



The activity of immunological indicators in chickens with the complex use of a symbiotic drug “Biomagn” and a disinfectant “Diolide”

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CO: Investigation.
VO: Conceptualization.
KV: Conceptualization; Writing — original draft.
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KS: Writing — original draft.
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None to declare.

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The goal of the research was to determine the effect of the complex use of symbiotic and disinfectant on immunological indicators in broiler chickens. The research was carried out on ROSS-308 cross broiler chickens. Chickens 1–41 days old (2 groups of 100 chickens) were kept in standard production premises with free access to feed and water, observing the technological parameters of cultivation. The chickens of the research group, in addition to the standard diet, were fed the synbiotic “Biomagn” (0.5 kg/t compound feed) according to the scheme and throughout the experiment they drank the drug “Diolide” with water (1 mg/l). Blood for immunological studies was collected on 10–27–34–41 days. The study was conducted without violating the guidelines of the EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Complex application of the proposed synbiotic and disinfectant to chickens contributed to an increase in the level of immunological reactivity, as indicated by higher ($P < 0.05$ – 0.01) bactericidal and lysozyme activity of blood serum and phagocytic activity of blood pseudoeosinophils of chickens compared to the control, an optimizing effect on the level of circulating immune complexes. So, the results of the research indicate the additive effect of the synbiotic drug “Biomagn” and the disinfectant “Diolide” on the functioning of the mechanisms of natural protection of broiler chickens during their growing period. This effect is due to the complex normalizing effect of the applied agents.

Key words: broiler chickens, symbiotic, chlorine dioxide, bactericidal, lysozyme and phagocytic activity

Introduction

Natural (or nonspecific) resistance of animal's organism and poultry is associated with immune system development and environmental factors: feeding, environmental, man-made. Deterioration of the ecological situation, increase in the number and influence forces of stress factors, including anthropogenic factors, have a negative influence on the health of poultry. All this contributes to the suppression of natural resistance,

reduced immune potential and productive qualities of poultry, as a result of which the organism is unable to produce the required number of immunocompetent cells and antibodies to form a full immune response [16, 18, 22, 29].

Moreover, presence of critical physiological periods and uncontrolled use of antibiotics, sulfonamides preparations, coccidiostats and carrying out numerous vaccinations leads to antibiotic resistance, immunodeficiency and death of poultry [5, 12, 23, 25].

Aiming to normalize the intestinal microflora, increase the immune potential and viability of poultry in Ukraine and abroad began to use probiotics and prebiotics.

Microbial preparations from associations of non-pathogenic bacteria demonstrate a positive influence on productivity and natural resistance of the organism. Literature data analysis demonstrates number of advantages of probiotics and prebiotics using over existing the effect of applied vitamin and antibiotic preparations [11, 20, 21]. Combined preparations from probiotics and prebiotics are called synbiotics [4, 5, 13, 16].

Biomagn is synbiotic preparation consisting of a complex: magnesium chloride, chitosan, and probiotic bacteria cultures of the following species: *Bacillus subtilis*, *B. licheniformis*, *Enterococcus faecium* and dried products of fermentation of microorganisms *Lactococcus lactis*, *B. subtilis*, *B. licheniformis* and other substances [7]. It shows antagonistic activity to different pathogenic bacteria and fungi.

Despite a long period of research and impressive results, the “probiotic concept” still remains hypothetical. And first of all because the mechanisms by which probiotic strains of bacteria exhibit their protective properties in vivo is not deeply studied. For today, the influence of probiotic preparations on the activity of protective systems of the poultry's organism has not been sufficiently studied. First of all, elucidation requires research of the influence of synbiotic preparations on the state of natural defense mechanisms in chickens in critical periods of their rearing [16, 23].

Currently the quality and safety of drinking water is especially relevant in the case of getting them from different water supply systems with high levels of chemical and biological pollution [1, 9, 10, 15]. The main reason for the low quality of drinking water from surface and ground-water sources in poultry farms is their unsatisfactory condition due to untimely replacement of filters, cleaning of pipelines and their rehabilitation. Scientists have accumulated extensive experience in the processing of water with chlorine dioxide at various stages of the technological process. Compared to chlorine, chlorine dioxide is characterized by lower doses and a higher rate of interaction with water components, such as 0.1–2.0 mg/L at a exposure of 30 min contact for complete disinfection. Dosages of 0.2–0.4 mg/L are effective bactericidal concentrations at which residual concentrations of chlorite are safe, prevent unpleasant odors and tastes in water [2, 9, 17, 24, 31].

At the post-disinfection stage, chlorine dioxide has a double action: bactericidal and virucidal effects as chlorine dioxide; bactericidal effects in chlorite ion form. As a bactericidal agent, chlorine dioxide can remain active in water for at least 48 hours, i.e. it has a prolonged action [3, 27].

That is why we performed study of the influence of “Biomagn” synbiotic preparation in combination with an aqueous solution of disinfectant “Diolide” on the indicators of cellular and humoral links of nonspecific resistance in broiler chickens during their rearing have high applied importance.

The purpose of the research was to determine the influence of the synbiotic preparation “Biomagn” in combination with an aqueous solution of the disinfectant “Diolide” on the activity of natural defense mechanisms in broiler chickens during the period of their rearing.

Materials and Methods

The research was performed in one of the farms of Lviv region on broiler chickens of ROSS-308 cross, 1-41 days after hatching. The study was conducted without violating the guidelines of the EU Directive 2010/63/EU on the protection of animals used for scientific purposes. Chickens were maintained in poultry houses with free access to feed-stuff and water, technological parameters of broiler breeding (temperature and light regime) under norms of ONTP-2005.

Two groups of broiler chickens were created for research: control and experimental groups, 100 heads in each. The control group of poultry received the standard compound feed-stuff (SCF) according to the existing norms recommended for ROSS-308 cross. The experimental chickens were similarly fed SCF and “Biomagn” synbiotic preparation at the rate of 0.5 kg per ton of combined feed. This preparation was used by such scheme: the first time at one day of age — seven days in a row, the next task — at 22 days of age, seven days in a row.

The preparation “Biomagn” represents probiotic bacteria mixture, involving the following species: *Bacillus subtilis*, *B. licheniformis*, *B. coagulans*, *Enterococcus faecium* and dried fermentation products of microorganisms: *Lactococcus lactis*, *B. subtilis*, *B. licheniformis*, as well as chitosan, magnesium chloride, xylanase, cellulase, protease, milk thistle meal, acidity regulator, betaine and emulsifier.

Along with this, the experimental group of poultry was supplied by water a solution of the preparation “Diolide” [7] where the main active substances: sodium chlorite and sodium chloride. For disinfection of drinking water, the preparation “Diolide” was used at a dose of 1.0 mg/L of chlorine dioxide. Diolide was added through the mediators. Pre-mother liquor is diluted to a concentration that allows it to be presented by the medicator in accordance with its technical characteristics. This preparation was developed in the State Research Institute for Laboratory Diagnostics and Veterinary Sanitary Expertise.

To carry out the immunological testing was performed on chicken of different ages: 10-; 27-; 34- and 41-days after hatching.

Phagocytosis was determined in heparin-stabilized blood. The phagocytic reaction of pseudoeosinophils in the blood was evaluated using a daily culture of *Escherichia coli* strain VKM-125 by phagocytic activity (FA), phagocytic index (FI) and phagocytic number (PN) by the method described in the reference book [2]. The content of circulating immune complexes (CIC) — by their precipitation with polyethylene glycol, lysozyme activity (LASK)

using *Micrococcus lysodeicticus* strain VKM-109, bactericidal activity (BASK) with the use of daily culture of *E. coli* strain VKM-125 were determined in the blood serum, according to these methods, which are described in the reference book [2].

Also, quality and safety of drinking water was tested by microbiological indicators. Samples of drinking water before and after disinfection of the water supply system were taken for the research. The study was carried out using microbiological research methods according to the orders and methodological recommendations of the Ministry of Health of Ukraine [32]. From 2 different dilutions of the test sample in the amount of 1 ml was made in 2 bacteriological cups and poured 15 ml of molten and cooled to 45°C MPA, the samples were cultured at temperature 37°C. The total number of microorganisms in 1 ml was determined by the method of colonies counting that grew from each dilution, multiplied the number of colonies by the degree of dilution and summed their number [26].

The experimental study was carried out under recommendations of "General Ethical Principles of Animal Experiments", approved by the National Congress on Bioethics (Kyiv, 2001) and agreed with the provisions of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1985).

Digital data were processed by biometric method of variational nonparametric analysis using *Microsoft Excel* spreadsheet package *Microsoft Office Professional XP* and *Origin 6.1* program. The differences between values were considered statistically significant: $P < 0.05$; $P < 0.01$ and $P < 0.001$.

The free software *Jamovi 2.3.28* (jamovi.org) was used for statistical processing. The Shapiro-Wilk test was applied to check the normality of the distribution, and the Levene's test was used to assess the equality of variances. In the case of normal distribution and equal variances, the Student's *t*-test was used to determine statistically significant differences between groups.

Results and Discussion

The key principle of evaluation the immune status of poultry involves the quantitative characterization of the functional activity of non-specific resistance factors.

Analysed the data in the compared groups met the criteria of normality of distribution (according to Shapiro-Wilk algorithm) and homogeneity of variances (according to Levene's test). The probability of difference was estimated by Student's *t*-test. As we can observe from the data in table 1, the tested indicators of the humoral link of nonspecific resistance in broilers of the control group are increased. At the same time, these changes were more pronounced in chickens when studying the content of circulating immune complexes. Thus, in 34 days chicken the content of the CIC was one and a half times higher than in 10 days. These data indicate an increase in the antigenic load on the organism

of chickens during this period, which may be due to a number of negative factors.

The "Biomagn" synbiotic preparation in combination with an aqueous solution of "Diolide" preparation caused an activating influence on the mechanisms of non-specific protection of the organism of broilers. It is known that the state of natural resistance of the organism fully characterizes the bactericidal activity of blood serum, which is an integral indicator of the humoral link of nonspecific resistance of the organism and is the ability to inhibit the growth of microorganisms. From the data shown in table 1 we see that the use of the studied preparations caused an increase in BASK in chickens of the experimental group compared with the control, especially at 16 days of age, where the differences were significant. Here with the lysozyme activity of serum in experimental group at 27th; 34th and 41th days after hatching was respectively 5.6 ($P < 0.01$); 5.0 ($P < 0.05$) and 6.6 % ($P < 0.05$) is higher in comparison with control group. These data indicate the activating effect of the tested preparations on humoral resistance markers in poultry.

Instead, the action of "Biomagn" in combination with "Diolide" solution in working concentration revealed a decrease in the level of circulating immune complexes in the serum of broiler chickens of the experimental group relative to the control (table 1). In particular, on the 10th and 27th day the content of CIC in the blood of broilers of the experimental group tended to increase, and at 34th and 41th days of age was 1.3 ($P < 0.001$) and 1.27 times, respectively ($P < 0.01$) is smaller than in the control group. Thus, the results of these investigations indicate the optimizing influence of the studied preparations on the level of circulating immune complexes in the serum of broiler chickens. It is known that significant and long-term circulation of CIC content in the organism can lead to organ pathology.

Phagocytes are the main active components of the cellular component of natural defense, starting from the embryonic period of development. They form the first line of defense of the cellular component of the organism's natural or nonspecific resistance. From the data shown in table 2 we can see, that synbiotic preparation application in combination with the disinfectant "Diolide" significantly affected the state of the cell unit of nonspecific resistance in broilers. In particular, phagocytic activity, which characterizes the level of pseudoeosinophils in peripheral blood at 27th; 34th and 41th days after hatching was higher ($P < 0.01$ – 0.001) than in control group of chicken.

Also, the direct coordination was found between phagocytic activity and phagocytic number, and especially the phagocytic index in the blood of experimental group of broilers. In all periods of the investigation, the phagocytic index in experimental group was higher ($P < 0.05$ – 0.001) than in the control group. It indicates the stimulating influence of the components of the investigated preparation on the activity of the cellular link of the natural defense mechanisms of the poultry.

Table 1. Nonspecific resistance humoral factors in broiler chicken under the action of the studied preparations ($M \pm m$; $n=7$)

Indicators	Groups	Research period (age of poultry), day			
		10 th	27 th	34 th	41 th
BASK, %	C	38.04±0.56	39.61±0.30	39.22±0.42	40.01±0.56
	E	40.87±0.52**	41.29±0.56	41.48±1.27	42.17±1.25
LASK, %	C	17.4±2.27	19.2±0.66	22.6±0.75	21.29±1.57
	E	20.29±1.32	24.8±1.16**	27.57±1.45*	27.8±1.77*
CIC, mmol/l	C	38.0±2.86	42.2±2.96	55.2±2.83	54.8±2.15
	E	43.14±2.56	45.14±2.16	42.4±1.13***	43.0±1.38**

Note. Here and further * — $P<0.05$, ** — $P<0.01$, *** — $P<0.001$ compared to the control group of chicken using Student's t -test.

Table 2. Phagocytosis and pseudoeosinophils' level markers in broiler chicken peripheral blood under the action of the tested preparations ($M \pm m$; $n=7$)

Indicators	Groups	Research periods, day			
		10 th	27 th	34 th	41 th
Phagocytic activity, %	C	31.6±0.81	32.0±0.83	33.4±0.51	33.80±0.37
	E	35.0±0.58	36.2±0.58**	37.5±0.50***	38.0±0.31***
Phagocytic index, un.	C	4.55±0.08	4.69±0.10	4.67±0.12	4.89±0.04
	E	5.14±0.04***	5.26±0.05**	5.33±0.03***	5.76±0.27*
Phagocytic number, un.	C	14.45±0.50	14.69±0.49	13.99±0.21	14.47±0.20
	E	14.62±0.26	14.55±0.26	14.19±0.21	15.15±0.62

Table 3. "Diolide" effect for the water supply system treatment

Concentration of working solution (for chlorine dioxide)		Consumption of mother liquor (ml) Diolide for the production of working solution in the amount of: 100 liters	Before disinfection, CFU/cm ³	After disinfection, CFU/cm ³ (norm <20 CFU/cm ³)
mg/L	percentage of the preparation, %			
1.0	0.0004	20.0	56	0

Other results of this experiment demonstrated presence of mesophilic, aerobic and facultative anaerobic microorganisms in the amount of 56 CFU/cm³ (at less than 20 CFU/cm³), does not correspond to sanitary and hygienic norms and rules (table 3) in the drinking water for poultry.

The use of disinfectant "Diolide" allowed to normalize bacteriological parameters of water.

These data, on the one hand, indicate the possible contamination of tested water with opportunistic pathogens for use from decentralized water supply. On the other hand, the effectiveness of tested disinfectant to normalize the bacterial microflora of water was demonstrated. It should be noted that opportunistic pathogens in large quantities can cause disease in humans, animals and poultry.

This is also indicated by the results obtained by [26] and [32], who noted that opportunistic pathogens in large scales in decentralized water supply can be a rea-

son of disease cases in humans, and animals (including poultry). However, [24] and other scientists recommend chlorine dioxide use for the improvement of disinfectant effectiveness, which has also been confirmed by other scientists. After disinfection of the water supply system in the samples of the investigated water, no growth of bacterial microflora was detected, which corresponds to DSTU 7525:2014 "Requirements and methods of quality control of drinking water". These data are also consistent with the results of research [6] conducted on laboratory animals, which indicates the safety of chlorine dioxide in the water supply system. The influence of chlorine dioxide on water quality is also noted by scientists [27]. At the same time, they pay attention to filters for water purification, because without control over the use of disinfectants, they can serve as a source of microorganisms, which is also consistent with our previous work [9, 17, 26].

Thus, the results of the research indicate a positive influence of the synbiotic "Biomagn" together with an aqueous solution of the preparation "Diolide" on the activity of natural defense mechanisms in broiler chickens during the period of their rearing. This influence is due to the complex additive action of factors containing the studied preparations. In particular, the active basis of the preparation "Biomagn" is a multicomponent symbiosis of probiotic strains, designed taking into account the synergistic complement of the unique properties of each of them, namely: antagonistic activity against a wide range of pathogenic and opportunistic microorganisms [11]. Thus, as noted [30], representatives of the genus *Bacillus*, containing the investigated preparation, having a clear antagonism against pathogenic microorganisms, produce a number of enzymes: xylanase, protease, cellulase which improve cleavage in the gastrointestinal tract (GIT) of arabinoxylans (pentosans) and starch into oligosaccharides and partially into mono- and trisaccharides, and reduce the viscosity of food in the digestive tract [28, 30]. In this case, the investigated symbiotic preparation contains the products of metabolism of the above microorganisms, and especially the genus *Bacillus*, providing the synthesis of various amino acids and antibiotics [14, 19].

At the same time, the synbiotic preparation contains the products of metabolism of the above microorganisms — enzymes: xylanase, protease, cellulase, which improve the breakdown in the gastrointestinal tract (GIT) of arabinoxylans (pentosans) and starch into oligosaccharides and partly on mono-, di- and trisaccharides, and reduce the viscosity of food in the digestive tract. Proteases (alkaline and acidic) improve the absorption of the protein they break down into peptides. It should be noted that the yeast cell wall contained in the preparation contains mannanol oligosaccharides (MOS), which are bound to bacterial receptors by mannose residues they are not destroyed by digestive enzymes and are firmly held on the surface of pathogenic bacteria. Pathogenic bacteria with blocked receptors cannot attach to the surface of epithelial cells and pass through the gastrointestinal tract. At the same time, MOS exhibits prebiotic properties and strengthens the non-specific protection of animals and poultry, improves their intestinal microflora which is confirmed in the works of many authors [11, 23, 28].

The multidirectional influence of probiotic preparations from aerobic bacilli may be due to bacterial translocation. This phenomenon, which was discovered only at the end of the last century, is the penetration of viable bacteria from the gastrointestinal tract through the blood into the internal organs. Translocation of representatives of the normal microflora and bacterial components of probiotics is a natural defense mechanism, one of the important factors in the activation of nonspecific resistance of the microorganism [13].

The preparation "Biomagn" has pronounced sorption properties due to the high content of aluminosilicates,

which allows you to effectively sorb mycotoxins or other toxins from food. Another component of the preparation, milk thistle flour, which contains the active substance — silymarin, which has hepatoprotective and antioxidant properties. Along with this there is a stimulation of mucin synthesis and strengthening of the intestinal mucosal protective barrier and supply microbiota stability in the intestinal tract.

The tested synbiotic preparation contains magnesium chloride. As noted by [28], magnesium ions are known to simultaneously activate natural resistance in chicken by participating in process of immune response as a cofactor in Ig synthesis, antibody-dependent cytotoxicity, lymphocyte binding to Ig M, macrophage responses to lymphokines. Magnesium is involved in the formation of adhesion molecules, in the interaction of phagocytes and the object of phagocytosis, is part of the properdin system. In the presence of Mg^{2+} ions, a C3b complex with factor B is formed. C3b triggers an alternative pathway for complement activation. Magnesium is also involved in the classical pathway of complement activation, in the formation of the C4b2a complex, which has converting activity against C3 [8, 32].

In general, the mechanism "Biomagn" influence is based due to the complex action of both probiotic microorganisms and products of their synthesis and other substances. The latter are known to be powerful regulators of immune and antioxidant function of the organism, and due to the disinfectant "Diolide" is the neutralization of microorganisms in the water supply system, which allows to get quality and safe water. This complex additive action of the investigated factors provides optimal microflora and acid-base balance, protects the intestinal mucosa, improves digestion of nutrients, enhances immune potential, and in particular natural defense mechanisms, which generally has a positive influence on the growth and viability of broilers.

Thus, we can note the positive effect of "Biomagn" synbiotic preparation together with the aqueous solution of the disinfectant "Diolide" on the activity of natural protective factors in broiler chickens during their growing period. This is evidenced by the higher lysozyme activity of blood serum and phagocytic activity of pseudoeosinophils in chickens of the experimental group compared to the control at 27th; 34th and 44th days after hatching ($P < 0.05 - 0.001$). In all periods of research, the phagocytic index in experimental group was higher than in the control chicken ($P < 0.05 - 0.001$).

Under the action of the investigated preparations in the blood serum of broiler chickens at 16 days after hatching recorded higher bactericidal activity of blood serum ($P < 0.01$), while at 34th and 44th days after hatching revealed a lower content of circulating immune complexes ($P < 0.01 - 0.001$), which indicates the activating influence of the investigated preparations on the activity of the humoral link of nonspecific resistance and reducing the antigenic load on the organism.

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Активність природних механізмів захисту в курчат-бройлерів за дії синбіотичного препарату «Біомагн» та дезінфекційного засобу «Діолайд»

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Підвищення збереження курчат та забезпечення високої інтенсивності їх росту на всіх стадіях вирощування є однією з найбільш актуальних проблем птахівництва. У зв'язку з цим, надзвичайно важливими є розробка і впровадження у виробництво різних засобів для підвищення імунобіологічної реактивності та життєздатності птиці. Значні перспективи у цьому напрямку відкриваються за використання пре- і пробіотиків та безпечних дезінфекційних засобів. З огляду на це, актуальним є дослідження комплексної дії синбіотика та дезінфектанта на функціонування природних механізмів захисту курчат-бройлерів упродовж періоду їх вирощування. Дослідження проводили в одному із господарств Львівської області на курчатах-бройлерах кросу РОСС-308, починаючи з 1- до 41-добового віку. Курчат утримували у пташниках з вільним доступом до корму і води, за технологічних параметрів вирощування бройлерів (температурний та світловий режим) відповідно до норм ОНТП-2005. Для досліджень сформували 2 групи курчат-бройлерів — контрольну і дослідну групи, по 100 голів у кожній. Бройлерам контрольної групи згодовували стандартний комбікорм (СК) згідно з чинними нормами, рекомендованими для кросу РОСС-308. Курчатам дослідної групи аналогічно згодовували СК і синбіотичний препарат «Біомагн» із розрахунку 0,5 кг на тонну комбікорму. Вказаний препарат застосовували за схемою: перший раз в одностововому віці — сім днів поспіль, наступне задавання — у 22-добовому віці, сім днів поспіль. Препарат «Біомагн» — це суміш пробіотичних бактерій *Bacillus subtilis*, *B. licheniformis*, *B. coagulans*, *Enterococcus faecium* та висушених продуктів ферментації мікроорганізмів *Lactococcus lactis*, *B. subtilis*, *B. licheniformis*, магнію хлориду, хітозану й інших речовин та емульгатора. Водночас бройлерам дослідної групи впродовж всього експерименту з водою випоювали розчин препарату «Діолайд», де основні діючі речовини — натрію хлорит, натрію хлорид, дозою 1,0 мг/л за двоокисом хлору. Для проведення імунологічних досліджень у курчат брали кров у різні вікові періоди: у 10-, 27-, 34- і 41-добовому віці. Застосування курчатам синбіотичного препарату «Біомагн», а також дезінфекційного засобу «Діолайд» позитивно впливало на досліджувані показники клітинної і гуморальної ланок неспецифічної резистентності організму. Про це свідчать вища ($P < 0,05-0,01$) бактерицидна і лізоцимна активність сироватки крові та фагоцитарна активність псевдоеозинофілів крові у курчат дослідної групи щодо контрольної. При цьому констатовано оптимізувальний вплив досліджуваних препаратів на рівень циркулюючих імунних комплексів. Результати проведених досліджень свідчать про адитивний вплив синбіотичного препарату «Біомагн» і дезінфекційного засобу «Діолайд» на функціонування механізмів природного захисту курчат-бройлерів упродовж періоду їх вирощування. Цей вплив зумовлений комплексною нормалізуючою дією досліджуваних чинників, що містить синбіотичний препарат і дезінфекційний засіб.

Ключові слова: курчата-бройлери, пре- і пробіотики, діоксид хлору, псевдоеозинофіли крові, неспецифічна резистентність