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Cytotoxicity effects determination of vitamin-mineral feed supplement for poultry «Ekstraselen-Vit» on broiler chickens liver cells.

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ABSTRACT

The purpose of the present research was to conduct a histological study of the broiler chickens' liver (cross "Ross-308") separated into two groups: control and experimental, both kept in the same conditions. Broiler chickens from the experimental group got a biologically active comprehensive vitamin-mineral feed supplement «Ekstraselen-Vit» with drinking water at the dose of 0.1 ml per 1 liter continuously from 15 to 42 days of cultivation. Found that in the control group observed morphological and functional changes characterized by fatty and protein dystrophy of hepatocytes, which is associated with age-related physiology in birds industrial areas. In the test group under the influence of the specimen a clear structural composition of liver acinus with a distinct beamed arrangement of polygonal hepatocytes with an even coloring of cell cytoplasm can be seen. That indicates a sufficient amount of the protein – an indicator of preventive hepatoprotective specimen effect on liver and the whole organism.

Keywords: bird, broilers, liver, liver acinus, cytotoxic effect, histology, feed supplement, selenium, vitamins.

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INTRODUCTION

No organ is affected by so many different toxins, as the liver, which is the central component of detoxification system that neutralizes toxins and prepares them for removal from the body. Biotransformation of highly toxic substances entering the body with food takes place namely in a liver, which involves the conversion of toxic chemical substances into new substances that are not harmful to the organism and can easily be removed therefrom. Liver is able to regenerate its own damaged cells, regenerate or replace them, maintaining their function in relative order. Unfortunately, in many cases even this perfect system of toxin neutralization has limits. If the further inflow of toxic substances does not fall down and there are no measures to protect the liver, its activity weakens, the structure changes [7, 8, 11, 14].

The most common pathology of this organ is fat and protein dystrophy. This is usually caused by long-term effect of cell toxins, potent drugs, vaccines requiring maximum work stress, as well as improper or poorly balanced feeding [16, 38]. Usually this is accompanied by hypodynamia of birds and animals, especially by cage housing. The greatest problem is that such changes in the liver are rarely accompanied by any symptoms. And often pathology is noticed only when the liver becomes very large. However, the alteration of cells conceals the danger of the gradual development of inflammation (hepatitis), hepatic fibrosis and its subsequent transition to cirrhosis [4, 39].

Joining to the WTO forced leading enterprises to increase the environmental safety requirements of animal and poultry breeding. This has led to reconsidering of many methodological approaches to the development of a new generation of environmentally sound products able to take their place in the system of measures to ensure biological protection of animals and birds [5, 6, 21]. Nowadays, the international scientific community studies are aimed at finding safe and non-toxic methods to fight with non-contagious and infectious birds disorders, ways to reduce the negative impact of various stress factors that are unavoidable in the conditions of intensive poultry farming, searching for means to increase the productivity [2, 3, 27].

In order to determine the effectiveness and perspectivity of developed specimen except complex pharmacognostic researches, the leading pharmaceutical industry manufacturers conduct clinical studies on animals to determine the tropism of specimen in the body, cumulative and cytotoxic effect indices [11].

In the laboratory we have developed a biologically active vitamin-mineral complex feed supplement of a new generation Extraselen-Vit based on selenium having a particle size of 20-60 nm with lipophilic complex (A, E, D, K) and hydrophilic (B_1, B_3, B_5, B_6) vitamins, characterized by bioavailability and has a positive effect on cellular and humoral immunity, can serve as a natural stimulant of growth and have a toxic-radioprotective effect reducing the impact of negative environmental factors [22, 23].

The main component of the feed supplement - selenium – has an antimutagenic, antiteratogennym, radioprotective effect, stimulates the antitoxic protection, normalizes metabolism of nucleic acids and proteins, participates in the photochemical reactions of light perception, and serves as a synergist of fat soluble vitamins, which accounts for about 5% of the composition of a complex feed supplement. In 1957 Schwartz and Foltz found out that lack of selenium in the animals' and birds' diet leads to the liver cirrhosis, muscular dystrophy and cardiomyopathy [33, 34]. Besides the lack of this microelement in the chickens' diet leads to a number of serious pathologies, such as exudative diathesis, subcutaneous edema, joints diseases, while the adult bird is marked by the reductions in egg production and deterioration in hatchability The Natural sources of selenium in the human, animals' and birds' diets are plants. However, it should be noted that the concentration gradient of this element by higher plants is comparatively low. Besides not all soil selenium is available for plants; for example: acidic, highly waterlogged soils and soils, where the bulk of the selenium is connected with ferric hydroxide have low bioavailability of this microelement, although the total concentration may be significant [33]. The compound of modern feed supplement, used in veterinary medicine, includes both organic and inorganic forms of microelements. A comparative study of the impact of different microelements on the animals' and birds' bodies is a priority for many scientific areas in the world [1, 5]. However, regarding to selenium it should be noted that each of its forms has its own advantages and disadvantages: on the one hand the organic compounds are less toxic, but inorganic selenium forms can be easily excreted from the body, and their consumption at the recommended doses is safer than the consumption of organic forms; digestibility of organic and inorganic selenium in the gastrointestinal tract of poultry is almost identical, but the further distribution in the body is different. Furthermore, the choice of

organic or inorganic form of selenium is an ultimate impact on the cost of drugs: the organic selenium is many times as expensive [8, 12]. However, it was found that the addition of selenium to the main poultry diet improves the antioxidant status, increases the productivity, expressed in weight of birds, the number of laid eggs and their hatchability, chickenfeedconversion ratio and also increases the concentration of selenium in poultry meat [6, 9].

Mutual protection from oxidative destruction is part of synergism of vitamin E and selenium. They are essential components of the mechanism, where the vitamin E is a kind of probe able to output from the membrane on its surface phospholipid molecule having undergone peroxidation for the destruction of the peroxide or hydroperoxide fragment by glutathione peroxidase. At the same time, it was found that introduction of tocopherol into the birds' diet in addition to the main feed from 200 to 400 IU results in a significant improvement in the oxidative stability of poultry meet at refrigeration storage [20]. Furthermore, selenium is able to activate in the liver, kidneys and other organs a system of oxidative degradation of xenobiotics, toxic metabolites. It is a powerful activator of methionine metabolism in the liver. It helps reduce the appearance of clinical symptoms and pathological alteration in the tissues. In turn, the vitamins A, D and E, are part of a complex specimen, affect immune status – increases the phagocytic, bactericidal and lysozyme activity of blood serum and content of immunoglobulins in it [15]. Rebel et al. have discovered that the addition of vitamins to the main diet of poultry with bowel pathology - malabsorption syndrome – marked the stimulation effect of the immune system, manifested by the increased leukocyte activity, and more rapid healing of the affected bowel [16]. The bioavailability of B vitamins, coming from the developed complex feed supplement, increases due to synergies with cholecalciferol: it improves the hydrophilic vitamin absorption in the intestine. Vitamin B1, or thiamine, amounts 0.1% of the components of developed feed supplement, participates in the oxidative decarboxylation of α-keto acids: pyruvate, α-ketoglutaric, γ-hydroxy-α-ketoglutaric and glyoxylic; it has antioxidant properties against ascorbic acid, pyridoxine and easily interacts with the hydroxy groups of polyphenols. Thiamine is actively involved in liver functioning, in case of its lack disorders occur in protein metabolism. Thiamine derivatives are involved in the processes of rephosphorylation, actively interact with nucleoside phosphates or directly with other carriers of macroergic phosphates. In turn, the feed supplement component, pantothenic acid, making 0.2% of the complex composition, is an indispensable part of the CoA, thus playing a fundamental role in the metabolism, taking part in biological processes such as oxidation and biosynthesis of fat acids, oxidative decarboxylation of keto acids, the Krebs cycle, biosynthesis of steroids, neutral fats, phosphatides, porphyrins, synthesis of acetylcholine, the acylation of aromatic amides, glucosamine synthesis hippuric acid. Approximately 0.9% of the feed supplement composition accounts for vitamin B_5, which is a part of codehydrogenases – coenzymes of NAD and NADP and together with apoenzyme activates redox reactions of cellular metabolism, participates in the processes of glycolysis, Krebs cycle and respiratory chain, biosynthesis of fat acids. Vitamin B_6, or pyridoxine, is in equal proportion with thiamine. It is found in more than 50 enzymes, predominantly involved in the processes of amino acid synthesis and metabolism as well as in phosphorylation of carbohydrates and fatty acid metabolism and membrane unsaturated lipids. In addition, it is inextricably linked to the synthesis of coenzyme A from pantothenic acid which is part of the key enzymes of glycogen metabolism. Lack of vitamin B_6 leads inevitably to a deep disruption of nitrogen metabolism [25, 31, 42].

The purpose of the study is to conduct the histological research of the broiler chickens' liver cross "Ross-308" for the cytotoxic effect of the developed vitamin and mineral complex «Ekstraselen-Vit» as a component of toxicological assessment. The conducted study will allow us to identify the intensity of the damage and the degree of reversibility of the structures for the further prediction of the sensitivity of target tissues and organs from its side effects.

MATERIALS AND METHODS

Studies were conducted in the vivarium of the Technology Management Faculty, Sci-diagnostic and treatment veterinary center of FSBEI HPE "Stavropol state agrarian university" according to the recommendations of All-Russian Research Institute Of Technology And Poultry Breeding for technology of bird keeping [13, 19].

30 apparently healthy broiler chickens of "Ross-308"cross at the age of 15 days were the target of the study. They were divided on the analogs basis into two groups of 15 animals in each. The first group was a control set; and the second group of chickens was injected with developed by vitamin-mineral selenium-

containing feed supplement "Extraselen-Vit» by mouth watering with drinking water at a dose of 0.1 ml per 1 liter water continuously from 15 to 42 days of cultivation.

On day 42 we euthanized chickens (by the strong and accurate blow to the head, causing serious brain damage) in accordance with Directive 2010/63/EU of THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION for Protection of animals used for scientific purposes, and then carried out the selection of the liver, from which we cut out pieces at the size of $1cm^3$ for histological studies.

The material was fixed in 10% buffered formalin, exposed to the effect of alcohol of increasing concentrations and xylene and then poured into histological medium “Gistomiks” using closed histological processor Tissue-Tek VIP™ 5 Jr and pouring paraffin station Tissue-Tek® TEC™ 5 (Sakura, Japan). We got histological sections of thickness of 5 - 7 microns from obtained blocks.

For review purposes histological sections were stained with hematoxylin and eosin, Mallory staining was used to identify collagen fibers (Biovitrum, Russia) on the automatic multistainer Prisma™ (Sakura, Japan) according to the recommendations from the manual [17].

10 digital images of every examined organ in randomly selected sight were made (in .jpg format, 3136×2352 pixels, 24 bit graphic pallet) with zoom 100, 200, 400 *и* 1000 on digital microscope Olympus BX45.

RESULTS AND DISCUSSION

In clinical studies of chickens in both groups overall health and behavioral reactions varied within the physiological norm. With the help of ante-mortem inspection and pathoanatomical autopsy of broiler chickens changes were not found, the degree of bleeding was good. Carcasses of chickens of both groups on nutritional status have a round shape chest, well-developed muscles and adipopexis in the lower abdomen. The liver is located behind the heart and underdeveloped diaphragm, in the bath-shaped recess of sternum, formed by rib, side and secondary processes of the keel bone with the intervening ligaments, covered inside by a serous cover. The color of the liver ranged from red to brown, it was of elastic consistency with sharp edges. Cut edges of the liver converge uniformly without bulging parenchyma.

Histological examination of the liver samples of control and test groups showed that capsule body is clearly visible; it is composed of dense irregular connective tissue, spliced with mesothelium. Intraorgan connective trabeculae are underdeveloped, there are large blood vessels.

In the control group of chickens liver acinus have a classic look and anastomose together, therefore lobed structure of the liver is not clearly visible. Between the acinus the presence of blood lacunae can be seen, with circulating blood. Around the lacunae and vascular connective trabeculae lymphoid follicles in the activation stage are located, characterized by a loose arrangement of cellular elements without clear structural framework (Fig. 1A, B).

In the experimental group portal liver acinus with a triangular shape are viewed, which include the segments of three classical adjacent liver acinus, surrounding the triad. In the corners of portal acinus veins are located. The trabeculae around triads perivascular lymphoid tissue forms clear round follicles (Fig. 2A, B).

Central vein, the triad system - artery, vein, and the gall duct - in the control and experimental groups are clearly visible. In the venous channel, along with the erythrocytes, macrophages, and single eosinophils can be seen. In the gall ducts of the experimental group there is a moderate amount of bile. In the control group of the bile duct lumen is increased in volume, epithelium is wrinkled and is in a state of active proliferation (Fig. 1C).

In both groups in the acinus the central vein can be seen. Hepatic beams are distinctly visualized only around the central veins. Further from these veins beams ramify and anastomose with each other, that gives the parenchyma a reticulate character (Fig. 2C).

In the control group we marked in hepatocytes moderate degenerative changes in the form of atomized fatty degeneration (Fig. 1D). In six samples of liver tissue sections parenchymal tissue had poor

cellular and nuclear polymorphism as evidenced by the different intensity of staining of nuclei and their compactness. The cytoplasm of hepatocytes with fine oxyphilic grit shows the development of granular dystrophy (Fig. 1E). The capillaries between the beams are widened and filled with erythrocytes.

Hepatocytes of the experimental group have a polygonal structure, clear lines of cells. Cytoplasm of cells is colored in pink evenly, indicating sufficient quantities of protein. The nuclei are uniformly stained, have the same size, the nucleoli and chromatin can be seen. Capillary channel is moderately filled with blood (Fig. 2D, E).

The knowledge of the morphofunctional characteristic of liver in the appliance of selenium-containing feed supplement which affect the cell structure of the organism taking into account the age of the bird, as well as housing conditions, nutrition and operation is important for morphologist. In solving this problem, in the scientific literature the information on the poultry liver morphology has incomplete, fragmented and contradictory nature [2, 5, 15].

It is well known that therapeutic doses of selenium have the essential action, but an overdose can cause a serious liver injury. In Nashwa Mostafa Saied and Alaa eldin Ahmed Hamza papers it is reflected that the application of selenium on the background of isotretinoin-induced liver injury reduces the toxic effect of the feed supplement [18, 26, 33].

In the S. J. Csi et al. work a positive description of the nanoselen application as an addition to the basic diet of poultry was given, the increase of liver enzymes activity and increase of selenium content in the liver was marked, however, a detailed description of the state of hepatic structures is not described [2]. The same conclusion was reached by scientists D. Yuan, X. A. Zhan, 1 and Y. X. Wang. In the article «Effect of selenium sources on the expression of cellular glutathione peroxidase and cytoplasmic thioredoxin reductase in the liver and kidney of broiler breeders and their offspring» they reflected the results of the study about the effects of different forms of selenium on the activity of liver enzymes [41].

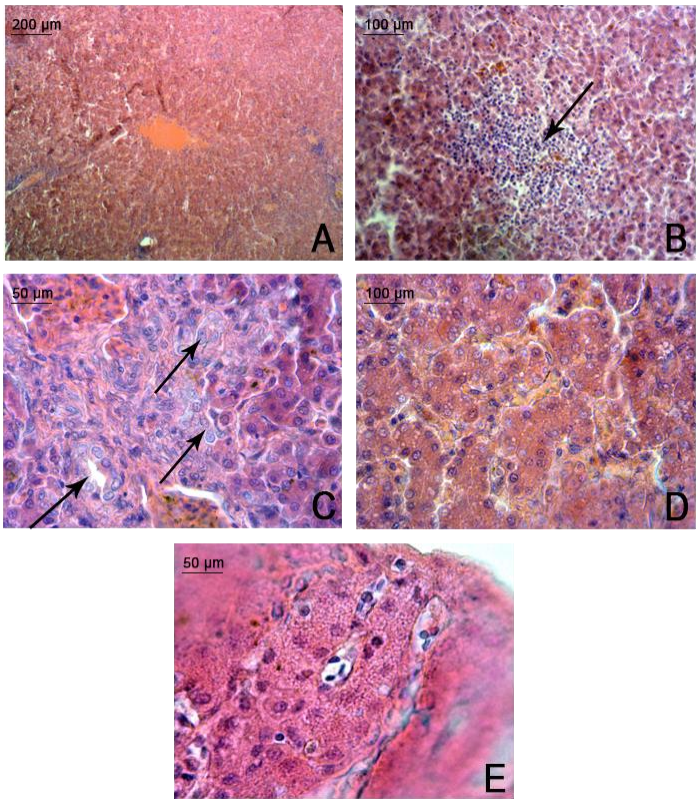

Figure 1: Microscopic structure of the control group liver. A – the classic liver lobule; B – activation of lymphoid follicles; C – active proliferation of the epithelium of ductus choledochus with false ducts appearance; D - atomizing fatty degeneration of hepatocytes; E - granular fatty degeneration of hepatocytes. A, B, C, D, E - H & E staining.

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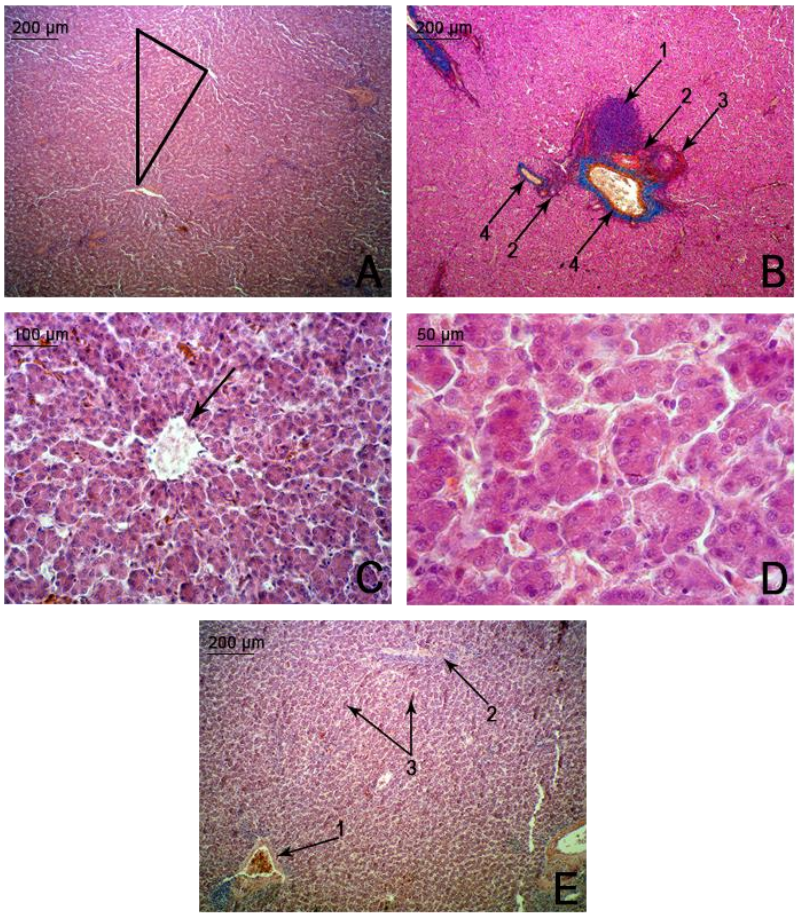

Figure 2: Microscopic structure of the experimental group. A – portal liver acinus; B - the triad liver system with lymphoid follicle (1 – lymphoid follicle, 2 – artery, 3 – biliary duct, 4- vein); C – central vein of liver acinus; D – hepatocytes without pathological lesions; E – a lobe with moderate hepatic vascularization of capillaries (sinusoids) (1 – the vein triad of the liver, 2 – central vein, 3- capillaries). A, C, D, E - H & E staining.. B – Mallory staining.

Study of Süheyla Gonca et al. about histopathological effects of cholesterol and protective effect of concurrent application of vitamin E and selenium on the structure of rat liver indicates that application of cholesterol causes an inflammatory infiltration of the liver, fibrosis and proliferation of bile ducts, while the use of vitamin E and selenium improved the histological and functional state of the liver of experimental animals: minimal hepatocellular damage was observed in the group using selenium and vitamin E. Süheyla Gonca's research has partly confirmed our results [36].

The conducted comparative histological research of the liver has showed that the intensity of the beam structure of the lobules and moderate hyperemia of vessels was observed in experimental and control groups of chickens. The main distinguishing feature was that chickens in the control group had the activation of lymphoid follicles in the liver. Proliferative cholangitis was expressed and these processes were not observed in the experimental chickens. In most cases, moderate hyperemia of vessels as capillary bed and vessels in the triad area. Also, in the experimental group of chickens the structure of liver is clearly expressed, the boundaries between liver cells are preserved, hepatic tubules can be easily seen, the nucleus of liver cells are of equal size, indicating that the functional activity of the liver under the influence of the specimens.

CONCLUSION

Comparative morphological characteristics of chickens liver in the control group showed that they had grain proteinosis and Fatty Liver Syndrom, which was not observed in the liver of experimental chicken group.

Thus, the morphological study of chicken liver in test and control groups has showed that in the group which was given a complex selenium-containing feed supplement "Extraselen-Vit» the processes of

physiological functioning of the body were found. Besides the feed supplement carries preventive action during fat and protein dystrophy appearance, which proves the absence of cytotoxic action of vitamin mineral complex on the bird's body.

The resulting effect of the complex feed supplement confirms one of the basic properties of selenium - its essentiality: it improves liver function by increasing the synthesis of primary bile acids, increasing bile acid conjugation with taurin, activating the cholesterol secretion. The effect of the specimen due to its complex composition of vitamins reduces toxicity and enhances positive effects of selenium. This makes it possible to carry out a targeted correction of postnatal morphogenesis of the chickens' liver - broilers and may be a significant substitute for chemicals which are foreign to the body.

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