a soft silicone probe, rats were given a 4% Solution of tetracycline hydrochloride daily intragastric administration at the rate of 0.5 g/kg of body weight for seven days. The animals developed tetracycline-induced hepatic steatosis, and since these rats did not receive therapy, a "Self-rehabilitation" group was formed from them (n = 5). Rats were intragastrically administered with the specified dietary supplement for seven days an hour before the use of tetracycline and additionally the next two days after the completion of the seed, (group "Correction", n = 4) to determine the corrective properties of BAS "FLP-MD "(the main active substance – phospholipids of milk) in relation to the content of triacylglycerols and free fatty acids in bile samples. The daily dose of BAS "FLP-MD" was 13.5 mg/kg of body weight [27]. Animals of the control group ("Control", n = 4) were injected intragastrically with an equivalent volume of distilled water. During the study, the animals' body weight was monitored, and the doses of drugs were determined individually, considering changes in body weight during the entire experiment period.

Bile samples were taken to determine the content of triacylglycerols and free fatty acids in it in acute experiments with bile duct cannulation. The day before the acute experiment, the rats were weighed and kept on a starvation diet with free consumption of drinking water. Animals' access to feed during the day before the end of the experiment was restricted to eliminate the influence of feed on the processes of bile formation and on the composition and secretion of bile. Before surgery, rats were drugged by intraperitoneal administration of thiopental sodium at the dose indicated above. After that, laparotomy and cannulation of the bile duct were performed using a plastic cannula, which was connected to a micropipette. Bile samples were taken every 30 minutes for three hours of acute experiment. Thus, at the end of the three-hour acute experiment, six half-an-hour bile samples (No. 1-6) were obtained from each animal. These samples were analysed for the content of triacylglycerols and free fatty acids by thin-layer chromatography in the author's modification [28] on standard plates of the company "Silufol" (Csech Republic) in the solvent system petroleum, ether-diethyl, ether-acetic acid (90 : 70 : 1).

Statistical processing of the obtained results was conducted using the software "Statistica 5.0" ("StatSoft Inc.", USA). As an indicator of the statistical significance of the identified differences in the values of the studied indicators in the experimental groups, the Student's t-test was

considered for the normal distribution of data. The normality of the distribution was evaluated using the Shapiro-Wilk test. The indicators obtained during the experiment had a normal distribution. The differences between the two indicators of the compared samples at $P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered statistically substantial [29].

Results and Discussion

As a result of the study of the concentration of triacylglycerols in rat bile, it was determined that in animals of the control group it averages 3.01 ± 0.28 mg%. Therewith, their concentration in bile samples from animals with tetracycline-induced liver damage on average corresponded to values of 1.39 ± 0.28 mg%, which is 53.8% less than in the control. But in laboratory rats that received BAS "FLP-MD" against the background of experimental tetracycline liver damage, the content of triacylglycerols in bile samples averaged 2.83 ± 0.69 mg%, that is, it did not differ from the values of this indicator in bile samples of animals of the control group.

During the three-hour acute experiment, bile samples were taken every half an hour. This allowed examining the dynamics of changes in the concentration of triacylglycerols in rat bile during the entire acute experiment.

During the first hour of the experiment in two half-an-hour bile samples (No. 1 and No. 2) obtained from animals with tetracycline-induced liver damage ("Self-rehabilitation" group), the concentration of triacylglycerols was 1.60 ± 0.25 mg% (samples No. 1) and 1.62 ± 0.31 mg% (samples No. 2), which is 49.5% ($P < 0.001$) and 46.0% ($P < 0.001$) less than their value in the control – 3.18 ± 0.26 (samples No. 1) and 3.00 ± 0.12 (samples No. 2). Similar differences in the concentration of triacylglycerols in bile samples were also observed in rats between the "Self-rehabilitation" and "Correction" groups. Namely, in bile samples No. 1 in sick animals ("Self-rehabilitation" group), the concentration of triacylglycerols was 44.8% ($P < 0.01$) less than in the bile of rats that, in addition to tetracycline load, received a course of BAS "FLP-MD" (group "Correction"), which corresponded to the values of 2.90 ± 0.61 ($P < 0.01$, samples No. 1). In addition, in bile samples No. 2 obtained from animals of the "Self-rehabilitation" group, the concentration of triacylglycerols was 45.5% ($P < 0.01$) less than in rats of the "Correction" group (2.98 ± 0.77, $P < 0.01$, samples No. 2). There were no statistically substantial differences between the concentration of triacylglycerols in bile samples No. 1 and No. 2 in animals of the control group and the "Correction" group (Fig. 1).

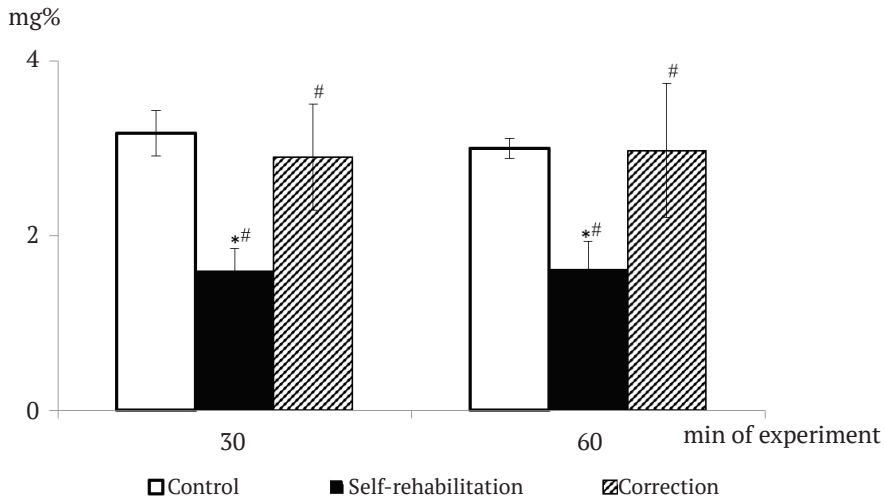

Figure 1. The concentration of triacylglycerols in bile samples of rats ("Control" group), with experimental hepatic steatosis ("Self-rehabilitation" group) and in sick animals that were additionally administered BAS "FLP-MD" ("Correction" group) obtained during the first hour of the acute experiment

Notes: * $P < 0.001$, a statistically substantial difference compared to the control; # $P < 0.01$, a statistically substantial difference between the indicators of the "Self-rehabilitation" and "Correction" groups

During the second hour of the acute experiment, a substantially lower concentration of triacylglycerols was recorded in bile samples (No. 3 and No. 4) in animals with experimental hepatic steatosis compared to the control indicators. Thus, in rats of the "Self-rehabilitation" group, the concentration of triacylglycerols in bile samples No. 3 and No. 4 was 1.46 ± 0.31 and 1.30 ± 0.25 mg%, respectively, which is 51.3% ($P < 0.001$) and 55.2% ($P < 0.001$) lower than their values in the control (3.00 ± 0.28 mg% and 2.90 ± 0.22 mg%, respectively). The concentration of triacylglycerols in similar bile samples in animals of the "Self-rehabilitation" group (1.46 ± 0.31 mg% and 1.30 ± 0.25 mg%) and the "Correction" group (3.03 ± 0.89 mg% and 2.75 ± 0.70 mg%) differed upwards in the latter, respectively, by 51.8% ($P < 0.01$) and 55.7% ($P < 0.01$) (Fig. 2).

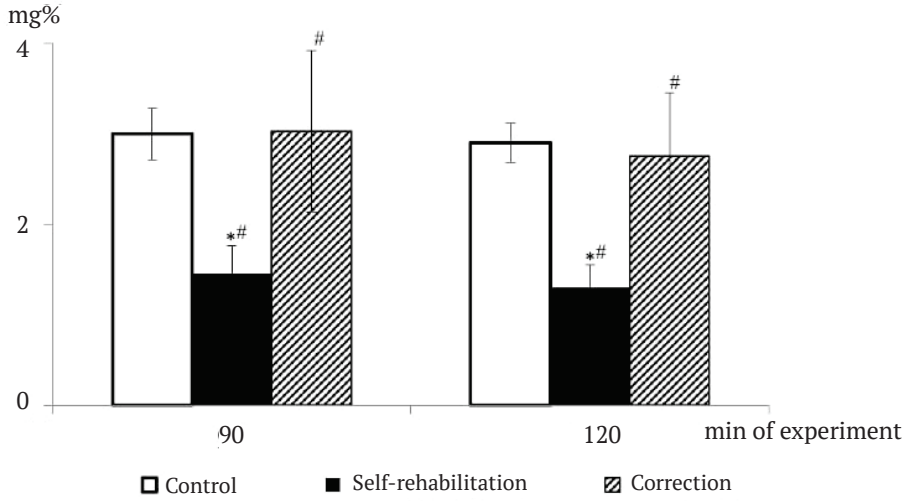

Figure 2. Concentration of triacylglycerols in bile samples of rats ("Control" group), with experimental hepatic steatosis ("Self-rehabilitation" group) and in sick animals that were additionally administered BAS "FLP-MD" ("Correction" group) obtained during the second hour of the acute experiment

Notes: * P < 0.001, a statistically substantial difference compared to the control; # P < 0.01, a statistically substantial difference between the indicators of the "Self-rehabilitation" and "Correction" groups

Notably, in comparison with the content of triacylglycerols in bile samples collected during the first and second hours of the acute experiment, further inhibition of their entry into the bile composition was observed. This may be due to insufficient activity of the corresponding transport systems of the canalicular domain of the plasma membrane of hepatocytes.

Thus, in bile samples taken during the third hour of acute experiment in rats with hepatic steatosis, the concentration of triacylglycerols remained substantially reduced compared to the corresponding indicators of the control group. In animals with hepatic steatosis ("Self-rehabilitation" group), it was 1.22 ± 0.29 mg% (samples No. 5) and 1.12 ± 0.26 mg% (samples No. 6) and was respectively lower by 58.6% (P < 0.001) and 63.0% (P < 0.001) than in the control, which corresponded to the values of 2.95 ± 0.33 mg% (samples No. 5) and 3.03 ± 0.50 mg% (samples No. 6). In animals that received a course of BAS "FLP-MD" under the conditions of modelling hepatic steatosis, the concentration of triacylglycerols was close to that in the control: 2.65 ± ± 0.53 mg% (samples No. 5) and 2.68 ± 0.61 mg% (samples No. 6). Therewith, the content of triacylglycerols in the bile of rats of the "Self-rehabilitation" group was characterised by values lower than those of the "Correction" group, respectively, by 54.0% (P < 0.01) in half-an-hour samples No. 5 and by 58.2% (P < 0.01) in half-an-hour samples No. 6 (Fig. 3).

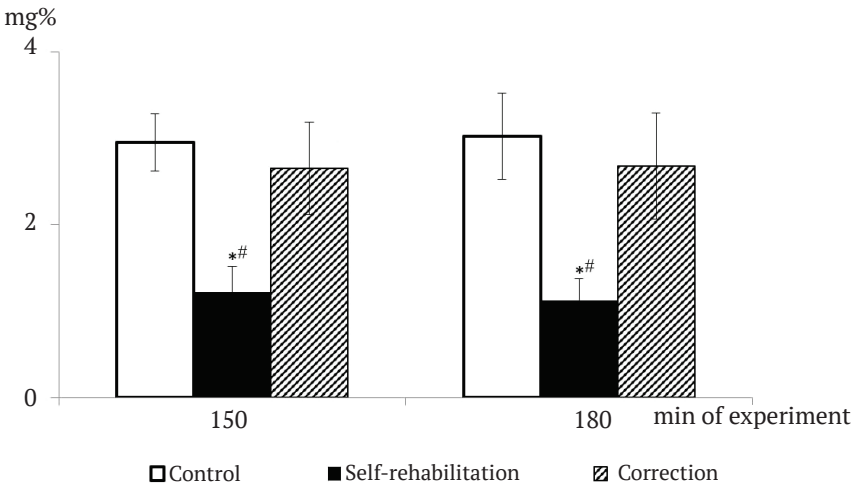

Figure 3. Concentration of triacylglycerols in bile samples of rats (control group), with experimental hepatic steatosis ("Self-rehabilitation" group) and in sick animals that were additionally administered BAS "FLP-MD" ("Correction" group), obtained during the third hour of an acute experiment

Notes: * P < 0.001, a statistically substantial difference compared to the control; # P < 0.01, a statistically substantial difference between the indicators of the "Self-rehabilitation" and "Correction" groups

Thus, under the conditions of experimental hepatic steatosis in experimental rats, a substantial decrease in the content of triacylglycerols in bile is observed. This indirectly indicates a disruption of the mechanisms of entry of triacylglycerols into the primary bile tubules with the accumulation of these lipids in liver cells and is one of the factors in the progression of fatty liver dystrophy. The use of the dietary supplement "FLP-MD" in animals under the conditions of modelling tetracycline-induced liver damage completely eliminates the negative effect of the applied toxic dose of the antibiotic on the structures of hepatocytes, which ensures the restoration of the processes of triacylglycerol intake to bile. Mainly, the corrective effect of phospholipid-containing dietary supplements is due to its membranotropic and membranoprotective properties [27]. It is known [30] that tetracycline in high doses has a cytotoxic effect on hepatocytes. Therewith, the result of this action is the development of fatty liver dystrophy, accompanied by the accumulation of triacylglycerols in liver cells [31]. This is the result of a disruption of the balance between lipid formation and catabolism, a decrease in the activity of mitochondrial beta-oxidation of fatty acids, an increase in the synthesis of endogenous fatty acids, and insufficient incorporation of triacylglycerols into low-density lipoproteins [4; 32]. In addition, due to the toxic effect of tetracycline on the body, activation of lipid peroxide oxidation reactions is also observed. This, in turn, leads to excessive formation of free radicals, covalent binding of electrophilic metabolites to proteins, a decrease in the content and oxidation of free glutathione [3].

Modelling of hepatic steatosis in rats also substantially reduces the concentration of free fatty acids in bile. The average concentration of free fatty acids in the bile of animals with toxic liver damage ("Self-rehabilitation" group) was 14.70 ± 1.89 mg%. While the average concentration of free fatty acids in the bile of animals in the control group was 21.88 ± 3.47 mg%. Therewith, the average concentration of free fatty acids in the bile of animals that received BAS "FLP-MD" ("Correction" group) against the background of modelling tetracycline-induced liver damage corresponded to the values of 39.69 ± 2.90 mg%.

Considering these changes in stages, in the bile samples obtained from animals of the "Self-rehabilitation" group during the first hour of the acute experiment, the concentration of free fatty acids was 16.50 ± 1.82 mg% (samples No. 1) and 16.26 ± 1.29 mg% (samples No. 2), which is lower than in animals of the "Control" group by 23.3% (21.53 ± ± 3.85, P < 0.05) and 21.4% (20.68 ± 3.55, P < 0.05), respectively. The concentration of free fatty acids in the bile of rats of the "Correction" group corresponded to the values of 41.15 ± 3.64 mg% (samples No. 1) and 42.95 ± 3.64 mg% (samples No. 2), which is 91.2% (P < 0.001) and 107.7% (P < 0.001), respectively, more than in the control. Notably, the concentration of free fatty acids in the first two half-an-hour samples of bile of rats that received a dietary supplement under the conditions of modelling hepatic steatosis exceeded the concentration of these components in the bile of animals with hepatic steatosis without correction by 2.5 and 2.6 times, respectively (Fig. 4). This result indicates a pronounced corrective and choleretic effect of the dietary supplement components on the hepatobiliary system of sick animals.

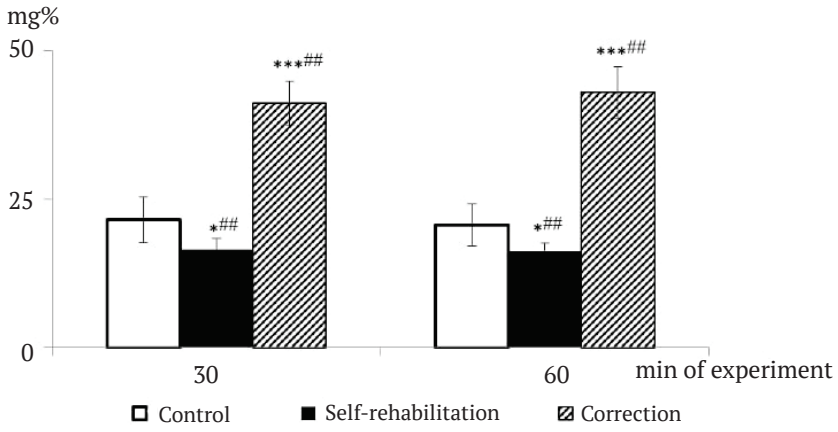

Figure 4. Concentration of free fatty acids in bile samples of rats ("Control" group), with experimental hepatic steatosis ("Self-rehabilitation" group) and in sick animals that were additionally administered BAS "FLP-MD" ("Correction" group) obtained during the first hour of the acute experiment

Notes: * P < 0.05 and * * * P < 0.001 statistically substantial difference compared to the control; ## P < 0.001 statistically substantial difference between the indicators of the "Self-rehabilitation" and "Correction" groups

At the second hour of the acute experiment, animals with hepatic steatosis ("Self-rehabilitation" group) demonstrated a further decrease in the concentration of free fatty acids, respectively, by 26.9% (P < 0.05) and 34.6% (P < 0.01), compared with the control – 21.15 ± 3.51 mg% (samples No. 3) and 21.88 ± 3.16 mg% (samples No. 4), which corresponded to the values of 15.46 ± 1.77 mg% (in samples No. 3) and 14.30 ± 2.22 mg% (in samples No. 4). Whereas in animals of the "Correction" group, the concentration of free fatty acids in bile was 42.28 ± 0.86 mg% (samples No. 3) and 39.13 ± 2.48 mg% (samples No. 4), which is 99.9% (P < 0.001) and 78.9% (P < 0.001) higher than in animals of the "Control" group, respectively. The difference in the content of free fatty acids in the bile of rats with hepatopathology ("Self-rehabilitation" group) and in rats who were treated with BAS "FLP-MD" ("Correction" group)

is even greater than during the first hour of the acute experiment. In particular, in both half-an-hour bile samples from animals of the "Self-rehabilitation" group, the concentration of free fatty acids was 2.7 times (P < 0.001) lower than in similar samples from rats of the "Correction" group (Fig. 5).

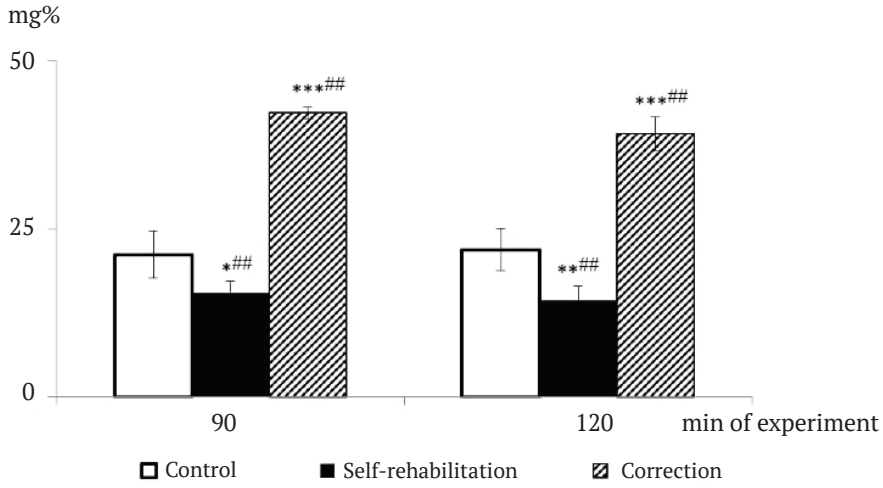

Figure 5. Concentration of free fatty acids in bile samples of rats ("Control" group), with experimental hepatic steatosis ("Self-rehabilitation" group) and in sick animals that were additionally administered BAS "FLP-MD" ("Correction group") obtained during the second hour of the acute experiment

Notes: * P < 0.05, * * P < 0.01 and * * * P < 0.001 statistically substantial difference compared to the control; ## P < 0.001 statistically substantial difference between the indicators of the "Self-rehabilitation" and "Correction" groups

In the bile samples during the final third hour of the acute experiment, the most pronounced differences in the concentration of free fatty acids were identified between animals of the "Control" group and rats with experimental hepatic steatosis ("Self-rehabilitation" group), and between animals that received BAS "FLP-MD" ("Correction" group) during the simulation of hepatic steatosis, and rats with tetracycline-induced hepatic steatosis without the specified correction ("Self-rehabilitation" group). Thus, the concentration of free fatty acids in the bile of animals with experimental hepatic steatosis was 13.24 ± ± 2.17 mg% (samples No. 5) and 12.46 ± 2.08 mg% (samples No. 6), which, respectively, was 41.1% (P < 0.01) and 47.2% (P < 0.001) lower than their concentration in the control (22.48 ± 3.40 mg% (samples No. 5) and 23.60 ± 3.37 mg% (samples # 6). In animals of the "Correction" group, the concentration of free fatty acids in bile samples was 37.10 ± 2.85 mg% (samples No. 5) and 35.55 ± 3.28 mg% (samples No. 6), i.e. it was 65.1% (P < 0.001) and 50.6% (P < 0.001) higher than in rats in the "Control" group. It was at the end of the acute experiment that the most substantial differences between the concentration of free fatty acids in the "Self-rehabilitation" and "Correction" groups were observed in bile samples No. 5 and No. 6. In particular, in animals that, in addition to tetracycline for modelling hepatic steatosis, were administered BAS "FLP-MD" ("Correction" group), the concentration of free fatty acids in bile samples No. 5 was 2.80 times (P < 0.001) higher than in similar bile samples obtained from animals of the "Self-rehabilitation" group. In bile samples No. 6 from animals of the "Correction" group, the concentration of free fatty acids was 2.85 times (P < 0.001) higher than in the corresponding half-an-hour bile samples of rats of the "Self-rehabilitation" group (Fig. 6).

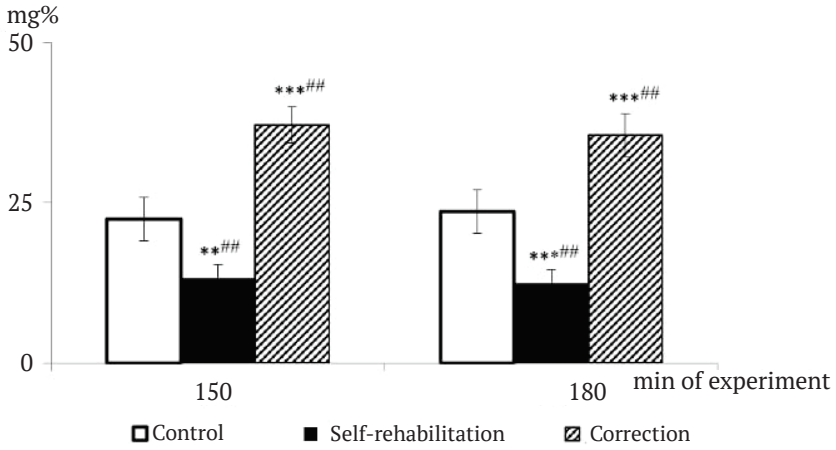

Figure 6. The concentration of free fatty acids in bile samples of rats ("Control" group), with experimental hepatic steatosis ("Self-rehabilitation" group) and in sick animals that were additionally administered BAS "FLP-MD" ("Correction" group), obtained during the second hour of the acute experiment

Notes: ** P < 0.01 and * * * P < 0.001 statistically substantial difference compared to the control; ## P < 0.001 statistically substantial difference between the indicators of the "Self-rehabilitation" and "Correction" groups

The described changes in the quantitative characteristics of free fatty acids in bile samples obtained from animals of different groups are marked by trends similar to the dynamics of the content of triacylglycerols and also indicate a pronounced choleretic effect of phospholipid-containing dietary supplement. This fact is especially important if the pathogenesis of hepatopathology indicates the development of intrahepatic cholestasis, which is also diagnosed with tetracycline-induced hepatosis, as described in the previous study [15].

A similar effect of phospholipid-containing drugs, regardless of the nature of the origin of the active substance, is noted in their works by other authors [7; 33]. Thus, according to the results obtained, the introduction of the dietary supplement "FLP-MD" (the main active substance – phospholipids of milk) to laboratory rats under experimental conditions allows not only successfully correcting the metabolic processes of the described lipid fractions disrupted by hepatic steatosis, but also substantially stimulating the mechanisms of their entry into bile.

Conclusions

Under the conditions of a drug-induced (tetracycline) form of hepatic steatosis in laboratory rats, inhibition of the processes that ensure the intake of triacylglycerols and free fatty acids in the bile is observed. The use of BAS "FLP-MD" (the main active substance is milk phospholipids) in rats simultaneously with the simulation of tetracycline-induced liver damage identifies a pronounced corrective effect on their content in bile samples during the entire 3-hour study period. Therewith, the concentration of triacylglycerols in the bile of animals that received BAS "FLP-MD" against the background of the development of hepatic steatosis was restored to values characteristic of animals of the "Control" group. The use of dietary supplements in rats in parallel with the simulation of tetracycline-induced hepatic steatosis stimulates the excretion of free fatty acids in the bile and leads to an increase in their concentration in hepatic secretions compared to the control and group of sick animals for Self-rehabilitation.

Considering the substantial disruptions of lipid homeostasis in hepatic steatosis and the identified effects of BAS "FLP-MD" on the content of triacylglycerols and free fatty acids in the bile of sick animals, it is possible to assert the prospects of using this supplement to correct the metabolism of the described individual lipid fractions in conditions of drug-induced liver damage, primarily with tetracycline group antibiotics. Especially relevant is the use of phospholipid-containing drugs in hepatopathology, accompanied by the development of intrahepatic cholestasis. Since bile secretory processes and physico-chemical properties of bile largely depend on the secretion of phospholipids and cholates by hepatocytes to the primary bile tubules, an important aspect of further investigation will be the experimental determination of the effects of BAS "FLP-MD" on the phospholipid and bile-acid composition of bile in the normal state and hepatopathology.

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Корекція вмісту триацилгліцеролів і вільних жирних кислот у жовчі щурів за експериментального жирового гепатозу

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Анотація. Актуальність наукового дослідження пов'язана із значним поширенням жирового гепатозу в свійських тварин (до 40 %) та розвитком небезпечних для здоров'я ускладнень у вигляді цирозу печінки, печінкової недостатності та онкології. Метою цієї роботи було визначити коригувальну ефективність біодобавки «FLP-MD» на основі фосфоліпідів молока щодо вмісту триацилгліцеролів і вільних жирних кислот у жовчі щурів із тетрациклініндукованим жировим гепатозом. Моделювання медикаментозної форми жирового гепатозу здійснювали шляхом внутрішньошлункового введення 4 %-го розчину препарату тетрацикліну гідрохлориду з розрахунку 0,5 г/кг маси тіла тварини протягом семи діб. В якості коригувальної терапії впродовж дев'яти діб тваринам внутрішньошлунково вводили біодобавку «FLP-MD» на основі фосфоліпідів молока у дозі 13,5 мг/кг маси тіла. На завершенні експерименту впродовж трьох годин через кожні 30 хв у щурів відбирали зразки жовчі, в яких визначали вміст триацилгліцеролів і вільних жирних кислот методом тонкошарової хроматографії. Встановлено, що концентрація триацилгліцеролів у жовчі хворих щурів на третю годину її відбору на 63,0 % нижча за контрольні показники. У лабораторних щурів, які на тлі моделювання медикаментозного гепатозу отримували фосфоліпідовмісну біодобавку, цей показник у жовчі відповідав значенням контрольної групи. Водночас концентрація вільних жирних кислот у зразках жовчі на третю годину її відбору в хворих щурів відзначалася зниженням на 47,2 % порівняно з контролем. Застосування досліджуваної біодобавки у хворих тварин викликало зростання концентрації вільних жирних кислот у жовчі в 2,85 раза порівняно з контролем, що зменшує інтенсивність їх використання на синтез триацилгліцеролів та запобігає розвитку жирової інфільтрації печінки. Отже, фосфоліпідовмісна біодобавка є високоефективним коригувальним засобом за порушення обміну триацилгліцеролів і вільних жирних кислот у щурів із медикаментозним жировим гепатозом. Це дає підстави рекомендувати її в якості коригувальної терапії та для профілактики розвитку жирового гепатозу, особливо у випадку застосування тваринам антибіотиків тетрациклінової групи

Ключові слова: фосфоліпіди молока, біологічно активна добавка, тетрацикліну гідрохлорид, коригувальна терапія

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Quality of Broiler Chicken Meat with the Addition of Chelated Compounds of Microelements to the Diet

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Abstract. At present, the current direction in poultry farming is the development of methods for raising poultry without the use of antibiotics to overcome antibiotic resistance. For this purpose, it is recommended to use mineral supplements of chelated forms of microelements of zinc, copper, and manganese in poultry diets. Chelated minerals are characterised by better digestibility during intensive broiler farming, which limits the use of minerals and reduces environmental pollution. The purpose of the study was to examine the quality of meat of broiler chickens of the Cobb-500 cross, provided that chelated forms of zinc, copper, and manganese are included in the diet. Experimental studies were conducted in 2021 on broiler chickens of the Cobb-500 cross. Two groups of 20 heads of poultry were formed to examine the chemical composition of meat. Poultry of the control group received a basic diet with zinc, copper, and manganese sulfates, and poultry of the experimental group – enriched with chelated compounds of these microelements. The addition of chelated compounds of zinc, copper, and manganese to the diet of broiler chickens leads to an increase in the amount of fat, calcium and zinc in white muscles by 69.6, 24.6% and 1.4 times, and in red muscles-by 41.1, 30.9% and 3.4 times, respectively. The content of copper and manganese increases by 48.0 and 95.5% in red muscles and by 28.1 and 15.2% in white muscles compared to the control group. Therewith, there is a decrease in the relative content of essential amino acids by 1.1-1.3% and an increase in non-essential amino acids by 2.6-2.7%. According to the overall assessment of organoleptic parameters of broiler chickens fed zinc, copper, and manganese chelates, the sum of points was 2.0 points higher in the femoral muscles and 1.5 points higher in the pectoral muscles. In addition, according to the tasting assessment of meat from the thigh muscles of broiler chickens, more points were obtained in terms of tenderness by 10.0%, taste by 12.2%, and aroma by 13.2%. According to the tasting assessment of meat from the pectoral muscles of broiler chickens, more points were obtained in terms of tenderness by 18.9% and aroma by 10.3%. According to the reaction with copper sulfate, the content of ammonium and ammonia salts, broiler chicken meat was fresh and obtained from healthy poultry. As a result of organoleptic, physico-chemical, and biochemical studies of broiler chicken meat under the conditions of adding microelement chelates to the diet, it was established that it belongs to fresh and high-quality for consumption. These studies argue for the use of chelated compounds of microelements in poultry farming and contribute to their further introduction into production

Keywords: poultry, white and red muscles, zinc, copper, manganese, chemical composition

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Introduction

Zinc, copper, and magnesium are important microelements that are necessary for poultry in small but sufficient quantities for normal biological functioning. Since drinking water is generally not considered the main source of minerals, feed and supplements are the main source of minerals for poultry. However, due to the potentially dangerous content of microelements and the difference in bioavailability among typical feed ingredients, micromineral additives, usually in the form of inexpensive oxides and sulfates, have become common practice, always providing a high supply of minerals in the body [1]. In addition, some microelements (such as copper and zinc) can be used at the pharmacological level to improve the health and productivity of poultry. Given the fact that the absorption of many minerals from the gastrointestinal tract is carefully regulated, and the effectiveness decreases with increasing mineral intake [2], most of the excess amount of microelements is simply released into the environment, which can be toxic to plants and microorganisms or contribute to the spread of microbial genes that give them resistance to antimicrobial substances [3; 4].

Microelements are essential components in the poultry diet. They are essential for the growth and development of bones and the formation of natural plumage. Microelements are part of enzymes, and also perform the function of appetite stimulants. Mineral microelements act as catalysts for many biological reactions in the body [1; 2; 4]. There are two sources of microelements: inorganic and organic. Common sulfates, oxides, chlorides, and carbonates are inorganic sources, but they do not have sufficient bioavailability. Due to the combination of amino acids and hydrolysed protein, organic microelements are formed by chelating a soluble salt. Through a coordinated bond, chelated minerals bind to the organic ligand. The relative bioavailability and efficacy of organic microelements may vary depending on the ligand attached to the mineral, the bond strength, and the ligand-to-mineral ratio [3].

It is well known [4] that organic chelates play a crucial role in the absorption of nutrients in the intestine and increase bioavailability. Iron, copper, zinc, manganese, chromium, and selenium are common organic microelements. The main function of zinc and copper is to improve the health and productivity of poultry. Organic manganese is important for enhancing bone growth, development, and reproductive function in animals and poultry [1]. Iron promotes the transport of oxygen and carbon dioxide in the body. In commercial practice, to meet the need for microelements, to avoid micronutrient deficiencies, and to help poultry reach its genetic growth potential, inorganic microelements are used in amounts that are 2-10 times higher than recommended by the National Research Council for Poultry Diet [5]. The use of inorganic microelements in the diet of poultry has two main disadvantages. Copper sulfate and zinc oxide are common inorganic sources of copper and zinc used in poultry diets, but these two sources are derived from the metallurgical industry and contain substantial amounts of pollutants such as fluorine and cadmium entering poultry feed [6]. Secondly, the antagonistic effect between inorganic microelements can reduce their metabolism and absorption rate. Chelated complexes of metals with amino acids exhibit inert properties since covalent and Ionic bonding occurs between the mineral and the ligand. Based on this, these forms remain under the influence of factors that lead to precipitation, similar to what happens with inorganic minerals after salt dissolution [5]. In addition, the sise and stability of chelated compounds of microelements can protect the latter during movement in the gastrointestinal tract and ensure their absorption unchanged, without any destruction of amino acids. Organic microelements have better bioavailability than their inorganic forms [2; 3; 7]. In the diet of poultry, a lower concentration of organic forms of minerals is required, which prevents negative effects on poultry productivity and the environment [7].

The purpose of the study is to determine organoleptic and biochemical parameters, examine the chemical and amino acid composition of meat of broiler chickens of the Cobb-500 cross, provided that chelated forms of zinc, copper, and manganese are included in the diet.

Literature Review

Different forms of organic minerals may differ in the degree of availability of chelated compounds, and therefore their effects on productivity, meat yield, and other indicators may also vary. Therefore, their effectiveness in the comparative aspect in relation to changes in the chemical composition of meat requires further study [2]. Common inorganic forms (sulfates) tend to easily separate from inorganic salts when exposed to acidic pH values in the gastrointestinal tract, which can increase the frequency of antagonism with other components of the poultry diet [3]. This may reduce their absorption and excretion in the faeces [4]. In scientific literature sources, it is noted [2; 5] that the use of chelated compounds in the form of premixes in the diet of industrial poultry contributes to the improvement of productivity, poultry health, and meat quality. Therefore, the researchers [7] comprehensively analysed chelates of minerals and amino acids, including copper. The productivity of laying hens and eggshell quality improved after the addition of copper methionine, compared to the performance of poultry that received additional copper sulfate.

Among chickens treated with chelated microelements, there was a substantial increase in body weight gain, mineral deposition in tissues, improved immunity, and an increase in feed conversion rate compared to chickens treated with inorganic microelements at the same dose [8]. Broiler chickens whose diet was supplemented with organic chromium (0.5 mg/kg) had an increased body weight compared to poultry whose diet was supplemented with chromium from inorganic sources [9]. The feed conversion rate increased in chickens fed organic minerals compared to chickens fed inorganic minerals (1.6 vs. 1.7). In addition, the introduction of mineral proteins to the broiler diet helped reduce ascites cases from 5% to 2% [9].

Zinc or copper methionine enhances humoral and cell-mediated immune functions [10]. Therefore, the researchers comprehensively analysed chelates of minerals and amino acids, including copper [9; 10]. Copper is considered a growth stimulator in poultry farming [8], especially given the fact that the use of antibiotics as growth stimulants is prohibited in the European Union [11]. In this regard, researchers [12-14] are searching for new alternative methods of treatment and prevention of animal diseases.

Copper is an extremely active catalyst for lipid peroxidation reactions [7]. Given the dominant degree of oxidation, zinc [10] is an integral part of several biochemical pathways as a catalytic or regulatory cofactor. It also plays a structural role in many other functional proteins [3]. Manganese is involved in various biological processes as a cofactor of superoxide dismutase, transferase, hydrolase, and lyase [5]. Manganese deficiency, especially in growing chickens, is closely associated with many side effects, manifested by growth retardation and skeletal abnormalities. Given that such chelates may soon be largely introduced into broiler feed, their impact on the nutritional value of meat should be considered.

In the course of the study, researchers identified [8] that the use of feed additives with chelated compounds of zinc, copper, and manganese stimulates an increase in the overall natural resistance of broilers, accompanied by an increase in bactericidal activity by 7.4%, phagocytic activity by 8.3%, and lysozyme activity by 6.6%. In this regard, it is advisable to involve chelated elements in industrial poultry farming, since they can be used as a powerful immunostimulator that ensures high quality of meat products without the use of antibiotics.

The use of zinc chelated supplements improves the antiviral response and systemic immunity, and also promotes specific suppression of viral replication or infection-related symptoms [15].

The use of chelated compounds of zinc, copper, and manganese increases the hatchability of chickens by 1.9%, egg production of chickens – by 5.8%, reduces feed conversion by almost 6.0%. Therewith, an increase in the ash level by 4.3% in the dry matter of the bones of day-old chickens indicates an improvement in the skeletal structure [8].

The dose of chelated compounds added to feed for broiler chickens can be reduced or increased, which does not create a negative impact on their productivity, the body's antioxidant defence system, hematological parameters, and meat quality [3; 6; 10].

Studies by F. Hussan, D. Krishna, V.C. Preetam, P.B. Reddy, S. Gurram [4] proved that the introduction of chelated minerals in the amount of 25% in the diet of poultry does not negatively affect productivity indicators, such as weight gain and feed intake. Therewith, the addition of up to 20% inorganic microelements also has no negative effect on weight gain and feed conversion rate, with the advantage of reducing environmental pollution due to lower mineral excretion.

Materials and Methods

Experimental studies were conducted in 2021 at the Department of Virology, Pathanatomy, and Poultry Diseases, and at the Department of Veterinary Expertise, Microbiology, Zoohygiene, and Safety and Quality of Livestock Products of Sumy National Agrarian University. Two groups of broiler chickens of the Cobb-500 Cross were formed according to the principle of analogues (control and experimental), 20 heads each to examine the chemical composition of poultry meat based on the vivarium of the Faculty of Veterinary Medicine of Sumy National Agrarian University. The bird was kept by the floor method in accordance with DSTU (National Standard of Ukraine) 8219:2015 [16]. Drinking of the bird was organised from drip drinkers and was not limited.

Poultry of the control group received a basic diet with zinc sulfates (100 mg/kg), copper (20 mg/kg), and manganese (100 mg/kg), and the experimental group – enriched with chelated compounds of zinc (56 mg/kg), copper (14 mg/kg), and manganese (56 mg/kg). For feeding broiler chickens, mixed feed with the addition of MINTREX® premix produced by Novus International was used. This premix contains mineral compounds, in particular chelated minerals zinc, copper, and manganese, which are distinguished by the presence of methionine hydroxyanalog as a ligand. In this case, two ligands covalently bind one metal atom. Experimental studies lasted 42 days.

All animal studies were conducted in accordance with Directive 2010/63/EU, as amended by Regulation (EU) 2019/1010 [17].

On the 42nd day of the experiment, poultry was slaughtered. Ten heads of broiler chickens from each group were selected for experimental studies. After 24 hours of cooling at 4°C, the meat from the carcasses was separated from the bones. White (pectoral muscles) and red muscles (thigh muscles) were used for the study. The samples were frozen at -20°C for further chemical analysis.

The content of dry matter, ash, protein, and fat in meat samples was determined by the standard AOAC method [18]. Total tissue lipids were extracted by Folch *et al.* method [19]. The content of calcium, zinc, copper, and manganese in meat samples was determined by the AAS flame method [20] on the Unicam 939 device (AA spectrometer Unicam, Great Britain) after ashing at a temperature of 550°C according to The AOAC method.

Standard ion solutions were purchased from Merck (Germany) for the production of calibration lines. Standard solutions of 20 mEq/L, 40, 60, 80, and 100 mEq/L were used for zinc, copper, manganese, and calcium.

The cholesterol content was determined by the colourimetric method [21]. The amino acid profile of meat samples was analysed by liquid hydrolysis in 6 M hydrochloric acid to obtain amino acids from protein using liquid chromatography on an LC-10ad chromatograph (Shimadzu Liquid Chromatograph, Japan), which was connected to an automatic sampler and a fluorimeter (JASCO-Intelligent Spectrofluorimeter 821fp, UK) [22].

The tasting assessment of boiled meat of broiler chickens of the experimental groups was conducted on a 5-point scale, where 1 point is the worst indicator, and 5 is the best indicator. In boiled meat, poultry was evaluated for taste, aroma, juiciness, tenderness; in meat broth – aroma, colour, taste, transparency, and richness. The research was conducted according to the methods set out in DSTU ISO 6658:2005 [23].

Veterinary and sanitary assessment of broiler meat was conducted using generally accepted methods [24]. The study of meat was conducted after its aging at a temperature of 0 to + 4°C. For the study, meat samples were taken from the pectoral and thigh muscles. Organoleptic and biochemical parameters were determined.

During the veterinary and sanitary assessment, the following organoleptic indicators were determined: the appearance of muscles, their smell, colour, the condition of muscles on the incision, the condition and elasticity of fat and muscle tissue, and the indicators of the cooking sample. For biochemical studies, an extract of meat was

prepared with water in a ratio of 1 to 3. Production of the extract for research was conducted separately from the red and white muscles of broiler chickens.

According to the reaction of measuring the activity of the peroxidase enzyme in meat, which is based on the oxidation of hydrogen peroxide by benzidine in the presence of the peroxidase enzyme to form paraquinondiamide, its quality was evaluated. With a positive reaction, a blue-green colour was formed, which after a while changed to brown.

A reaction with Nessler's reagent was used to determine the presence of ammonia and ammonium salts in meat. As a result of this reaction, a compound of dimercuramonium iodide is formed, namely its complex salt, which provides the appearance of a yellow-orange colour.

A reaction with copper sulfate was used to determine such an indicator as the freshness of meat. During this reaction, meat proteins are deposited during heating and copper sulfate complexes are formed with the products of primary protein breakdown.

Isolation of volatile fatty acids from broiler chicken meat was conducted by distillation with water vapour and titration with 0.1 N potassium hydroxide solution. The analysis was conducted using water vapour distillation equipment, which consists of a round-bottomed flask, a flask heater, a flat-bottomed flask, a safety tube, a steam-removing tube, a drop trap, a refrigerator and a conical flask. For a control study, distilled water with reagents was used, without the use of meat extracts.

The obtained digital results were processed using variational and statistical methods. The arithmetic mean (M) and statistical error of the arithmetic mean (m) were determined. The probability of difference was determined by the reliability criterion (td) and by the Student's method [25]. The difference between the two values was considered probable for *P < 0.05.

Results and Discussion

The study began with an assessment of the condition of broiler chicken carcasses in the experimental and control groups. It was established that broiler chickens had a shiny beak, the oral mucosa was shiny, pale pink in colour and characterised by little moisture, and the eyeballs are convex in shape and have a shiny cornea.

In broiler chicken carcasses, the skin surface had a dry consistency, white-yellow colour, with a reddish tinge. The muscles of broiler chickens on the cut are moistened, pale pink in colour, elastic in consistency, with a specific characteristic smell of fresh meat. No changes in internal organs or adipose tissue were observed. Therewith, the tendons in the carcasses of broiler chickens are dense, elastic, and the surface of the joints is smooth and glossy. According to the sample of cooking meat, a clear and aromatic broth was obtained, with a characteristic specific smell and taste.

According to some indicators of the chemical composition of red and white muscles of broiler chickens of the experimental and control groups, namely: in terms of humidity, dry matter, relative protein, and ash content, no statistically substantial difference was established (Table 1).

Table 1. Chemical composition of broiler chicken meat, M ± m, n = 10

Indicator	Research group		Control group	
	Red muscles	White muscles	Red muscles	White muscles
Humidity, %	72.37 ± 0.12	70.78 ± 0.13	74.14 ± 0.14	73.05 ± 0.21
Dry matter, %	27.63 ± 0.12	29.22 ± 0.13	25.86 ± 0.14	26.95 ± 0.21
Protein, %	22.91 ± 0.12	23.02 ± 0.21	20.91 ± 0.22	19.94 ± 0.31
Fat, %	6.84 ± 0.14*	10.23 ± 0.31*	2.08 ± 0.21	6.03 ± 0.22
Cholesterol, mg/100 g	41.32 ± 0.26*	46.10 ± 0.32*	54.31 ± 0.43	56.12 ± 0.24
Mineral substances, %	1.13 ± 0.32	1.14 ± 0.18	0.98 ± 0.11	1.02 ± 0.1
Calcium, mg/100 g	8.02 ± 0.53*	4.52 ± 0,36*	5.54 ± 0.61	3.41 ± 0.32
Zinc, mg/100 g	6.32 ± 0.24*	1.94 ± 0.12*	1.84 ± 0.12	1.37 ± 0.10
Copper, mg/100 g	0.037 ± 0.005*	0.041 ± 0.001*	0.025 ± 0.001	0.032 ± 0.001
Manganese, mg/100 g	0.043 ± 0.002*	0.038 ± 0.002*	0.022 ± 0.002	0.033 ± 0.001

Note: * P < 0.05, compared to the control group

However, the relative amount of fat in the white muscles of broiler chickens in the experimental group was higher by 69.6% (P < 0.05), and in red – by 41.1% (p < 0.05) compared to the control group (Table 1). Fat has a positive effect on the texture and taste of poultry meat [19]. In turn, the cholesterol content in the white muscles of broiler chickens in the experimental group was lower by 23.9%, and in red – by 17.9% compared to the control group.

The addition of chelated compounds to the feed of chickens in the experimental group contributed to a 30.9% increase in calcium content in red muscle and 24.6% in white muscle compared to the control group. In the study group, an increase in zinc content was also noted, 3.4 times higher for red muscles and 1.4 times higher for white muscles. The content of copper and manganese increased by 48.0 and 95.5% (P < 0.05) in red muscles, respectively, and by 28.1 and 15.2% (p < 0.05) in white muscles compared to the control group.

The enriched mineral composition of meat provides an increase in the nutritional value of the product, which also indicates a decrease in the release of microelements by poultry in faeces [3].

In addition, the amino acid composition of broiler chicken meat was investigated (Table 2).

Table 2. Comparative assessment of muscle tissue protein usefulness in broiler chickens, %, n = 10

Amino acid	Control group		Research group	
	Red muscles	White muscles	Red muscles	White muscles
Essential amino acids				
Valine	4.36 ± 0.02	4.30 ± 0.08	4.11 ± 0.06*	4.54 ± 0.07*
Isoleucine	3.92 ± 0.03	3.74 ± 0.05	3.68 ± 0.07*	3.96 ± 0.10
Leucine	7.32 ± 0.24	7.48 ± 0.32	6.82 ± 0.08	7.31 ± 0.09
Lysine	9.02 ± 0.09	8.71 ± 0.12	9.18 ± 0.18	7.41 ± 0.12
Methionine	1.69 ± 0.03	1.79 ± 0.02	2.41 ± 0.06*	1.87 ± 0.09
Threonine	3.52 ± 0.05	3.55 ± 0.03	3.24 ± 0.08	3.12 ± 0.10
Tryptophan	1.42 ± 0.02	1.32 ± 0.03	1.45 ± 0.02	1.63 ± 0.02*
Phenylalanine	3.96 ± 0.12	3.58 ± 0.09	3.24 ± 0.07	3.36 ± 0.08
Total	35.21 ± 0.60	34.47 ± 0.74	34.13 ± 0.62	33.20 ± 0.67
Non-essential amino acids				
Alanine	5.18 ± 0.21	5.34 ± 0.12	6.56 ± 0.18*	5.94 ± 0.26
Arginine	6.40 ± 0.16	6.17 ± 0.18	7.03 ± 0.20*	6.72 ± 0.31
Aspartic acid	7.98 ± 0.18	7.09 ± 0.17	8.69 ± 0.17*	5.53 ± 0.19*
Histidine	2.26 ± 0.09	2.01 ± 0.10	2.53 ± 0.21	2.37 ± 0.22
Glycine	4.98 ± 0.17	4.78 ± 0.23	5.02 ± 0.09	4.68 ± 0.18
Glutaric acid	14.7 ± 1.21	13.7 ± 1.13	14.14 ± 1.11	14.59 ± 1.24
Oxyproline	0.19 ± 0.02	0.16 ± 0.03	0.26 ± 0.08	0.34 ± 0.06*
Proline	3.88 ± 0.21	3.59 ± 0.19	3.83 ± 0.15	3.96 ± 0.21
Serine	3.57 ± 0.15	3.99 ± 0.24	4.28 ± 0.20*	3.94 ± 0.23
Tyrosine	2.43 ± 0.21	2.15 ± 0.17	2.03 ± 0.08	2.48 ± 0.14
Cysteine	1.57 ± 0.14	1.27 ± 0.18	1.35 ± 0.12	1.36 ± 0.17
Total	53.14 ± 2.75	50.25 ± 2.74	55.72 ± 2.59	52.91 ± 3.21

Note: * P < 0.05, compared to the control group

Based on the results presented in Table 2 it follows that from the investigated essential amino acids in red muscles, the relative content of valine and isoleucine decreases by 1.1 times, respectively (P < 0.05). However, in white muscles, on the contrary, the relative valine content increases – by 1.1 times (P < 0.05) compared to the control group. Therewith, such methionine increases 1.4 times (P < 0.05) in red muscles and tryptophan increases 1.2 times (P < 0.05) in white muscles.

Among the investigated non-essential amino acids, an increase in the relative content of alanine by 1.3 times was noted (P < 0.05), arginine by 1.1 times (P < 0.05), aspartic acid, and serine, respectively, by 1.1 and 1.2 times (P < 0.05) in red muscles compared to the control group. In white muscles, the oxyproline content increased by 2.1 times (P < 0.05), and the aspartic acid content decreased by 1.3 times (P < 0.05) compared to the control group. The results of the tasting evaluation of boiled broiler chicken meat are shown in Table 3.

Table 3. Tasting assessment of boiled broiler chicken meat, M ± m, n = 10

Indicator	Juiciness	Tenderness	Taste	Aroma	Total points
Femoral muscles					
Control group	4.2 ± 0.3	4.0 ± 0.1	4.1 ± 0.2	3.8 ± 0.1	19.8
Research group	4.5 ± 0.2	4.4 ± 0.1*	4.6 ± 0.1*	4.3 ± 0.2*	17.8
Pectoral muscles					
Control group	3.7 ± 0.6	3.7 ± 0.2	4.1 ± 0.2	3.9 ± 0.1	15.4
Research group	3.9 ± 0.5	4.4 ± 0.2*	4.3 ± 0.3	4.3 ± 0.1*	16.9

Note: * P < 0.05, compared to the control group

According to the tasting assessment (see Table 3), boiled meat (thigh muscles) of broiler chickens of the experimental group had an increase in the indicators of tenderness by 10.0% ($P < 0.05$), taste by 12.2% ($P < 0.05$), and aroma by 13.2% ($P < 0.05$) compared to the indicators of the control group. According to the tasting assessment (Table 3), boiled meat (pectoral muscles) of broiler chickens of the experimental group demonstrated an increase in tenderness indicators by 18.9% ($P < 0.05$) and aroma by 10.3% ($P < 0.05$) compared to the control group. For other investigated indicators, no statistically substantial difference was identified (Table 3). Therewith, there are no foreign odours or taste in the meat of the experimental and control groups.

In addition, the total amount of points for the tasting assessment of broiler chicken meat was analysed and it was identified that the indicators in the femoral muscles exceed by 2.0 points those in the control group. Instead, the scores assigned to the tasting assessment of the pectoral muscles of broiler chickens were 1.5 times higher than in the control group.

During the tasting of broiler chicken broth, the experimental group received 0.2-0.3 points more than the control group.

Thus, boiled meat and broth from it of broiler chickens fed microelements in chelated form had better results on tasting assessment than boiled meat and broth obtained from broiler chickens of the control group.

According to the biochemical parameters of broiler chicken meat as the acid number of fat, the amount of volatile fatty acids, the peroxide number of fat and PH, no statistically substantial difference was identified (Table 4).

Table 4. Biochemical parameters of broiler chicken meat, M ± m, n = 10

Indicator	Control group	Research group
The acid number of fat, mg AV	0.53 ± 0.11	0.48 ± 0.21
Amount of volatile fatty acids, mg AV/g	2.7 ± 0.1	2.8 ± 0.1
Peroxide number of fat, g of iodine	$9.02\ 10^{-3} \pm 0.02 \cdot 10^{-3}$	$9.06 \cdot 10^{-3} \pm 0.02 \cdot 10^{-3}$
PH value of white meat	5.6 ± 0.1	5.5 ± 0.2
PH value of red meat	5.9 ± 0.2	6.0 ± 0.1
Reaction with copper sulfate	Negative	Negative
Qualitative reaction to ammonia and ammonium salts	Negative	Negative
Qualitative response to peroxidase activity in white meat	Negative	Negative
Qualitative response to peroxidase activity in red meat	Positive	Positive

As can be seen from the results in Table 4, the reaction with copper sulfate is negative, which indicates the absence of protein breakdown products in the broiler chicken broth of the experimental group.

Therewith, the absence of ammonia and ammonium salts in the meat of broiler chickens of the experimental group indicates the freshness of the meat and the fact that it is obtained from healthy poultry. According to the qualitative reaction to determine the activity of the peroxidase enzyme, its presence was established in the red meat of broiler chickens of the experimental and control groups. In turn, in the white muscles of broiler chickens of the experimental and control groups, this enzyme is absent.

The addition of zinc nanoxide to the diet of broilers in experiments conducted by the authors [4] did not substantially affect the amount of fat in poultry carcasses, pH values, and cholesterol, but according to their own studies, a substantial increase in the amount of fat was by 69.6% ($P < 0.05$) in the white muscles of broiler chickens, and in red – by 41.1%. Studies have shown that the cholesterol content in the white muscles of poultry, to the diet of which chelated elements were added, was lower by 23.9%, and in red – by 17.9% compared to the control group.

According to the conducted experimental studies, an increase in the zinc content in the red muscles of broiler chickens was by 3.4 times, and in white ones – by 1.4 times. Therewith, the red muscles of broiler chickens increased the content of copper and manganese, respectively, by 48.0 and 95.5%, and in white – by 28.1 and 15.2% ($P < 0.05$) compared to the control group. The results obtained are consistent with the results of other researchers [6].

Therefore, adding chelated minerals in to the diet of broiler chickens is advisable for obtaining high-quality meat. H.A. Ghasemi [26] and other authors also note a positive effect of chelated compounds on growth, mineral absorption, skeletal bone condition, and antioxidant status in broiler chickens. C. Feng *et al.* [7] discovered that chelated minerals are characterised by better digestibility. Therefore, they can be used 75% less than the recommended level, without substantially reducing the production efficiency and slaughter yield of poultry, which reduces the ingress of minerals into the environment [8]. Today, the use of minerals is limited in the intensive cultivation of broiler chickens, which accordingly reduces environmental pollution.

Conclusions

The use of chelated compounds of zinc, copper, and manganese in the diet of broiler chickens does not affect the humidity level and relative content of dry matter, protein, and ash in meat. The amount of fat in the white muscles of broiler chickens increases by 69.6%, and in red – by 41.1% compared to the control. The calcium content of broiler chicken meat increases by 30.9% in red muscles and 24.6% in white muscles, and the zinc content in red and white muscles increases by 3.4 and 1.4 times, respectively. There is an increase in the content of copper and manganese by 48.0 and 95.5% in red muscles and by 28.1 and 15.2% in white muscles, respectively, compared to the control group.

In the red muscles of broiler chickens, when microelement chelates are added, the relative content of essential amino acids, namely valine and isoleucine, decreases by 1.1 times, respectively, and methionine increases by 1.4 times. Therewith, the relative content of valine and tryptophan in white muscles increases by 1.1 times.

In addition, red muscles show an increase in the relative content of non-essential amino acids: alanine by 1.3 times, arginine by 1.1 times, aspartic acid and serine, respectively, by 1.1 and 1.2 times. In white muscles, the relative content of oxyproline increases by 2.1 times and aspartic acid decreases by 1.3 times.

In addition, the indicators of organoleptic assessment of the femoral muscles of broiler chickens, which were added premix with chelated compounds of zinc, copper, and manganese to the main diet, predominate by 2.0 points, and the pectoral muscles – by 1.5 points.

The addition of premix with chelated compounds of microelements to the diet of broiler chickens does not affect the quality indicators of broiler chicken meat: acid and peroxide fat number, volatile fatty acid content, and pH value. Therewith, the content of ammonium and ammonia salts and the reaction with copper sulfate confirmed the freshness of meat and its receipt from healthy poultry. Thus, the results obtained indicate the prospects of using chelated compounds of microelements in poultry farming.

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Якість м'яса курчат-бройлерів за додавання до раціону хелатних сполук мікроелементів

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Анотація. Нині актуальним направленням у птахівництві є розробка методів вирощування птиці без застосування антибіотиків задля подолання антибіотикорезистентності. Для цього рекомендовано використання в раціонах птиці мінеральних добавок хелатних форм мікроелементів цинку, міді та марганцю. Хелатні мінерали характеризуються кращою засвоюваністю під час інтенсивного вирощування бройлерів, що обмежує використання мінералів і зменшує забруднення навколишнього середовища. Метою роботи було дослідження якості м'яса курчат-бройлерів кросу Кобб-500 за умови включення до раціону хелатних форм цинку, міді та марганцю. Експериментальні дослідження проводилися протягом 2021 року на курчатах-бройлерах кросу Кобб-500. Для дослідження хімічного складу м'яса було сформовано дві групи по 20 голів птиці. Птиця контрольної групи отримувала основний раціон із сульфатами цинку, міді та марганцю, а птиця дослідної групи – збагачений хелатними сполуками цих мікроелементів. Додавання до раціону курчат-бройлерів хелатних сполук цинку, міді та марганцю призводить до збільшення кількості жиру, кальцію та цинку в білих м'язах на 69,6, 24,6 % і в 1,4 раза, а в червоних – на 41,1, 30,9 % і в 3,4 раза, відповідно. При цьому, підвищується вміст міді та марганцю на 48,0 і 95,5 % у червоних м'язах і на 28,1 і 15,2 % – у білих порівняно з контрольною групою. Водночас спостерігається зниження відносного вмісту незамінних амінокислот на 1,1-1,3 % і збільшення – замінних амінокислот на 2,6-2,7 %. За загальною оцінкою органолептичних показників м'яса курчат-бройлерів, яким згодовували хелати цинку, міді та марганцю, сума балів була більшою на 2,0 бали в стегнових м'язах і на 1,5 – в грудних. При цьому, за дегустаційною оцінкою м'яса з стегнових м'язів курчат-бройлерів отримано більше балів за показниками: ніжності на 10,0 %, смаку на 12,2 % і аромату на 13,2 %. Водночас за дегустаційною оцінкою м'яса з грудних м'язів курчат-бройлерів отримано більше балів за показниками: ніжності на 18,9 % і аромату на 10,3 %. За реакцією з міді сульфатом, вмістом солей амонію та аміаку м'ясо курчат-бройлерів належало до свіжого та отриманого від здорової птиці. В результаті проведених органолептичних, фізико-хімічних і біохімічних досліджень м'яса курчат-бройлерів за умов додавання до раціону хелатів мікроелементів встановлена приналежність його до свіжого та якісного для споживання. Ці дослідження аргументують використання хелатних сполук мікроелементів в птахівництві і сприяють їх подальшому впровадженню у виробництво

Ключові слова: птиця, білі та червоні м'язи, цинк, мідь, марганець, хімічний склад

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Exposure to Disinfectants of Various Chemical Nature on the Culture of Pathogenic Leptospira

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Abstract. Infectious diseases cause substantial economic damage to livestock farms, so there is a constant search for new means of preventing diseases, especially disinfectants. Analysis of the scientific literature indicates a substantial problem of leptospirosis in Ukraine and there are virtually no data on the use of complex oxidising drugs for its prevention. The purpose of the work was to examine the effect of Biolide (active substances hydrogen peroxide, lactic and supralactic acids) and Diolide disinfectants (active substances sodium chlorite and sodium chloride) on the causative agents of leptospirosis. The stability of eight pathogenic Leptospira cultures of different ages circulating in Ukraine and their growth properties were tested by adding different concentrations of these disinfectants to them. The results obtained were statistically analysed in the Epitools – Epidemiological Calculators software. Effective concentrations and exposures of Biolide and Diolide for use in preventive and forced disinfection in leptospirosis were determined. As a result of studies on the effect of both disinfectants on 7-, 10- and 15-days Leptospira test cultures, no differences were recorded between the indicators of their accumulation (number of microbial cells/cm^3). Therefore, the results obtained for cultures of different ages were considered as repeatability. It is proved that for preventive and forced disinfection in leptospirosis, a 0.55% solution of Biolide is recommended for use at an exposure of 30 minutes at a temperature of 24°C. If the exposure period is increased to 60 minutes, it is allowed to reduce the concentration of the product to 0.185%. Regarding the drug "Diolide", it is recommended to use it in this zoonosis in a dilution of 200 mg/l (concentration of 0.08% of the active substance) during exposure for 15 minutes at a temperature of 24°C. If the exposure period is increased to 30 minutes, it is allowed to reduce the dilution of the drug to 50 mg/dm^3 (concentration of 0.02% of the active substance). In addition, it was determined that both disinfectants completely inhibit the growth of pathogenic cultures of Leptospira. The practical value of the study is to prove the possibility of using complex disinfectants based on oxidising agents for the prevention of leptospirosis

Keywords: veterinary medicine, leptospirosis, disinfection, Biolide, Diolide, bactericidal

Introduction

In January 1915, Japanese researchers Inada and Ido announced the discovery of the causative agents of Weyl's disease, which later became known as leptospirosis [1]. Leptospirosis is an infectious disease with a global spread, which is one of the most common (registered on all continents except Antarctica) and substantial in socio-economic terms natural focal zoonoses and poses a substantial danger to human and animal health (affects about 150 species of mammals) [2; 3]. Thus, about 0.5 million cases of human leptospirosis per year are registered globally, and the mortality

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rate ranges from 5% to 15% [4; 5]. Animals with leptospirosis develop non-sterile immunity. In addition, the prevalence of the disease is facilitated by the variability of its etiological structure among different animal species, both within countries and regions, districts, etc. [6]. This fact is primarily explained by the extreme variability of pathogens (there are about 250 serovars of Leptospira) [2; 7].

All of the above contributes to the constant search for new effective measures for the prevention of leptospirosis, namely vaccines and disinfectants that would destroy the causative agent of the disease during its stay in the environment after isolation from the host (sick animal or human) [8].

For effective prevention, priority should be paid to the biology of the causative agent of the disease. Thus, according to recent studies, Leptospira are tightly coiled spirochetes, usually ranging in size from 0.1 by 6 microns to 0.1 by 20 microns, but a separate culture may contain longer cells [9]. Microorganisms are mobile, have rounded ends, which are usually bent in the form of hooks. Leptospira, like other spirochetes, have a typical double membrane structure, in which the cytoplasmic membrane and the peptidoglycan cell wall are closely connected and overlapped by an outer membrane. These microorganisms are Gram-negative obligate aerobes with an optimal growth temperature of 28 to 30°C [9; 10]. Given the hydrophilicity of the pathogen (the vital activity of Leptospira substantially depends on the availability of water sources), the number of outbreaks of the disease increases substantially after natural disasters (hurricanes, floods, etc.) [11]. This indicator also depends on the climate – the wetter it is, the more outbreaks there are [12].

It has been experimentally proven that pathogenic Leptospira cannot circulate and survive in nature without the involvement of host organisms, and the main transmission routes are contaminated environmental objects [13; 14]. For example, in soil and water, these microorganisms can survive for 16-28 days, after which molecular genetic diagnostic methods begin to give a negative result [15]. Therefore, to interrupt the epizootic chain of infection, Leptospira must be destroyed on contaminated surfaces, bedding, feeders, drinkers, etc.

Since Leptospira do not form spores and are relatively unstable in the environment, according to the recommendations of the World Organisation for Animal Health, the following chemicals/disinfectants affect them: 1.0% sodium hypochlorite, 70% ethanol, formaldehyde, detergents, quaternary ammonium compounds, iodine-based compounds, glutaraldehyde, and hydrogen peroxide [8]. Analysis of the scientific literature indicates that oxidising disinfectants, which include halogens, chlorine, iodine, bromine, and chlorine dioxide, and oxygen-releasing substances such as peracetic acid and hydrogen peroxide, are most common in animal and poultry farming [16]. The relevance of such drugs is promoted by their cost-effectiveness combined with the ease of their use, especially in the presence of animals. Most often, complex drugs based on lactic acid, quaternary ammonium compounds, hydrogen peroxide, and other non-toxic oxidising agents are used. These substances are cheap compared to other active substances, have a wide spectrum of bactericidal action and low corrosion activity [17]. Therewith, there are no complex drugs based on these compounds on the Ukrainian market for the disinfection of livestock premises from pathogens of leptospirosis.

However, effective use of the disinfectant in production conditions is possible with its through examination at the stage of laboratory and production tests. For the purpose of preventive and forced disinfection in the event of dangerous infectious diseases, a detailed analysis of the bactericidal effect of new findings in disinfectants, including in relation to leptospirosis, is necessary.

Considering all the above, *the purpose of the study* was to determine the effective concentrations and exposure of complex disinfectants "Biolide" and "Diolide" for use in preventive and forced disinfection in leptospirosis.

Materials and Methods

All studies, the results of which are presented in the study, were fully conducted based on the State Scientific and Research Institute of Laboratory Diagnostics and Veterinary and Sanitary Expertise (DNDILDVSE, Kyiv) during January-February 2022.

As a test culture for experiments, strains of eight serogroups of Leptospira were used, which most often cause leptospirosis among animals in Ukraine and are included in the diagnostic series of Leptospira for setting up a microagglutination reaction (PMA) on the territory of the state (Table 1).

Table 1. List of Leptospira strains and their serogroup and serovariant correspondence

No.	Serogroup	Serovar	Strain
1.	Sejroe	Polonica	493 Poland
2.	Hebdomadis	Kabura	Kabura
3.	Tarassovi	Tarassovi	Perepelicyni
4.	Pomona	Pomona	Pomona
5.	Grippotyphosa	Grippotyphosa	Moskva V
6.	Canicola	Canicola	Hond Utrecht IV
7.	Icterohaemorrhagiae	Copenhageni	M 20
8.	Australis	Bratislava	Yez bratislava

The experiment used 7- ("young" culture), 10- ("mature" culture), and 15-days ("old" culture) Leptospira test cultures with an accumulation of at least 80-100 million microbial cells/cm^3, typical morphology of mobile microorganisms.

The objects of research were Biolide disinfectants with hydrogen peroxide, lactic, and supralactic acids in the composition, and Diolide, the active ingredients of which are sodium chlorite and sodium chloride. The developer of both disinfectants is DNDILDVSE.

Preparation of working solutions of these disinfectants, and the selection of their concentrations and exposures necessary for research, was conducted in accordance with the leaflets-tabs to them. The method of sequential multiple dilutions was used to obtain the required concentrations [18].

Thus, for studies on the effect of Biolide disinfectant on Leptospira culture, 2.0 cm^3 of the environment of the Terskikh were introduced into each of the five glass test tubes 1.0 cm^3 of 10% disinfectant solution was added to the first test tube and thoroughly mixed. After that, 1.0 cm^3 was transferred from the first test tube content to the second, from the second to the third, etc. 1.0 cm^3 was removed from the fifth test tube. As a result, multiple dilutions were obtained 1 : 2, 1 : 4, 1 : 8, 1 : 16 and 1: 32 with a drug concentration of 3.33%, 1.11, 0.37, 0.123 and 0.041%. At the next stage, they were transferred to 5 sterile test tubes of 1.0 cm^3 each from contents from each dilution and added to them 1.0 cm^3 of culture of Leptospira of a certain age. Thus, working dilutions of the product in the mixture of 1.67%, 0.55, 0.185, 0.062, and 0.02% were obtained, respectively.

Regarding the study of the effect of Diolide on the stability of these crops, a uterine solution of a disinfectant with a chlorine dioxide content of 5000 mg/cm^3 was first prepared, for which 4 g of the drug was dissolved in 100.0 cm^3 of distilled water. An initial experimental concentration of 400 mg/cm^3 was then prepared, by adding 8.0 cm^3 of the uterine solution up to 92.0 cm^3 of distilled water. A series of multiple dilutions in 5 glass tubes was prepared as follows: 2.0 cm^3 was added to the first tube of solution with a working concentration of 400 mg/cm^3 and to the next 4 test tubes 1.0 cm^3 of the environment of the Terskykh was added. Then 1.0 cm^3 was transferred from the first test tube contained in the second, from the second to the third, etc. 1.0 cm^3 was removed from the fifth test tube. As a result, a series of multiple dilutions of 0.16%, 0.08, 0.04, 0.02, and 0.01% were obtained with a concentration of 400 mg/cm^3 of chlorine dioxide in them, 200, 100, 50, and 25 mg/cm^3. At the final stage, 1.0 cm^3 was added to each test tube with a culture of Leptospira of a certain age. After applying the culture, the working dilutions of the product in the mixture were 0.08%, 0.04, 0.02, 0.01, and 0.005% (200 mg /cm^3, 100, 50, 25, and 12.5 mg/cm^3 accordingly).

For the experiment on testing disinfectors for the growth of Leptospira culture, a Terskykh nutrient environment (pH 7.2-7.4) was prepared with the addition of 10.0% rabbit blood serum, which ensures optimal growth of these microorganisms. The accumulation of Leptospira was recorded visually in the dark field of view of the microscope at a magnification of 10x40 in accordance with international requirements [8].

All manipulations of the experiments were performed three times. The minimum bactericidal activity of the investigated agents was determined by the lowest concentration of drugs that inhibit the growth of Leptospira. The study was performed at exposures of 15, 30 and, if necessary, 60 minutes at room temperature.

Statistical processing of the obtained results in the course of the study was performed in the software Epitools – Epidemiological Calculators [19]. It was used to calculate confidence intervals (CI) for the obtained values at the probability level (P) of 0.95. Therewith, the Student's test was not calculated due to substantial deviations in numerical ranges (for example, the accumulation of Leptospira could be recorded in the range of 10-30 million microbial cells/cm^3).

Results and Discussion

As a result of studies on the effect of both disinfectants on 7-, 10-, and 15-days-old Leptospira test cultures, no difference was recorded between the indicators of their accumulation (number of microbial cells/cm^3). Therefore, the results obtained in the context of cultures of different ages were considered repeatability.

Systematised and visualised results of studies on the effect of various concentrations of Biolide on the resistance of Leptospira strains are presented in Table 2 and in Figure 1.

Table 2. Results of the effect of Biolide disinfectant on Leptospira culture at different concentrations and exposures (n = 9)

Exposure, min.	Biolide concentration, %	Leptospira serogroups (mil/mL)								Control (mil/mL)
		Sejroe	Hebdomadis	Tarassovi	Pomona	Grippotyphosa	Canicola	Icterohaemorrhagiae	Australis	
15	1.67	–	–	–	–	–	–	–	–	80-100
	0.55	–	5-10	–	0-5	–	–	0-5	10-20	80-100
	0.185	0-20	15-40	5-15	0-20	15-40	0-20	20-30	30-40	80-100
	0.062	5-40	50-60	15-20	30-50	30-50	30-40	40-60	50-60	80-100
	0.02	15-40	50-70	20-30	30-50	40-50	40-50	50-70	60-70	80-100

30	1.67	–	–	–	–	–	–	–	–	80-100
	0.55	–	–	–	–	–	–	–	–	80-100
	0.185	–	5-10	–	–	0-10	–	–	5-10	80-100
	0.062	0-30	40-50	15-20	20-40	20-40	20-30	40-50	50-60	80-100
	0.02	15-30	40-60	20	20-40	20-50	30-40	50-70	60-70	80-100
60	1.67	–	–	–	–	–	–	–	–	80-100
	0.55	–	–	–	–	–	–	–	–	80-100
	0.185	–	–	–	–	–	–	–	–	80-100
	0.062	0-20	30-40	0-10	10-40	10-30	0-30	20-50	30-60	80-100
	0.02	5-20	40-60	10-15	10-40	10-50	30	40-60	40-70	80-100

Note: in the table, the values of confidence intervals (Cl) relative to the number of Leptospira in the field of view of the microscope with a probability level of 0.95 are given, "–" – the absence of Leptospira in the field of view of the microscope

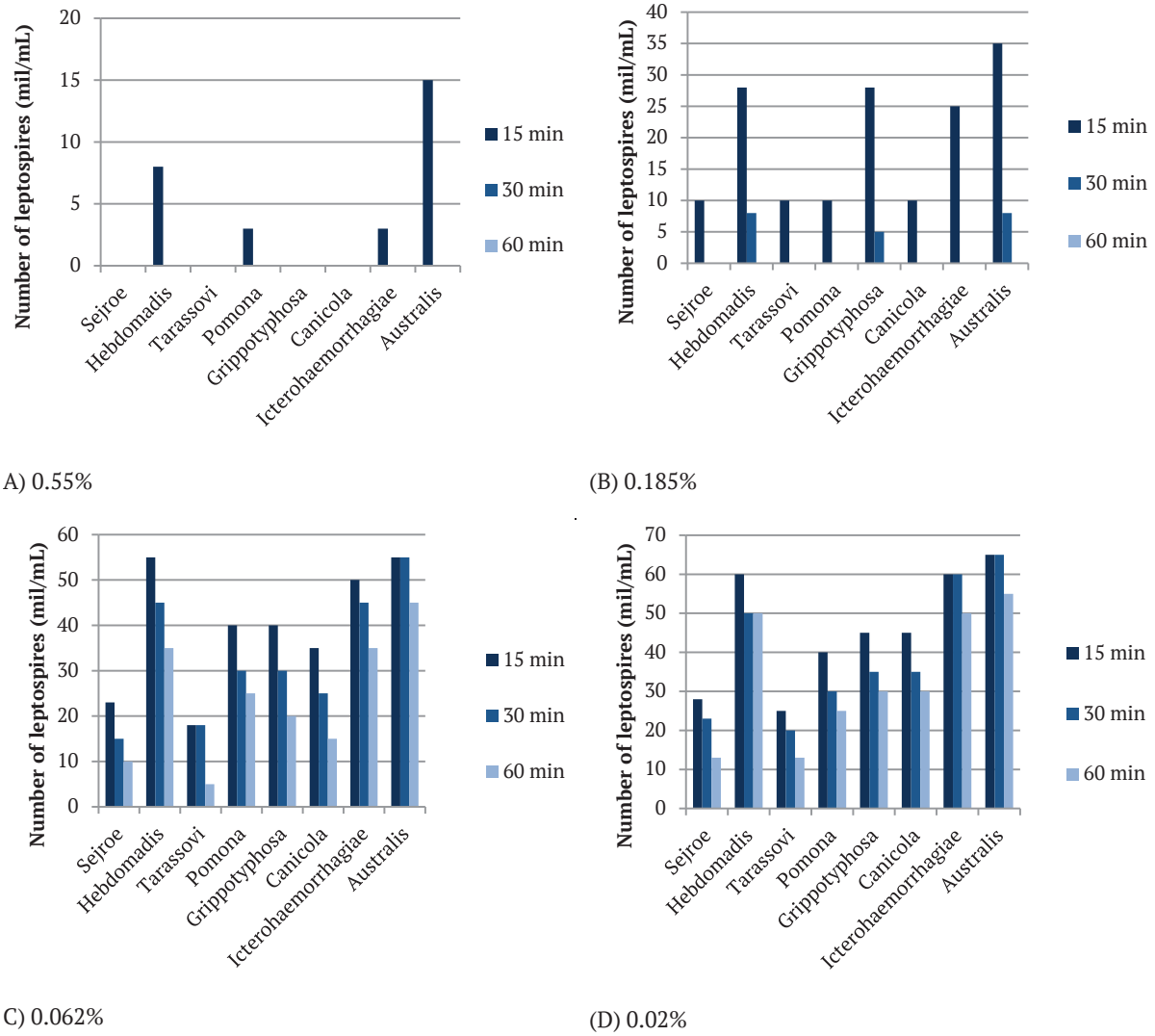


Figure 1. Dynamics of Leptospira registration due to exposure to different Biolide concentrations at exposures of 15-120 minutes

Analysing the obtained resistance indicators, it was determined that at drug concentrations of 1.67%, no microorganisms were registered in all the investigated cultures after 15 minutes of exposure. In the case of using a 0.55% solution, the accumulation of Leptospira in certain serogroups was 4-16 times less than in the control (native culture of Leptospira of 7-15 days of age without adding a disinfectant) or was not recorded at all. Thus, serogroups were the most sensitive to the action of a 0.55% solution of the drug "Biolide" under this exposure *Sejroe*, *Tarassovi*, *Grippotyphosa*, and *Canicola* (the micro-organisms were not visible at all in the field of view of the microscope.) In cultures *Pomona* and *Icterohaemorrhagiae* up to 5 million microbial cells/cm^{3}were registered. Therewith, the most persistent were *Hebdomadis* (5-10 million) and *Australis* (10-20 million microbial cells).

With a decrease in the concentration of the drug, the number of microbial cells in Leptospira cultures increased accordingly. Thus, when using a 0.185% solution – the average accumulation was 10-30 million microbial cells/cm,^{3}. In experimental cultures with 0.062 and 0.02% concentrations of the drug "Biolide" during 15-minute exposure, the accumulation of pathogenic cultures was quite substantial. Thus, in cases with serogroups *Icterohaemorrhagiae*, *Hebdomadis*, and *Australis* – this indicator was generally at the level of controls.

During exposure for 30 minutes, microorganisms were not recorded at all in all experimental samples with drug concentrations of 1.67 and 0.55%. In general, during this exposure, the amount of Leptospira in all cultures decreased slightly at different concentrations. In particular, the number of microorganisms substantially decreased in cultures when using the drug "Biolide" in the form of a 0.185% solution (Leptospira was detected only in samples with serogroups *Hebdomadis*, *Grippotyphosa*, and *Australis* (up to 10 mil/mL). Even in samples with a 0.02% solution, the accumulation rates were lower than in the controls.

Considering the data obtained, it was decided to continue the experiment with exposure for 60 minutes. As a result, pathogens were also not recorded in samples with a concentration of the drug "Biolide" of 0.185%. Therewith, with smaller dilutions of the drug, the accumulation indicators generally almost did not differ from those with previous exposure. Considering this, it was decided to stop the experiment.

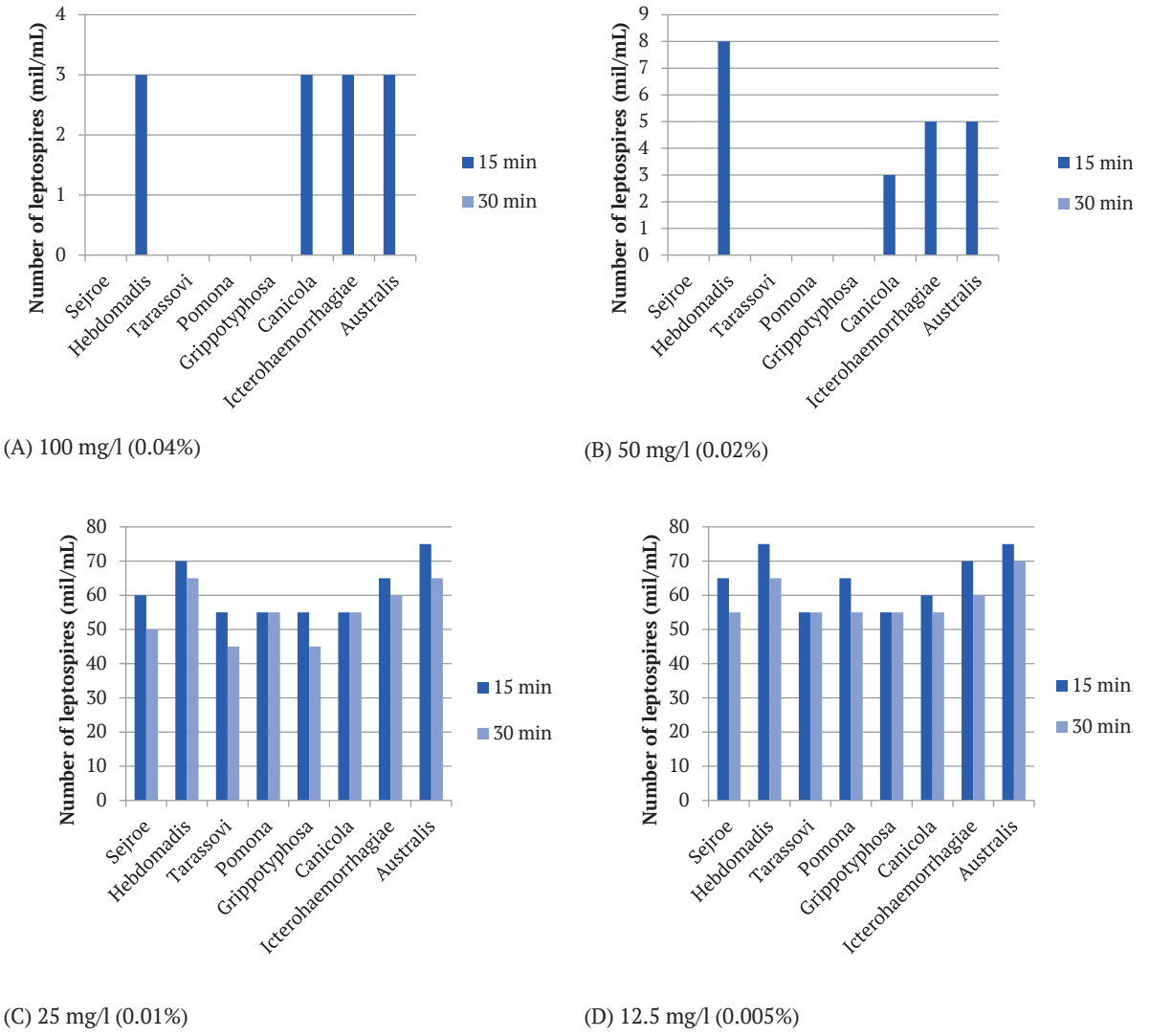
After systematising all the results obtained, it was found that serogroup cultures were the most resistant to low concentrations of the drug "Biolide" (0.02-0.062%). *Hebdomadis*, *Icterohaemorrhagiae*, and *Australis*.

The data obtained during the systematisation and visualisation of the results on the effect of various concentrations of Diolide on the resistance of Leptospira strains are presented in Table 3 and in Figure 2.

Table 3. Results of the effect of Diolide disinfectant on Leptospira culture at different concentrations and exposures (n = 9)

Exposure, min.	Dilution, mg/l (concentration, %)	Leptospira serogroups (mil/mL)								Control (mil/mL)
		Sejroe	Hebdomadis	Tarassovi	Pomona	Grippotyphosa	Canicola	Icterohaemo-rrhagiae	Australis	
15	200 (0.08)	–	–	–	–	–	–	–	–	80-100
	100 (0.04)	–	0-5	–	–	–	0-5	0-5	0-5	80-100
	50 (0.02)	–	5-10	–	–	–	0-5	0-10	10	80-100
	25 (0.01)	50-70	60-80	50-60	50-60	50-60	40-70	60-70	70-80	80-100
	12.5 (0.005)	60-70	60-90	50-60	60-70	50-60	50-70	60-80	70-80	80-100
30	200 (0.08)	–	–	–	–	–	–	–	–	80-100
	100 (0.04)	–	–	–	–	–	–	–	–	80-100
	50 (0.02)	–	–	–	–	–	–	–	–	80-100
	25 (0.01)	40-60	60-70	40-50	50-60	40-50	40-70	60	60-70	80-100
	12.5 (0.005)	40-70	60-70	50-60	50-60	50-60	40-70	60	60-80	80-100

Note: the table shows the values of confidence intervals (Cl) relative to the number of Leptospira in the field of view of the microscope with a probability level of 0.95, "–" – the absence of Leptospira in the field of view of the microscope



(A) 100 mg/l (0.04%)

(B) 50 mg/l (0.02%)

(C) 25 mg/l (0.01%)

(D) 12.5 mg/l (0.005%)

Figure 2. Dynamics of Leptospira registration due to exposure to different Diolide concentrations at 15 and 30 min exposures

Analysing the indicators of resistance of Leptospira culture to Diolide, its more pronounced inactivating effect was established than that of Biolide. This result indicates a substantial sensitivity of Leptospira to chlorine-containing compounds.

In particular, already at an exposure of 15 minutes when using the drug in a dilution of 200 mg/dm^3 (concentration of 0.08% for the active substance), no microorganisms were detected in any of the investigated cultures. In dilutions of Diolide 100 mg/dm^3 and 50 mg/dm^3 (0.04 and 0.02% active substance concentrations, respectively) single microorganisms were detected (5-10 mil/mL) in serogroup cultures *Hebdomadis*, *Canicola*, *Icterohaemorrhagiae*, and *Australis*. Therewith, when used during this exposure, smaller dilutions of the drug (12.5-25.0 mg/dm^3) indicators of microbial cell accumulation were within the limits of those in the controls.

After further exposure for 30 minutes, Leptospira was not detected at all in samples with Diolide dilutions of 50-200 mg/dm^3. As for the lower concentrations of the drug, as in the previous case, the accumulation of these microorganisms did not change substantially (on average, 50-60 mil/mL). Considering the result, it was decided not to continue the experiment.

Systematising the results obtained, it was found that the most resistant to low concentrations of Diolide (dilution 12.5-25.0 mg/dm^3, concentration 0.005-0.01%) identified serogroup cultures *Hebdomadis*, *Canicola*, and *Australis*.

In both experiments, the culture was incubated only at a temperature of 24°C, because Leptospira is sensitive to heat, which can inactivate them. Thus, according to the recommendations of the World Organisation for Animal Health, this type of microorganism is sensitive even to an increase in ambient temperature to a temperature of over 34°C [8]. Incubation was conducted at room temperature, since this allows the use of drugs in selected concentrations in livestock and poultry premises without the use of additional equipment.

Similar experiments with Leptospira culture have previously been conducted in Ukraine using disinfectants geocide (active ingredients are polyhexamethylene guanidine hydrochloride, benzalkonium chloride, and deltamethrin) and argicide (polyhexamethylene guanidine hydrochloride,

colloidal solutions of silver, and copper nanoparticles). Thus, it was experimentally established that the optimal concentration for the prevention of leptospirosis is a 0.55% solution of geocide with an exposure of 15 minutes. If the exposure period is increased to 30 minutes, it is allowed to reduce the concentration of the product to 0.02%. As for argicide, its optimal concentration for use is a 0.1% solution with an exposure of 60-75 minutes [20].

According to the latest data of foreign researchers, due to the influence of heat on the culture of pathogenic Leptospira (temperature 32°C) together with ultraviolet radiation, its accumulation is halved. Therewith, drugs acting with the release of chlorine completely destroy Leptospira during exposure for up to 27 minutes. In addition, the impact assessment was conducted not visually but using cultural and molecular methods [21]. Preservatives such as formaldehyde and paraffin instantly destroy the culture [22].

However, to destroy the antigen of pathogenic cultures of Leptospira, the action of more aggressive factors is necessary. Thus, according to literature sources, it can be inactivated at a temperature of 121°C or using phenol, formalin, or thiomersal heated to 50°C [23].

Additionally, a study was conducted on the effect of disinfectants "Diolide" and "Biolide" on the growth of cultures of pathogenic Leptospira. These crops were placed in an environment with the addition of these drugs in concentrations, and when checking the resistance indicators. As a result, on the 10th day of the experiment, no growth was found in all the investigated cultures. This result is associated with changes in the reaction of the environment (ph) and osmotic pressure in test tubes with culture since leptospirosis pathogens are extremely sensitive to changes in these indicators [24].

Conclusions

The possibility of using complex disinfectants of various chemical natures, which have oxidising properties, for preventive and forced disinfection in leptospirosis was justified. Thus, the drug "Biolide" based on hydrogen peroxide, lactic and super-lactic acids has a bactericidal effect on the culture of pathogenic Leptospira in the form of a 0.55% solution when exposed for 30 minutes at a temperature of 24°C. In addition, it is allowed to use this product at a concentration of 0.185%, provided that the exposure is increased to 60 minutes. It was found that the disinfectant "Diolide" (active ingredients sodium chlorite and sodium chloride) destroys Leptospira when it is used in a dilution of 200 mg/dm^3 (concentration of 0.08% of the active substance) during exposure for 15 minutes at the same temperature. It is possible to apply a lower concentration of 0.02% with an increase in exposure to 30 minutes. It was also discovered that both disinfectants have a pronounced bacteriostatic effect on Leptospira, since they inhibit the growth of these microorganisms even in minimal concentrations.

The next stage of this study will be to examine the possible effect of Biolide and Diolide disinfectants on other types of microbial test cultures.

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Вплив дезінфектантів різної хімічної природи на культуру патогенних лептоспір

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Анотація. Інфекційні хвороби завдають тваринницьким господарствам значних економічних збитків, тому проводиться постійний пошук нових засобів профілактики захворювань, а особливо дезінфектантів. Аналіз наукової літератури вказує на значну проблему лептоспірозу в Україні та фактично відсутні дані щодо застосування комплексних окиснювальних препаратів для його профілактики. Метою роботи було дослідити вплив дезінфектантів біолайд (діючі речовини перекис водню, молочна та надмолочна кислоти) та діолайд (діючі речовини натрію хлорит і натрію хлорид) на збудників лептоспірозу. Для цього, перевіряли стійкість восьми, циркулюючих в Україні, патогенних культур лептоспір різного віку та їхні ростові властивості, шляхом додавання до них різних концентрацій зазначених дезінфектантів. Одержані результати статистично аналізували в програмному забезпеченні Epitools – Epidemiological Calculators. З'ясовано ефективні концентрації та експозицію біолайду та діолайду для застосування при профілактичній та вимушеній дезінфекції при лептоспірозі. В результаті проведених досліджень щодо впливу обох дезінфектантів на 7-ми, 10- та 15-добові тест-культури лептоспір, не було зареєстровано різниці між показниками їхнього накопичення (кількість мікробних клітин/см^3). Тому, одержані результати по культурам різного віку враховували як повторюваність. Обґрунтовано, що для профілактичної та вимушеної дезінфекції при лептоспірозі рекомендовано до використання 0,55 % розчин засобу «Біолайд» за експозиції 30 хв за температури 24 °С. У разі збільшення терміну експозиції до 60 хв, допускається зниження концентрації засобу до 0,185 %. Щодо препарату «Діолайд», то його при цьому зоонозі рекомендовано застосовувати у розведенні 200 мг/л (концентрація 0,08 % за діючою речовиною) впродовж експозиції 15 хв за температури 24 °С. У разі збільшення терміну експозиції до 30 хв, допускається зниження розведення засобу до 50 мг/дм^3 (концентрація 0,02 % за діючою речовиною). До того ж, встановлено, що обидва дезінфікуючі засоби повністю пригнічують ріст патогенних культур лептоспір. Практична цінність роботи полягає у доведенні можливості застосування комплексних дезінфектантів на основі окиснювачів для профілактики лептоспірозу

Ключові слова: ветеринарна медицина, лептоспіроз, дезінфекція, біолайд, діолайд, бактерицидність

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