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The Intensity of Lipid Peroxide Oxidation Processes and the System State of Antioxidant Protection of Broiler Chicken Due to the Action of the Synbiotic Preparation in Complex with the Disinfectant

Lipid Peroksit Oksidasyon Proseslerinin Yoğunluğu ve Dezenfektanla Kompleks İçinde Sinbiyotik Preparatın Etkisine Bağlı Broyler Tavuğun Antioksidan Korumasının Sistem Durumu

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ABSTRACT

Developing methods for increasing the immune reactivity and antioxidant potential of the bird's body during critical periods of growth is an urgent task today. The purpose of the research was to find out the influence of the synbiotic "Biomagn" in combination with the disinfectant "Diolide" on the intensity of the processes of peroxide oxidation of lipids and the activity of the system of antioxidant protection in the organism of chickens. The research was carried out on 2 groups of chickens, 100 in each, starting from 1 to 41 days of age: the control group was fed with standard compound feed (SCF); the chickens of the experimental group were fed with SCF, and the synbiotic preparation "Biomagn" based on 0.5 kg per ton of compound feed. The experimental group received a solution of the preparation "Diolide" with water. For conducting immunological research, blood was taken from chickens at different age periods: from 10-, 27-, 31-, and 41-day-old chickens. The use of the synbiotic preparation "Biomagn" in combination with the disinfectant "Diolide" in the chickens of the experimental group normalized the intensity of the processes of lipid peroxidation and oxidative modification of proteins in the poultry's organism - a decrease was established ($P < .05–.001$) in GPO content and TBK-active products and aldehyde derivatives oxidative modification of proteins in the blood compared to the control. The detected event was determined by increased activity of the enzyme link of the antioxidant protection system of the organism of chickens.

Keywords: Antioxidant protection, chickens, chlorine dioxide, lipid peroxide oxidation, probiotics.

ÖZ

Büyüme sürecinin kritik dönemlerinde kanatlılarda vücudun bağışıklık tepkisini ve antioksidan potansiyelini artırmaya yönelik yöntemler geliştirmek günümüzde acil bir görevdir. Araştırmanın amacı, "Biomagn" sinbiyotiğinin "Diolide" dezenfektanı ile birlikte tavukların organizmasındaki lipid peroksit oksidasyon süreçlerinin yoğunluğu ve antioksidan koruma sisteminin aktivitesi üzerindeki etkisini belirlemektir. Araştırma, 1 günlük yaştan 41 günlük yaşa kadar yetiştirilen ve her birinde 100 tavuk bulunan 2 grup üzerinde gerçekleştirilmiştir: kontrol grubu standart karma yem (SKY) ile beslenmiştir; deney grubundaki tavuklar ise SKY ve 0,5 kg/ton oranında "Biomagn" sinbiyotik preparatı ile beslenmiştir. Deney grubuna "Diolide" preparatının su ile çözeltisi verilmiştir. İmmünolojik araştırmalar için tavuklardan farklı yaş dönemlerinde (10-, 27-, 31- ve 41 günlük tavuklardan) kan alınmıştır. Sinbiyotik preparat "Biomagn" ile dezenfektan "Diolide"nin birlikte kullanımı, deney grubundaki tavukların organizmasındaki lipid peroksidasyon süreçlerinin yoğunluğunu ve proteinlerin oksidatif modifikasyonunu normalleştirmiştir. Kanlarındaki GPO içeriği ve TBK-aktif ürünler ile aldehit türevleri oksidatif modifikasyonu kontrol grubuna göre azalmıştır ($P <$,05–,001). Tespit edilen etki, tavukların organizmasının antioksidan koruma sisteminin enzim bağlantısının aktivitesindeki artışla belirlenmiştir.

Anahtar Kelimeler: Antioksidan koruma, klor dioksit, lipid peroksit oksidasyonu, probiyotikler, tavuk

INTRODUCTION

Modern intensive production technologies in poultry farming are characterized by the presence of many factors that do not comply with the evolutionary poultry physiology, especially in broiler chickens. In most cases, this causes a stressful situation, which leads to significant violations of the biochemical homeostasis in the poultry's organism, which is explained by the action of catabolic hormones, the release of which increases under conditions of stress. At the same time, free radical processes and peroxidic oxidation of lipids increase, which contributes to a decrease in their productivity and the occurrence of immunodeficiency.[1,2]

The prerequisite for the development of oxidative stress is the accumulation of active oxygen species and free radicals, which influences the development of pathologies of various genesis.[3]

Processes of peroxide oxidation, which are required for the normal functioning of biochemical, biophysical, and physiological systems, occur in all cells of living organisms. The formation of products of lipid peroxidation (LPO) and oxidative protein modification (OPM) are normal functional processes in the organism, with which vital functions are connected. The intensity of changes in free radical processes in the poultry's organism depends on the concentration of oxygen in the tissues, therefore LPO is a physiological process since mitochondria membranes maintain a stationary level of LPO that has a certain functional value and reflects the degree of influence of molecular oxygen on mitochondrial lipids under normal physiological conditions.[4,5]

An increase in the content of LPO products in membranes weakens their barrier function and increases permeability to organic substances, and ions, and as a result, sulfhydryl groups are destroyed, which causes enzyme inactivation; thus, LPO processes are considered as one of the mechanisms of underlying cellular pathology at the basis of many negative effects, such as cytotoxic, genotoxic, mutational and oncogenic effects.[6] The physicochemical stability of eukaryotic cell membranes is ensured by the balanced processes of POL and ORM and the rotation of protein and lipid components and is associated with the protective and adaptive reactions of the body. The pathogenesis of many diseases is accompanied by the activation of peroxide oxidation processes.[7]

Given this, complex preparations have been developed in recent years to ensure the pro-oxidant-antioxidant balance of the organism and prevent its disturbance. As the most physiologically adaptogenic substances that are part of these preparations, compounds of an antioxidant nature have become more and more widely used. Various preparations can have antioxidant activity according to the mechanism of action, affecting both the central mechanisms of regulation and exhibiting a local effect.[8] The products being developed should effectively regulate the level of peroxide processes and indirectly affect the oxidative metabolism of the organism as a whole, and in immunocompetent cells, in particular. Given this, in the field of veterinary medicine, the research was directed at the search for biologically active substances with immunocorrective and antioxidant properties.[9]

The results of the search in the literature show that over the last few years, the number of data on the beneficial effects of probiotics has increased, especially those that are important for mediating reactions to oxidative stress. It became known that probiotics can modulate the redox status of the recipient due to their ability to chelate metal ions, and antioxidant systems, thus regulating signaling pathways and enzymes that produce reactive forms of oxygen and intestinal microbiota.[10,11]

There is scientific interest in finding potential probiotic strains that may exhibit powerful antioxidant properties along with health benefits. *In vitro* and *in vivo* research have ascertained that probiotics exhibit antioxidant potential.[12]

Despite the existing data on the influence of probiotics on the antioxidant and immune defense systems of the organism, the feasibility and safety of their use require additional research and scientifically based analysis.

Manufacturers offer a wide selection of probiotics with different compositions, quality, action, and use. However, sometimes, but not always, probiotics meet the claimed properties. However, practice shows that the use of probiotics is promising in the prevention and treatment of poultry diseases, especially in combination with disinfectants that are used to disinfect premises and the water supply system. Disinfection in the presence of poultry is carried out carefully and cautiously because the poultry is in close contact with fences, inventory, and equipment.[13,14,15]

Along with this, it is important to clean and disinfect the water supply system of drinking water, because microorganisms can be fed in the sediment with vitamins and other additives to drinking water and as a result, a "biofilm" is formed. One of the efficacious active substances of the disinfectant, which is effective for water disinfection, is chlorine dioxide.[16,17,18]

The research conducted by many scientists has established that excessive concentrations of chlorine dioxide in drinking water did not show any toxicity in the subchronic oral toxicity test. At the same time, it demonstrated favorable disinfecting activity and a tendency towards a higher safety profile.[19,20]

Therefore, the creation and use of complex preparations with immunomodulatory and antioxidant properties based on probiotics will provide an opportunity to increase the immunobiological reactivity antioxidant potential and resistance of the poultry organism to technological stresses. At the same time, the use of safe disinfectants in the water supply system is an urgent problem in modern conditions of industrial poultry farming.

The purpose of the research was to find out the influence of the synbiotic preparation "Biomagn" in combination with the solution of the disinfectant "Diolide" on the intensity of the processes of peroxide oxidation of lipids and the activity of the antioxidant defense system of the organism of broiler chickens during their growing period.

MATERIALS AND METHODS

The study was conducted at the poultry farm, located in Lviv oblast on broiler chickens from ROSS-308 cross. Chicken aged 1 to 41 days after hatching were used in the experiments. Poultry was held in the coops with free access to feedstuff and water supply, under the technological conditions, recommended for broiler breeding (temperature and insolation levels) by local standard - ONTP-2005. Two groups of broiler chickens were formed for the experiment (control and experimental), 100 chickens per group. The control group of the poutry was fed with standard compound feed-stuff (SCF) recommended for the ROSS-308 cross of broilers. The experimental group was similarly fed with SCF and supplied with "Biomagn" synbiotic preparation at a dose of 0.5 kg per ton of feedstuff. The preparation was used by the following scheme: for the first time on 1st day after hatching for seven days, and the next treatment was performed on the 22nd day, for seven days also.

Biomagn synbiotics include *Bacillus subtilis, Bacillus licheniformis, Bacillus coagulans*, fermentation products of *Lactococcus lactis, Bacillus subtilis, Bacillus licheniformis*, as well as betaine, acidity regulator, thistle meal, cellulose, emulsifier, magnesium chloride, chitosan, xylanase, and protease. The chickens of the experimental group were given a solution of the drug "Diolide" with water throughout the experiment (41 days) (active substances sodium chlorite and sodium chloride) at a dose of 1 mg/l per chlorine dioxide in accordance with its technical reglementation. This preparation was developed by employees of the State Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Examination (Kyiv).

Biochemical researches of blood were performed by samples from chickens after decapitation at various ages: 10-, 27-, 31-, and 41 days after hatching. The blood samples were examined for the following: content of reduced glutathione (RG; Butler E., 1963); content of lipid hydroperoxides (LHP; V.V. Myronchuk, 1998); concentration of TBK-active products according to the method of E.N. Korobeynikov (1989); activity of superoxide dismutase (EC SOD; 1.15.1.1) according to the method of E.E. Dubinina with co-authors. (1983); activity of glutathione peroxidase (GP; EC 1.11.1.9; Moin V.M., 1986); and content of ketone and aldehyde derivatives of oxidative modification of proteins (OPM_{370}, OPM_{430}) according to the method described by Levine et al. (1990). Biochemical researches were carried out according to the specified methods, which are described in the handbook.[2]

Concentration of Lipid Hydroperoxides

The measurement of LHP (lipid hydroperoxides) level was performed in accordance with the methods of trichloroacetic acid-induced protein precipitation and ethanol-induced lipid extraction. Ammonium thiocyanate interacts with lipid ethanol extracts and initiates the colour reaction. Extinction recording of the colored product was performed spectrophotometrically (λ 480 nm). LHP level (EU/ml) was calculated as the difference between the control and experimental samples.

Concentration of Thiobarbituric Acid Reactive Substances

Evaluating the concentration of TBARS (thiobarbituric acid reactive substances) is based on the principle of malondialdehyde and thiobarbituric acid interaction under the conditions of acidity and a high temperature. The result of malondialdehyde and thiobarbituric acid interaction is the colour reaction. The coloured product extinction recording was performed spectrophotometrically (λ 535 nm, and λ 580 nm) and TBARS level was calculated as μmol/ml.

Activity of GP (EC 1.11.1.9)

The measurement of GP (glutathione peroxidase) enzymatic activity is performed in the presence of GSH before and after adding tertiary butyl hydroperoxide. Evaluating the GP activity is based on the principle of GSH oxidation rate. SH-groups of GSH molecule are oxidized in the presence of 2-nitrobenzoic acid. Dinitrophenyl anion is formed as a result of GSH oxidation. Extinction recording of the coloured product was performed spectrophotometrically (λ 412 nm)

and GP activity was calculated in mmol GSH/min×mg of protein.

Concentration of GSH

The evaluation of the GSH (reduced glutathione) level is based on the principle of thionitrophenyl anion formation (coloured product) after binding of 2-nitrobenzoic acid to SH-group of GSH molecule. The value of GSH concentration depends on the intensity of the colour reaction. The coloured product extinction recording was performed spectrophotometrically (λ 412nm) and GSH content was calculated as μmol/ml.

Activity of SOD (EC; 1.15.1.1).

The measurement of SOD (superoxide dismutase) enzymatic activity was performed in the presence of NADH and phenazine methosulfate. The evaluation of the SOD activity is based on the principle of nitroblue tetrazolium reduction. The intensity of inhibition of the nitroblue tetrazolium reduction process indicates the intensity of enzyme activity. The absorbance recording was performed spectrophotometrically (λ540nm) and SOD activity was calculated as Units/mg of protein*min.

The content of aldehyde and ketone derivatives of oxidative modification of proteins.

The level of intensity of oxidative destruction of proteins was evaluated by the reaction of carbonyl derivatives of the amino acid reaction with dinitrophenylhydrazine. The content of carbonyls was calculated by measuring the optical absorption at 370 nm and 430 nm, taking into account the absorption coefficient of 22000 M-1 cm-1. Carbonyl groups were determined spectrophotometrically by the difference in absorbance at 370 nm (aldehyde derivatives, OMP370) and 430 nm (ketone derivatives, OMP430). During the determination, after adding 0.9 ml of THO and 1 ml of 2,4 dinitrophenylhydrazine to 0.1 ml of serum, incubation was carried out at room temperature and the mixture was centrifuged for 45 min at 3000 rpm. Next, the mixture was washed with ethanol-acetate mixture 3 times. After adding urea and heating for 5 minutes in a boiling water bath, measurements were made at the indicated wavelengths. The concentration of aldehyde and ketone derivatives of oxidative modification of proteins was expressed in nmol/mg of protein.

The experiment plan was designed taking into account Council Directive 2010/63/EU (Council Directive 2010/63/EU, 2010) on the protection of animals used for scientific purposes and the European convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasbourg, 1986),[22-24] and was approved by the Bioethics Commission dated November 7, 2022.

Digital data were processed by the biometric method of variational non-parametric analysis using Microsoft Excel program of the Microsoft Office Professional XP table editor package and Origin 6.1 program. The differences between values were considered statistically significant: $P < .05$; .01 and .001.

RESULTS

Free-radical processes caused by active forms of oxygen are the basis of lipid peroxidation (LPO). Lipid peroxidation is a physiological process. LPO occurs most intensively in biological systems, where the rate of metabolism is particularly high. In particular, a large number of free radicals are formed in mitochondria, which contain electron transport systems. However, the formation of ROS also occurs in microsomes, in the nuclear and plasma membranes, as well as in the cytoplasm.[25]

The process of formation of free radicals, hydroperoxides, peroxides, and diene conjugates ensures the renewal of cell membrane lipids, thereby maintaining structural homeostasis, and also forms a protective mechanism in the body at the physiological and biochemical levels under the influence of stress factors. According to modern ideas, the activation of the process of lipid peroxidation in biological membranes and fluids serves as a trigger (primary mediator) for starting a stress reaction. The intensity of LPO in body tissues is assessed by their content of diene conjugates, lipid hydroperoxides, and TBC-active products, respectively, the initial, intermediate, and final products of LPO, as well as by the degree of chemiluminescence, which correlates with the content of TBC-active products in them.[3,4,25]

The research demonstrated (Table 1) that the content of intermediate and final products of LPO in the broiler chicken blood plasma of the control group was increased during the growing of poultry. The most intensive growth of LPO processes was recorded in chickens during the period of active growth. At the same time, in the experimental group of 27-, 34-, and 41-day-old chicken, the content of GPO and TBC-active products in the blood plasma had the level of, respectively, 7.1, 19.5 and 28.0% ($P < .001$) that was less than in the control group of chicken (12.6 ($P < .01$); 20.5 and 28.2% ($P < .001$). This indicates the inhibitory influence of developed synbiotic preparation in complex applications together with disinfectant on the content of intermediate and final products of LPO.

Table 1. Lipid peroxide oxidation products levels in the blood plasma of broiler chickens (M±m; n=5)

Indicator	Groups	Periods of research			
		10th day	27th day	34th day	41th day
TBK-active products, μmol/ml	C	1.61±0.029	1.74±0.046	1.85±0.052	1.95±0.035
	E	1.61±0.034	1.52±0.041**	1.47±0.0408***	1.40±0.054***
LHP, unit E/ml	C	0.41±0.016	0.42±0.014	0.47±0.012	0.50±0.010
	E	0.40±0.012	0.39±0.013	0.38±0.013***	0.36±0.010***

*: statistically probable differences between the investigated indicators in chickens of the experimental group, compared to the control group: *p<0.05; **P < .01; *** P < .001. TBK-active products: thiobarbituric acid reactive substances; LHP: lipid hydroperoxides.

Similar changes were detected, but to a lower extent, in the content of products of protein oxidation in the chicken blood serum. The data, presented in Table 2, demonstrate that synbiotic preparation use in combination with disinfectant produces a reduction in oxidation modification of proteins in chicken blood serum. OPM aldehyde and ketone derivatives' content was lower in the blood of poultry from experimental group; however, the differences with control group were detected only in OPM aldehyde derivatives content in 41-day-old chicken.

Table 2. Aldehyde (OPM_{370}) and ketone (OPM_{430}) derivatives of oxidative modification of protein levels in chicken blood serum (M±m; n=5)

Indicator	Group	Periods of research			
		10th day	27 th day	34 th day	41st day
OPM_{370} nmol/mg protein	C	4.93±0.38	5.31±0.56	4.91±0.14	5.12±0.33
	E	4.91±0.45	4.58±0.46	4.09±0.35	3.87±0.34*
OPM_{430} nmol/mg protein	C	12.82±1.16	13.10±0.51	10.40±0.96	9.84±0.94
	E	12.18±0.97	11.39±1.05	9.46±0.48	8.76±0.87

*: statistically probable differences between the investigated indicators in chickens of the experimental group, compared to the control group: * P < .05; ** P < .01; *** P < .001. OPM: oxidative modification of proteins.

At the same time, the results of this study indicated the inhibitory influence of tested compounds on the intensity of protein oxidative modification accumulation in chicken blood serum.

It is known that OPM also causes the formation of ROOH in the body, followed by ROH (o- and m-tyrosine), R(OH)2, carbonyl, and other oxidized derivatives; autooxidative glycosylation of proteins also occurs. It is believed that the negative effect of oxidatively modified proteins in cells is due to the fact that oxidized proteins are a source of free radicals that deplete the reserves of cellular antioxidants [32]

The decrease of LPO and OPM products' content in the blood of the experimental group of poultry was probably associated with the complex action of the developed product on the enzyme link of the antioxidant defense system (ADS). In particular, when studying indicators characterizing the glutathione link of ADS, attention is drawn to higher glutathione peroxidase activity in chicken blood in the experimental group in all periods of testing compared to the control group of poultry (Table 3). At the same time, at 34 and 41-day-old chickens, the activity of this enzyme was, respectively, 17 (P <.05) and 21.6% (P < .05) higher than in the control. At the same time, in the experimental group chicken blood, compared to the control group, a tendency of increase of reduced glutathione content was detected in all dates of testing.

Glutathione peroxidase (GPx) is an antioxidant enzyme that catalyzes in the body the reduction of hydrogen peroxide and organic hydroperoxides with reduced glutathione (GSH) to water or to hydroxy-derived organic compounds.[7, 8, 12, 25]

The activity of the glutathione system of antioxidant protection in the cell limits reduced glutathione (GSH), which is oxidized (GSSG) in the process of H_2O_2 reduction. At physiological concentrations of peroxides and a high level of glutathione reduction in cells, glutathione peroxidase is found mainly in reduced forms.[3,4,12,25]

Table 3. Reduced glutathione, glutathione peroxidase, and superoxide dismutase activity levels in broiler chickens' blood (M±m; n=5)

Indicator	Group	Period of research			
		10th day	27th day	34th day	41st day
GP GSH/min per mg protein	C	21.10±0.37	21.42±0.53	21.57±0.54	21.56±0.43
	E	21.79±0.36	24.78±1.55	25.88±1.44*	26.21±1.35*
RG, µmol/ml	C	0.26±0.018	0.27±0.008	0.28±0.009	0.28±0.005
	E	0.28±0.01	0.31±0.016	0.31±0.012	0.32±0.019
SOD, unit act./mg of protein*min	C	20.75±1.97	20.54±0.57	19.56±1.04	19.92±0.78
	E	21.86±0.69	24.04±1.27*	23.33±1.26	23.89±1.19*

*: statistically probable differences between the investigated indicators in chickens of the experimental group, compared to the control group: *p<0.05; **p<0.01; ***p<0.001. GP GSH: enzymatic activity of glutathione peroxidase in the presence of glutathione, RG: reduced glutathione, SOD: superoxide dismutase.

From the data presented in Table 3, we can see that the use of the synbiotic preparation in combination with a disinfectant in the chickens of the experimental group caused an increase in superoxide dismutase activity, an enzyme of the primary link of the antioxidant defense system.

Superoxide dismutase (SOD) is a key enzyme of the antioxidant system. It neutralizes superoxide radicals, turning them into less toxic hydrogen peroxide. Three forms of superoxide dismutase are known: Su/Zn-SOD; Mn-SOD and Fe-SOD. Metals perform a catalytic function, being successively reduced and oxidized in the active center of the enzyme.[3,4,6,8,25]

Research results showed that on days 27- and 41, the activity of this enzyme in the blood of broiler chickens of the research group was, respectively, 20% ($P < .05$) and 19.9% ($p<0.05$) higher than in the control group.

Among other research results obtained in this study, the complex use of the "Biomagn" synbiotic preparation and the "Diolide" disinfectant in broiler chickens are worthy of attention after demonstrating a stimulating influence on chicken growth intensity. During the growing period of poultry, the weight of broiler chickens treated with our products surpassed the growth of the counterparts of control group. At the same time, the average daily growth parameter of chickens treated with the developed product was 6.7% higher than in the control group of poultry. It was noticed that in chickens of the experimental group, the intensity of growth increased until the end of the experiment.

Therefore, based on the results of our research, it can be stated that the use of the "Biomagn" synbiotic preparation and the "Diolide" disinfectant regulated the intensity of oxidative processes in the organism of chicken. The products ensured a pro-oxidant-antioxidant balance, and increased the immune potential of chicken organisms, as it was noted in our previous works,[26,27]and had a positive effect on chicken growth intensity.

DISCUSSION

The results of our research were confirmed by the literature data about the adaptive and metabolic processes that occur in the organisms of broiler chickens during their growing period. In particular importance is the clarification of the role of the tested synbiotic product and disinfectant used in broiler chickens in the regulation of metabolic homeostasis, in particular, the pro-oxidant-antioxidant balance of the body during the period of decrease of the body's immune potential, both under normal and stress conditions.[28-31]

As noted earlier, the processes of LPO and OPM are largely associated with protective and adaptive reactions of the organism. Enhancement of peroxide oxidation processes plays a significant role in the pathogenesis of many diseases. The analysis of literature data showed that for an integral evaluation of the functional activity of the poultry's organism, it is necessary to investigate the intensity of LPO processes and the activity of enzymes of the antioxidant protective system. Therefore, the functioning of the

antioxidant system provides an appropriate level of protection, and products of free radical peroxide oxidation can act as indicators of tissue damage since their content can be used to analyze the intensity of free radical processes in various organism systems.[32,33] The research of LPO is widely used in the investigation of oxidative stress.[34,35.]

Our studies are also consistent with data[36-38], which found that certain strains of *Bacillus* exhibit antioxidant activity, which is assumed to promote the synthesis of antioxidant enzymes that protect the host from oxidative stress.

At the same time, Yu et al.[39] report that the probiotic *Bacillus coagulans* promotes the activation of antioxidant defense system enzymes (SOD, CAT, GP) and reduces MDA levels in the blood serum of broilers. However, the probiotic *Lactobacillus plantarum* in this case does not promote the activation of antioxidant defense system enzymes and causes a less pronounced decrease in MDA levels in the broiler bloom serum.[39]

Studies by other authors also indicate a positive impact of probiotics (*Bacillus subtilis*, *Bacillus licheniformis*) on the antioxidant defense system, which is associated with the activation of SOD, CAT, GP enzyme in the bloom serum [40,41], liver, ileum [41,43], and a decrease in the concentration of MDA in the bloom serum [40] and ileum [41,42] of broilers, both under normal conditions and under conditions of *Clostridium perfingens* – induced subclinical necrotizing enteritis.[40,41] Instead, Ji et al.[42] report only a decrease in MDA levels in the mucosa of the ileum of broilers under the influence of the probiotic *Bacillus subtilis M6*, while the MDA content in the mucosa of the jejunum and the activity of antioxidant defense system enzyme in the mucosa of the ileum and jejunum of broilers remained unchanged.[42] The authors suggest that the enhancement of antioxidant status under the influence of probiotics may be associated with the stimulation of the expression of Nrf-2, HO-1, SOD, and GP genes and an increase in the concentration of non-enzymatic antioxidants under the influence of the studied probiotics,[40] as well as with the chelating properties of probiotics relative to metal ions.[41]

In summary, these results suggest that *Bacillus* may play a useful role in oxidative defense due to its inherent antioxidant activity.

The results of our studies are consistent with the fact that probiotics (*Bacillus coagulans*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Bacillus licheniformis*) have a positive effect on broiler growth performance and improve such animal parameters as total body weight, and average daily feed intake (ADFI), average daily gain (ADG) and feed to gain ratio (F/G).[29,30,37-42] The authors suggest that the improvement in broiler growth performance in this case is associated with increase in the number of *Ruminococcaceae* bacteria and a decrease in the number of *Desulfovibrio* bacteria in the broiler intestinal flora, which in turn may help reduce inflammation, anxiety, and depression.[39] Improvement of broiler growth performance under the influence of probiotics may be associated with increased immunity, regulation of metabolic functions, and composition of the intestinal flora, as well as increase in beneficial metabolites (extracellular digestive enzymes, lysozyme, antifungals proteins, antibiotics) produced by *Bacillus subtilis* and *Bacillus licheniformis.*[40]

In conclusion, the present findings demonstrate that synbiotic preparation "Biomagn" and "Diolide" disinfectant use in broiler chickens caused an inhibitory influence on LPO and OPM processes intensity, as evidenced by a decrease in the LPO intermediate and final products' content in blood plasma and aldehyde derivatives of OPM, which was accompanied by a positive effect on the growth of chickens. Under the influence of the studied drugs, a higher activity of the key enzymes of antioxidant protection - superoxide dismutase and glutathione peroxidase - was recorded in the blood of chickens against the background of the revealed tendency to increase the content of reduced glutathione, which helped ensure the pro-oxidant-antioxidant balance of the body.

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REFERENCES

1. Shevchuk M, Stoyanovskyy V, Kolomiiets I. Technological stress in poultry. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. *Series: Vet Sci*. 2018; 20(88): 63-68.
2. Osadcha YU, Shuliar A, Sydorenko O, Dzhus P, Shuliar A. Biochemical parameters of chicken blood under the influence of technological stimuli of various etiologies. *Sci Horizon*. 2023; 26(9): 70-80.
3. Lavryshyn Y, Varkholyak I., Martyschuk T, Guta Z, Ivankiv L. The biological significance of the antioxidant defense system of animals body. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. *Series: Vet Sci*. 2016:*18*(2):100-111.
4. Antonyak GL, Babych NO, Sologub LI, Snitynsky VV. Formation of reactive oxygen species and the system of antioxidant protection in the animal organism. Anim Biol. 2000;2(2):34-42.
5. Kalyuzhin VA. Thermoresistance in a yeast Saccharomyces cerevisiae. *Zh Obshch Biol*. 2011;72(2):140-149.
6. Regoli F, Gorbi S, Frenzilli G, et al. Oxidative stress in ecotoxicology: from the analysis of individual antioxidants to a more integrated approach. *Mar Environ Res*. 2002;54(3-5):419-423.
7. Lushchak VI, Bagnyukova TV, Luzhna LI. Indicators of oxidative stress.2. peroxides of lipids. *Ukr Biochim Zh*. 2006;78(6):113-120.
8. Mylostyva DF, Farafonov SJ, Puzniak OM, et al. Antioxidant system of the body of young Ukrainian beef cattle under the action of microelements. *Reg Mech in Bio.* 2023;14(1); 106-111.
9. Romanovych L, Kurtyak B, Mudrak D. The intensity of the processes of oxidative modification of proteins and the activity of enzymes of the antioxidant protection system of broiler chickens under the action of vitamins E and C. *Ukrainian J Vet Agric Sci.* 2019:*2*(3);19-22.
10. Klaenhammer TR, Kleerebezem M, Kopp MV, Rescigno M. The impact of probiotics and prebiotics on the immune system. *Nat Rev Immunol*. 2012;12(10):728-734.
11. Stoyanovskyy VG, Shevchuk MO, Kolomiiets IA, Kolotnytskyy VA. Dynamics of individual indicators of protein metabolism in the body of broiler chickens on the background of combined stress when included in the diet “Reasil Humic Vet” + “Laktin” and “Reasil Humic Health”. *Ukrainian J Vet Agric Sci. 2020*;*3*(2):42-46.
12. Mishra V, Shah C, Mokashe N, Chavan R, Yadav H, Prajapati J. Probiotics as Potential Antioxidants: A systematic review. *J Agric Food Chem*. 2015; 63(14):3615-3626.
13. Irshad A. Effect of probiotics on broilers performance. *Int J Poultry Sci*. 2006;5(6):593-597.
14. Sander JE, Hofacre CL, Cheng IH, Wyatt RD. Investigation of resistance of bacteria from commercial poultry sources to commercial disinfectants. *Avian Dis.* 2002;46(4):997-1000.
15. Wu BQ, Zhang T, Guo LQ, Lin JF. Effects of Bacillus subtilis KD1 on broiler intestinal flora. *Poultry Sci*. 2011;90(11): 2493-2499.
16. Horiuk Y, Kukhtyn M, Kovalenko V, Kornienko L, Horiuk V, Liniichuk N. Biofilm formation in bovine mastitis pathogens and the effect on them of antimicrobial drugs. *Independent J Man Prod*. 2019;10(7):897-910.
17. Chechet O, Kovalenko V, Haidei O, Polupan I, Rudoi O. Toxicity and virucidal activity of chlorine dioxide disinfectant. *Sci Horiz*. 2022;25(5):30-39.
18. Du Y, Lv XT, Wu QY, et al. Formation and control of disinfection byproducts and toxicity during reclaimed water chlorination: A review. *J Environ Sci (China)*. 2017;58:51-63.

19.Ma JW, Huang BS, Hsu CW, et al. Efficacy and safety evaluation of a chlorine dioxide solution. *Int J Environ Res Public Health*. 2017;14(3):329.
20.Park HW, Chen G, Hwang CA, Huang L. Effect of water activity on inactivation of Listeria monocytogenes using gaseous chlorine dioxide - A kinetic analysis. *Food Microbiol*. 2021;95:103707.
21. Mannetje L 't, Jones RM. *Field and Laboratory Methods for Grassland and Animal Production Research*. CABI; 2000. http://books.google.ie/books?id=yftDgmHAxqwC&printsec=frontcover&dq=Field+and+laboratory+methods+for+grassland+and+animal+production+research.+L.+%27t+Mannetje+(Editor),+R.M.+Jones.+Research+output:+452,+2000.+Academic,+10.1079/9780851993515.0000&hl=&cd=1&source=gbs_api
22.Buckmaster C. Shifting the culture of lab animal care. *Lab Anim (NY)*. 2012;41(7):205.
23.Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF
24. European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes. https://rm.coe.int/168007a67b
25.Studenok A, Shnurenko E, Karpovskyi V, Trokoz V, Gutyj B. Indicators of protein metabolism and intensity of lipid peroxide oxidation in chickens with different vegetative status. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies. *Series: Vet Sci.* 2020:23(102):110-118.
26.Chechet OM, Kovalenko VL, Vishchur OI, Haidei OS, Liniichuk NV, Krushelnytska OV. The activity of T- and B-cell links of specific protection of chicken-broilers under the influence of synbiotic preparation "Biomagn" and "Diolide" disinfectant. *Ukrainian J Vet Agric Sci*. 2022;5(1):46-52.
27.Chechet OM, Kovalenko VL, Gaidei OS. Preclinical tests of the preparation "Biomagn" on laboratory animals and with the use of Tetrahymena pyriformis. *Med Clin Chem*. 2021;23(3):48-56.
28.Jiang S, Yan FF, Hu JY, Mohammed A, Cheng HW. Bacillus subtilis-based probiotic improves skeletal health and immunity in broiler chickens exposed to heat stress. *Animals*. 2021;11(6):1494.
29. Aluwong T, Kawu M, Raji M, et al. Effect of yeast probiotic on growth, antioxidant enzyme activities and malondialdehyde concentration of broiler chickens. *Antioxidants*. 2013;2(4):326-339.
30.Wang J, Ishfaq M, Miao Y, et al. Dietary administration of Bacillus subtilis KC1 improves growth performance, immune response, heat stress tolerance, and disease resistance of broiler chickens. *Poultry Sci*. 2022;101(3):101693.
31.Ahmed-Farid O, Salah AS, Nassan MA, El-Tarabany MS. Effects of chronic thermal stress on performance, energy metabolism, antioxidant activity, brain serotonin, and blood biochemical indices of broiler chickens. *Animals*. 2021;11(9):2554.
32.Belenichev IF, Kovalenko SI, Dunaev VV. Antioxidants: modern idea, prospects of creation. *Medicines*. 2002;1:25-29.
33.Simonov MR, Kulyk MM. Changes in the activity of antioxidant system of chickens ISA BROWN CROSS depending of age. *Biol Tvarin*. 2019;9(1-2):56-61.
34.Gudyma V YU, Yanovych VG. The content of lipid peroxidation products and the activity of antioxidant enzymes in the tissues of laying hens at different levels of vitamin D3 in the ration. *Sci Bull Stepan Gzhytsky LNUVMBT*. 2010;2(44):55-58.
35.Romanovich NN, Kurtyak BM, Romanovich MS, Vishchur OI, Mudrak DI, Matiukha IO. Dynamics of intensity of the protein oxidative modification processes and the state of the antioxidant protection system in chicken-broilers under the effect of bps-44 and the dried preparations of Saccharomyces cerevisiae. *Biol Tvarin*. 2019;21(1):48-54.
36.Kim K, He Y, Xiong X, et al. Dietary supplementation of Bacillus subtilis influenced intestinal health of weaned pigs experimentally infected with a pathogenic E. coli. *J Anim Sci Biotech*. 2019;10(1):1-12.
37.Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. *Curr Biol.* 2014;24(10):R453-R462.
38.Zhao Y, Zeng D, Wang H, et al. Dietary probiotic Bacillus licheniformis H2 enhanced growth performance, morphology of small intestine and liver, and antioxidant capacity of broiler chickens against Clostridium perfringens-induced subclinical necrotic enteritis. *Probiotics Antimicrob Proteins*. 2020;12(3):883-895.
39. Gong L, Wang B, Mei X, et al. Effects of three probiotic Bacillus on growth performance, digestive enzyme activities, antioxidative capacity, serum immunity, and biochemical parameters in broilers. *Anim Sci J.* 2018;89(11):1561-1571.
40. Yu Y, Li Q, Zeng X, et al. Effects of probiotics on the growth performance, antioxidant functions, immune responses, and caecal microbiota of broilers challenged by lipopolysaccharide. *Front Vet Sci*. 2022;9:846649.
41. Xu Y, Yu Y, Shen Y, et al. Effects of Bacillus subtilis and Bacillus licheniformis on growth performance, immunity, short chain fatty acid production, antioxidant capacity, and cecal microflora in broilers. *Poultry Sci*. 2021;100(9):101358.
42. Zhao Y, Zeng D, Wang H, et al. Dietary probiotic Bacillus licheniformis H2 enhanced growth performance,

morphology of small intestine and liver, and antioxidant capacity of broiler chickens against Clostridium perfringens-induced subclinical necrotic enteritis. *Probiotics Antimicrob Proteins*. 2020;12(3):883-895.

43. Ji L, Zhang L, Liu H, et al. Bacillus subtilis M6 improves intestinal barrier, antioxidant capacity and gut microbial composition in AA broiler. *Front Nutr*. 2022;17(9):965310.