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EU renewable energy strategy as a roadmap for Ukraine (101085755 – JM RE – ERASMUS-JMO-2022-HEI-TCH-RSCH), and European Union policies and best practices in academic project management (101085243 – ProEU-ERASMUS-JMO-2022-HEI-TCH- RSCH

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FATTY ACID COMPOSITION IN THE QUAIL'S BLOOD PLASMA UNDER NANOSELENIUM AND PROBIOTICS DIET

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Abstract. *For the prevention and treatment of oxidative stress, adaptogenic and anti-stress drugs are used, aimed at removing or neutralizing the pathogenic factor. Of particular interest is the study of omega-3 and omega-6 long-chain polyunsaturated fatty acids and their transformation into bioactive lipid mediators. Polyunsaturated fatty acids are structural components of biological membranes, substrates of lipid peroxidation and precursors of eicosanoids, the composition of which changes during oxidative stress. According to the gas chromatographic analysis of the blood plasma fatty acid composition, it was established that in birds receiving standard compound feed, an increase in the saturation of serum lipids was observed due to an increase in the content of palmitic ($p<0.01$) and myristic ($p<0.001$) fatty acids, and the sum of unsaturated fatty acids was probably reduced ($p<0.001$) due to the reduced content of essential fatty acids. By adding bionano-selenium to the diet, the relative content of saturated fatty acids decreases in the fatty acid composition of quails' blood plasma phospholipids compared to intact quails. A decrease in the content of monounsaturated fatty acids of the ω-9 family was also recorded. Since phospholipids are the basis of lipoproteins, their fatty acid composition listed above may indicate an improvement in the transport function of blood plasma. At the same time, the amount of cholesterol esterified with fatty acids decreased in the blood plasma of the experimental birds, which in the complex allows us to conclude about the positive effect of the complex drug on the fatty acid formula of blood serum lipids.*

Introduction. The introduction of effective means of prevention and treatment, the etiological factors of which are alimentary factors, is an integral condition for increasing the profitability of poultry farming (Цехмістренко et al., 2019). For the prevention and treatment of oxidative stress, adaptogenic and anti-stress drugs are used, which leads to the optimization of the process of free radical oxidation of lipids (Ahmadi et al., 2018).

The urgency of creating new complex drugs is determined by the insufficient therapeutic effectiveness of existing drugs, as well as a number of side effects from their use. This is, in particular, the occurrence of oxidative stress, which is a non-specific universal appropriate response of the body to damage and promotes the mobilization of protective systems aimed at removing or neutralizing the pathogenic factor, mobilization of antioxidant defense systems and adaptation of metabolism to extreme conditions. Such a reaction is associated not only with the activation of neurohumoral regulation links during homeostasis disturbances, but also with the fact that active oxygen metabolites are signaling molecules that induce the activity of compounds, in particular lipids, involved in biosynthesis and antioxidant protection.

In recent decades, the development of new technologies applied to lipidomics has increased the interest in the study and analysis of lipid profile changes and the understanding of the basic molecular mechanisms of lipid metabolism together with their involvement in the development of human and animal diseases. Of particular interest is the study of omega-3 and omega-6 long-chain polyunsaturated fatty acids (LC-PUFA), in particular EPA (eicosapentaenoic acid, 20:5n-3), DHA (docosahexaenoic acid, 22:6n-3), and ARA (arachidonic acid, 20:4n-6), and their transformation into bioactive mediators of lipids. In this sense, new families of PUFA-derived lipid mediators, including EPA- and DHA-derived resolvins and DHA-derived protecins and maresins, are increasingly being investigated for their active role in "return to homeostasis" and resolution of inflammation (Zhang et al., 2021).Lipids are necessary elements of the diet for providing energy, in particular for beta-oxidation of fatty acids (FA). Fatty acids are the simplest lipids, which, in turn, are components of other, more complex lipids. FAs contain a hydrophilic carboxyl group attached to a hydrocarbon chain ranging from C6 (six carbon atoms) to C32, with an additional terminal methyl group. Most naturally occurring FAs have an even number of carbon atoms and linear hydrocarbon chains, although some of them, found mainly in bacteria, may contain branched or even cyclic structures (Afonyna, Kuyun, 2000; Tsekhmistrenko et al., 2022; Koval′ova & Pasiyeshvili, 2021). In addition, these chains can be presented in two main forms, saturated without the presence of double bonds, or unsaturated, containing one or more double bonds, the latter being more physiologically important due to their medicinal properties. Of particular interest in the unsaturated family are long-chain polyunsaturated fatty substances (LC-PUFA), which can be divided into two main groups - omega-3 (n-3) and omega-6 (n-6), depending on the position of the first double bond from the methyl terminal group of FA. Common LC-PUFAs are EPA (eicosapentaenoic acid, 20:5n-3), DHA (docosahexaenoic acid, 22:6n-3), and ARA (arachidonic acid, 20:4n-6). The optimal substrate for the processes of lipid peroxidation (LP) are unsaturated fatty acids (PUFA) (Bityutskyy et al., 2021; Tsekhmistrenko et al., 2020), and the immediate target of the attack of oxidizing radicals is double bonds in the molecules of these acids (Afonyna, Kuyun , 2000; Tsekhmistrenko et al., 2022; Koval′ova & Pasiyeshvili, 2021).

Reactions of free radical oxidation and the antioxidant defense system (AODS) form the LP–AOD system, which is based on the maintenance of cell homeostasis. In the case of an imbalance of the AOD system, homeostasis disturbances occur, the nature of which depends on the intensity of LP processes, the state of cell membranes, the structural components of which are higher fatty acids and free cholesterol. Polyunsaturated fatty acids are structural components of biological membranes, substrates of LP and precursors of eicosanoids. Studies by many authors indicate changes in the fatty acid composition of blood plasma lipids and erythrocytes during oxidative stress (Afonyna, Kuyun, 2000; Tsekhmistrenko et al., 2022; Koval′ova & Pasiyeshvili, 2021).

Materials and Methods. In the case of studying the biological effect of nanoselenium, 120 Pharaoh breed quails were selected at the age of one day and 2 groups of 60 heads each were formed by the method of analogues. The conditions of keeping quails were the same and corresponded to zootechnical standards. Poultry of the control group (group 1) received complete compound feed. A probiotic preparation with nanoselenium (group 2) was added to the combined feed of experimental groups of poultry by multi-stage mixing. Probiotic and Selenium dosages correspond to established effective amounts according to previous scientific studies. Probiotic and biogenic nanoselenium were synthesized together with scientists of the Department of Interferon Problems and Immunomodulators of the Institute of Microbiology and Virology named after D.K. Zabolotny of National Academy of Sciences of Ukraine. The material for research was blood and blood serum. Lipids were extracted from blood plasma using a chloroform-methanol mixture. Separation of lipids into fractions was carried out on thin-layer silica gel plates in the solvent system hexane : diethyl ether : glacial acetic acid (85 : 15 : 1). Detection of individual fractions of lipids on both plates was carried out in iodine vapor. The identification of lipid fractions on the plates was carried out using standard lipids with the degree of purification of ChC. According to the results of thin-layer chromatography, the content of individual classes of lipids of the first plate was calculated according to the formula with correction coefficients for each studied fraction.

Results and Discussion. It is known that during pathological processes accompanied by peroxidation processes of biomolecules, the degree of saturation of fatty acids (FA) of lipids changes, so the ratio of saturated and unsaturated fatty acids in membrane lipids is of great importance for ensuring the functional state of cells (Danchuk et al., 2004). **The aim of our research** was to evaluate the fatty acid composition of blood lipids of birds after the introduction of a complex drug (Table 1).

Table 1.

Fatty acid composition of quail blood plasma phospholipids, % M±m, n=3

Fatty acids, their code and family	Control group	For the addition of bionano-selenium
Caprylic, 8:0	0,12±0,01	0,09±0,01*
Capric, 10:0	0,24±0,01	0,20±0,01*
Lauric, 12:0	0,34±0,01	0,28±0,01*
Myristic, 14:0	0,56±0,01	0,50±0,01*
Pentadecanoic, 15:0	0,38±0,01	0,32±0,01*
Palmitic, 16:0	8,85±0,04	8,25±0,17*
Palmitoleic, 16:1 ω-7	0,91±0,02	0,88±0,01
Stearic, 18:0	10,24±0,14	9,23±0,23*
Oleic, 18:1 ω-9	33,15±0,84	30,44±0,88*
Linoleic, 18:2 ω-6	14,98±0,45	15,34±0,49
Linolenic, 18:3 ω-3	6,68±0,21	6,97±0,23
Arachinic, 20:0	0,28±0,01	0,23±0,01*
Eicosaenoic, 20:1 ω-9	0,24±0,01	0,22±0,01
Eicosadienoic, 20:2 ω-6	0,30±0,01	0,32±0,01
Eicosatrienoic, 20:3 ω-6	1,17±0,01	1,31±0,04*
Eicosatetraenoic (arachidonic), 20:4 ω-6	5,34±0,06	5,96±0,14*
Eicosapentaenoic, 20:5 ω-3	1,65±0,02	1,82±0,04*
Docosadienoic, 22:2 ω-6	0,97±0,03	1,22±0,05*
Docosatrienoic, 22:3 ω-3	1,26±0,02	1,43±0,05*
Docosatetraenoic, 22:4 ω-6	2,37±0,10	3,05±0,11*
Docosapentaenoic, 22:5 ω-3	4,47±0,18	5,45±0,16*
Docosahexaenoic, 22:6 ω-3	5,50±0,16	6,48±0,17*

The gas chromatographic analysis data of the blood plasma fatty acid composition show that in the birds of the 1st group (control) there is an increase in the saturation of serum lipids due to an increase in the content of palmitic ($p<0.01$) and myristic ($p<0.001$) fatty acids, and the sum of unsaturated fatty acids was probably reduced ($p<0{,}001$). An increase in the content of palmitic acid indicates the intensification of LP processes and the accumulation of lysoforms of the lecithin fraction of phospholipids. The level of PUFA was probably reduced in comparison with the indicators of the birds of the 2nd group (experiment) due to the reduced content of essential fatty acids (linoleic and linolenic).

Therefore, the fatty acid formula of blood serum lipids of quails of the 1st group is characterized by increased hydrogen saturation of the lipid complex against the background of a deficiency of certain PUFAs, which is a compensatory reaction, because it is saturated fatty acids that are less susceptible to peroxidation processes (Lyzohub et al., 2003).

It was recorded (Fig. 1) that addition of bionano-selenium in comparison with intact quails changes the fatty acid composition of the quails blood plasma phospholipids by decreseing of the relative content of FA with even (caprylic, capric, lauric, myristic, palmitic, stearic and arachinic) and odd (pentadecanic) number of carbon atoms in the chain.

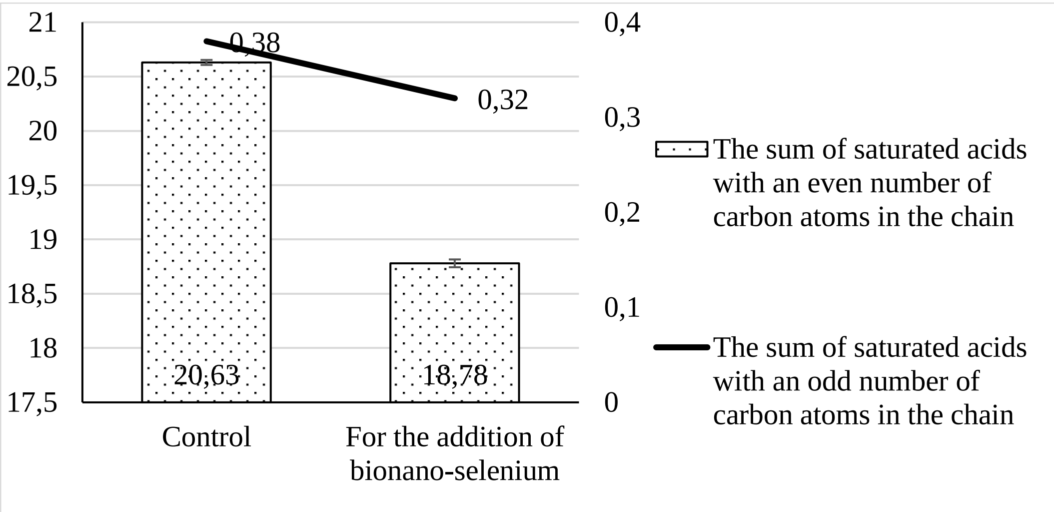

Fig. 1. Content of saturated acids in quail blood plasma phospholipids with even and odd number of carbon atoms in the chain

A decrease in the content of monounsaturated fatty acids of the ω-9 (oleic) family was recorded (fig. 2), but an increase in polyunsaturated fatty acids of the ω-6 (eicosatrienoic, eicosatetraenoic, arachidonic, docosatetraenoic) and ω-3 (eicosapentaenoic, docosatrienoic, docosapentaenoic, and docosahexaenoic) families.

At the same time, the content of long-chain and unsaturated derivatives of linoleic (1.48 vs. 1.29) and linolenic (0.52 vs. 0.46) acids increases in the fatty acid composition of quail blood plasma phospholipids due to the addition of bionanoselenium. Since phospholipids are the basis of lipoproteins, their fatty acid composition listed above may indicate an improvement in the transport function of blood plasma (Hopanenko & Rivis, 2015).

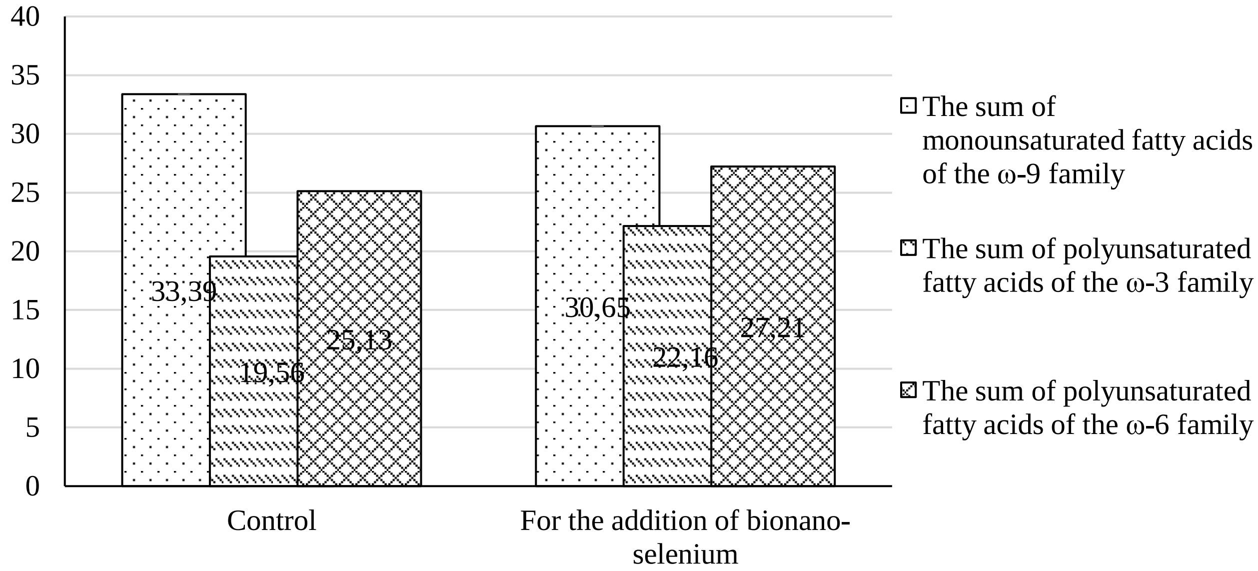

Fig. 2. The content of unsaturated acids of the ω-3 ω-6 and ω-9 families in the composition of quail blood phospholipids

The detected state of the fatty acid composition of lipoproteins in the blood serum of quails injected with sodium selenite is a consequence of the intensification of LPO processes and leads to a violation of the metabolism of fatty acids at the stage of eicosanoid formation, which is consistent with literature data (Afonyna, Kuyun, 2000; Tsekhmistrenko et al., 2022; Koval′ova, & Pasiyeshvili, 2021). Under stress, the concentration of individual PUFAs in cell membranes increases significantly. One of the important functions of PUFA is the synthesis of eicosanoids, the substrate of which is arachidonic and linoleic fatty acids. Metabolites of arachidonic acid – prostaglandins, thromboxanes and leukotrienes have high biological activity with a wide range of regulatory action.

After the drug introduction, the lipids fatty acid composition normalized due to a decrease in the content of palmitic acid by 18.9% ($p<0.05$), myristic acid content by 21.4%, ($p<0.01$) and arachidonic acid content by 19.6%, ($p<0.01$) acids. Research and analysis of data on the content of individual fatty acids in the erythrocyte membranes of birds of the experimental and control groups allowed to establish an increase in the percentage content of fatty acids 22:5 (ω-3) and 22:6 (ω-3) in the membranes of erythrocytes ($p<0.05$) of the 1st group of poultry, which may be a protective factor against peroxidic damage of membrane phospholipids, since ω-3 fatty acids are an imperfect substrate for cyclo- and lipoxygenases (Jump, 2004).

The imbalance of fatty acids in the blood erythrocytes of birds injected with sodium selenite is characterized by an increase in the content of certain polyunsaturated fatty acids (arachidonic and docosahexaenoic, $p<0.05$). The effectiveness of using the drug consists in normalizing the level of fatty acids and restoring their metabolism at the stage of eicosanoid formation.

The nature of the disorders caused by the introduction of pro-oxidant sodium selenite depends on the intensity of LPO processes, the state of cell membranes, the structural components of which are higher fatty acids and free cholesterol. Polyunsaturated fatty acids – structural components of biological membranes, substrates of LPO and precursors of eicosanoids are the connecting link of these processes. The studies of many authors indicate changes in the fatty acid composition of blood plasma lipids and erythrocytes during oxidative stress, which accompanies most diseases. Arachidonic acid, like other

PUFAs, interacts with the components of the respiratory chain of mitochondria, inhibits chain complexes and disrupts electron transfer processes, which leads to the formation of free radicals in mitochondria. Under physiological conditions, when the concentration of PUFAs in mitochondrial membranes is normal, this effect is insignificant. During stress, the concentration of PUFAs in cell membranes increases significantly.

In the fatty acid composition of the esterified blood plasma cholesterol, the content of saturated fatty acids with steam (caprylic - by 5.88%, capric - by 8.33%, myristic - by 5.77%, palmitic - by 1.93%), decreased lauric - by 6.25% and arachinic - by 5.41%) and odd (pentadecane - by 6.25%) number of C atoms in the chain and monounsaturated acids of the ω-7 family (palmitoleic - by 3.57%) (Table 2).

Table 2

Fatty acid composition of esterified cholesterol in quails' blood plasma, %, M±m, n = 5

Fatty acids and their code	Control group	Experiment
Caprylic, 8:0	0,17±0,01	0,16±0,01
Capric, 10:0	0,24±0,01	0,22±0,01
Lauric, 12:0	0,32±0,01	0,3±0,01
Myristic, 14:0	0,52±0,01	0,49±0,01
Pentadecanoic, 15:0	0,32±0,01	0,3±0,01
Palmitic, 16:0	8,62±0,11	8,95±0,12
Palmitoleic, 16:1 ω-7	1,12±0,03	1,16±0,02
Stearic, 18:0	8,99±0,39	9,76±0,31
Oleic, 18:1 ω-9	31,39±1,14	32,66±1,11
Linoleic, 18:2 ω-6	15,03±0,4	15,37±0,38
Linolenic, 18:3 ω-3	5,93±0,06	5,44±0,09**
Arachinic, 20:0	0,37±0,01	0,35±0,01
Eicosaenoic, 20:1 ω-9	0,19±0,01	0,21±0,01
Eicosadienoic, 20:2 ω-6	0,33±0,01	0,30±0,01
Eicosatrienoic, 20:3 ω-6	1,80±0,04	1,74±0,04
Eicosatetraenoic (arachidonic), 20:4 ω-6	5,13±0,13	5,42±0,13
Eicosapentaenoic, 20:5 ω-3	1,94±0,05	1,53±0,09**
Docosadienoic, 22:2 ω-6	1,02±0,02	0,98±0,02
Docosatrienoic, 22:3 ω-3	1,36±0,03	1,15±0,05**
Docosatetraenoic, 22:4 ω-6	3,22±0,07	2,98±0,07*
Docosapentaenoic, 22:5 ω-3	5,54±0,16	4,71±0,11**
Docosahexaenoic, 22:6 ω-3	6,44±0,06	5,82±0,14**

At the same time, there was an increase in the content of stearic (by 5%) and oleic (by 4.05%) acids, as well as polyunsaturated fatty acids of the ω-6 family: linoleic (by 2.26%), eicosadiene (by 10.53%), eicosatetraenoic arachidonic (by 5,6 %).

At the same time, in the experimental group of quails, with the addition of bionano-selenium, the content of acids of the ω-3 family decreased sighnificantly: linolenic (by 8.36%), eicosapentaenoic (by 21.13%), docosatric, penta- and hexaenoic acids (by 15.44%) , 14.98 and 9.63%, respectively) and ω-6 fatty acids - docosadienoic and docosatetraenoic (by 3.92 and 7.45%) and eicosadienoic and eicosatrienoic acids (by 9.09 and 3.33%). At the same time, the addition of bionanoselenium does not change the inclusion of long-chain and unsaturated derivatives of linoleic acid in the esterified blood plasma cholesterol of quails, but the inclusion of long-chain and unsaturated derivatives of linolenic acid increases (fig. 3).

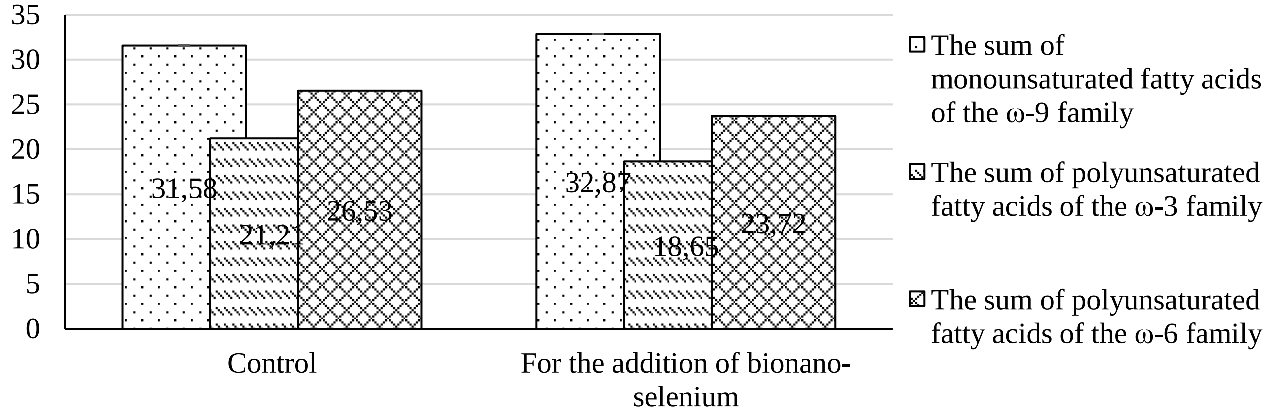

Fig. 3. The content of unsaturated acids of the ω-3 ω-6 and ω-9 families in the composition of esterified cholesterol in quail blood plasma

At the same time, with the addition of bionanoselenium in the blood of quails, it was established encreasing of the amount of saturated fatty acids with an even number of carbon atoms in the chain increased by 1.32%, the amount of monounsaturated fatty acids of the ω-9 family by 6.62%, and the amount of polyunsaturated fatty acids of the ω-6 family by 10.17%.

At the same time, there was a decrease in the amount of saturated fatty acids with an odd number of carbon atoms in the chain by 6.25 %, the amount of monounsaturated fatty acids of the ω-7 family by 4 %, the amount of polyunsaturated fatty acids of the ω-3 family by 2.07 %, and the ratio of ω-3/ ω-6 by 20.19 %.

A decrease in cholesterol esterification of quails' blood plasma with saturated and monounsaturated fatty acids due to the consumption of bionanoselenium as part of the diet may indicate a decrease in its crystallinity and an improvement in interstitial transport (Загайко et al, 2008). Cholesterol, which contains a large amount of saturated and monounsaturated fatty acids, can easily be deposited on the walls of blood vessels and exhibit atherogenic properties (Смоляр, 2003). At the same time, due to the presence of a relatively large number of polyunsaturated fatty acids in the composition of cholesterol, it is easily transported by blood and is not deposited on the walls of blood vessels (Смоляр, 2003).

Conclusions. The results obtained by us indicate an improvement in the transport and anti-inflammatory function of the blood plasma of quails fed with bionanoselenium as part of compound feed due to an increase in the content of polyunsaturated fatty acids in phospholipids. At the same time, the amount of cholesterol esterified with saturated and monounsaturated fatty acids in blood plasma decreases.

One of the important functions of PUFA is the synthesis of eicosanoids, the substrate of which is arachidonic and linoleic fatty acids. Metabolites of arachidonic acid - prostaglandins, thromboxanes and leukotrienes have high biological activity and belong to pro-inflammatory factors that contribute to the aggregation of platelets and the formation of pro-inflammatory cytokines.

The conducted studies indicate a positive effect of the complex drug on the fatty acid formula of blood serum lipids of experimental birds. The drug under study normalizes the lipid composition of blood serum, contributing to the optimization of the level of saturated and unsaturated fatty acids.

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