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**STRENGTHENING HEALTH AND BIOPRODUCTIVITY OF
LAYING HENS AS A RESULT OF USING THE
AUTOCHTONOUS PRODUCT ZOOBIOR**

**Scientific specialty: 431.01 Veterinary therapeutics, pharmacology and
toxicology**

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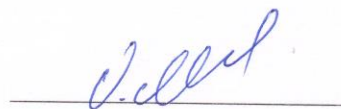
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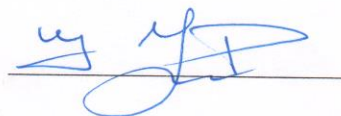


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RESEARCH CONCEPTUAL FRAMEWORKS

Relevance of the investigated topic

Industrial poultry farming is one of the main branches of the agri-food sector which is under constant attention of specialists and scientists, due to its substantial contribution to supplying the population with food of high biological value as well as to ensuring food security for the country (Baltag 2020, p. 137-189; Macari ş. a. 2014; Putin, Macari şi Rotaru 2020, p. 6-8; Кальницкая и Уша 2010; Мартынова и Корниенко 2020).

The chicken egg production, in particular, as a sector of the agri-food complex is of essential significance in ensuring the country's and the population's food security with complete animal protein. Eggs, without any religious or cultural barriers to consumption, are the most promising source of animal protein, highly assimilated by the organism (93-97 %), and from a functional perspective, eggs have a moderate calorie content (around 150 kcal/100 g), also representing an excellent source of animal protein (6 g per egg), being relatively rich in fat-soluble compounds (Мартынова., Корниенко., 2020; Marco. şi Moal., 2019).

Research field situation description and research issues identification

Although poultry farming is considered an essential branch of the livestock sector, this priority can currently only be achieved by applying intensive rearing and exploitation methods. The main aim is to maximize production, which inevitably leads to higher burden on the animal organism. Animal rearing and farming intensification has many advantages, but also some negative consequences, a major problem being the impact of technological stress, including oxidative stress, nutritional stress, etc., on health, productivity and, ultimately, on the quality of animal products (Voiniţchi ş a. 2018; Macari A 2015; Macari V ş.a., 2014).

As an important part of the human diet eggs must be safe and harmless. This is a very important imperative, the appropriateness of which is confirmed both by general knowledge and by the literature, which shows the consequences that may occur if growth promoters, which until recently were accepted for such purposes, are administered to animals. For poultry, the problem is extremely pressing, both in terms of the rapid digestibility and metabolization of feed by their bodies and in terms of eggs being used as food by the human body.

In the light of the above, in order to replace chemical growth stimulants with frequent side effects, it is worth introducing into the poultry technological cycles natural products which have beneficial properties: adaptive, biostimulatory, anti-stress and hepatoprotective, with the capacity to fortify the health and bioproductivity of laying hens.

Research aims and objectives

Research aim was to clarify the nature of local product ZooBioR's action on laying hens in order to improve their health and strengthen their bioproductive potential, as well as on their physiological-metabolic status (blood, blood serum, muscle, liver, eggs), and to develop the process of strengthening the health and bioproductive potential of these birds.

Research objectives: (1) Tolerance and clinical hematologic status assessment in laying hens receiving ZooBioR supplemented feed; (2) Analysis of evolutionary changes in protein, carbohydrate, lipid and mineral metabolism during the early laying period in hens receiving ZooBioR-supplemented feed; (3) Evaluation of the trypsin-antitrypsin system profile during the early laying period in hens receiving ZooBioR-supplemented feed; (4) Analysis of evolving changes in the liver's functional state during the early laying period in hens receiving ZooBioR-supplemented feed; (5) Determination of changes in liver and muscle tissue markers in young laying hens treated with ZooBioR; (6) Estimation of bioproductive performance of hens supplemented with ZooBioR and feed conversion per 1 kg egg mass and per 10 eggs; (7) Determination of morphometric changes in eggs from hens under the study; (8) Optimal strategy selection for ZooBioR product use, determination of economic efficacy and egg production efficiency index when implementing this product in laying hens.

Research hypothesis. The local product ZooBioR obtained from cyanobacterium *Arthrospira platensis* (spirulina) has the ability to fortify the health and well-being of laying hens, their physiological-metabolic status, their productive capacity in poultry under controlled conditions of modern poultry farms, where the negative impact of technological stress is inevitable. This beneficial capacity is due to presence of several bioactive components in this product, such as essential amino acids, sulphated polysaccharides, phospholipids and the microelements zinc and selenium. The product formula ensures a very high bioavailability for the animal body and facilitates the maintenance of healthy, viable and resistant chickens under the conditions of the intensive rearing and farming system.

Scientific research methodology. Two clinical experimental studies were conducted in accordance with the scientific criteria and ethical principles applied in research. Research location: Department of Food Safety and Public Health, Faculty of Veterinary Medicine of TUM, zootechnical unit „*Acustic Tehnologie*”, Floreni village, Anenii Noi, Biochemistry Laboratory of the USMF "Nicolae Testemitanu". The investigations were carried out on young laying hens of the Braun-Nic hybrid, exploited under industrial conditions in a poultry farm. The research methods applied were general (inspection of the birds, discussions with the keepers, clinical examination of the hens) and specific (general blood tests, hemoleukogram parameters, biochemical determination of blood serum, liver and muscle tissue). Egg morphometry was

performed. Specific methods were conducted on blood samples, blood serum, liver and muscle tissue homogenates, and allowed to assess the clinical-hematological status, protein, carbohydrate, lipid, mineral metabolism markers, trypsin-anthritripsin system state, as well as to evaluate some quantitative and qualitative parameters of eggs.

Scientific novelty and originality. For the first time, within two complex studies, one scientific-practical and one for implementation, the tolerance level of laying hens to the local biological active product of natural origin ZooBioR was determined, the action nature of this product on some clinical-hematological status and biochemical indices in the following biological substrates: serum, liver tissue and muscle, egg production and morphometric indices of eggs were determined, as well as the optimal dose of administration of this product to laying hens was outlined. An innovative procedure for strengthening the health and productive potential of laying hens and improving the quality of eggs was developed and proposed for implementation.

Scientific problem addressed in the work consists in the scientific justification of using the local product ZooBioR in laying hens by establishing its action on health and bioproductive potential, physiological-metabolic status (blood, blood serum, muscle, liver, eggs), which has stimulated the optimal dose determination for administration of the researched product and the development of the process for strengthening the health and bioproductive potential of poultry.

Main results submitted for defence

1. New scientific-practical data on the effects of the local biological active product ZooBioR from *Arthrospira platensis* (spirulina) on laying hens, exploited under intensive poultry farm conditions;
2. The impact of ZooBioR on the clinical and hematologic status of laying hens kept under physiological conditions in poultry farms;
3. Effects of ZooBioR on the functional state of the liver and on the protein, carbohydrate, lipid and mineral metabolism, as well as on the trypsin-antitrypsin system in laying hens kept in poultry farm conditions;
4. ZooBioR properties as an adaptogen, anti-stress, health fortifier and stimulator of productive potential in laying hens;
5. Advanced metabolic processes in biological substrates (muscle, liver) in laying hens and those treated with ZooBioR.

Work approval at national and international scientific fora. The thesis materials were presented at various national and international scientific forums: Conferința științifică națională, consacrată jubileului de 95 ani din ziua nașterii academicianului Boris Melnic, Chișinău, R. Moldova (2023); Conferința științifică națională cu participare internațională „Integrare prin cercetare și inovare”, Chișinău, R. Moldova (2022); 5th International Scientific Conference on

Microbial Biotechnology, Chişinău, R. Moldova (2022); International, scientific and practical conference „Azerbaijan at a new stage of development - Food and nutrition security in the period of globalalization and post-pandemic period: modern situation, challenges, and prospectives”, Baku-Lankaran, Azerbaijan (2021); National Scientific Symposium with International Participation „Modern biotechnologies – Solutions to the challenges of the contemporary world”, Chisinau, R. Moldova (2021); 10th International Conference of Zoologists Sustainable Use and Protection of Animal World in the Context of Climate Change, dedicated to the 75th anniversary from the creation of the first research subdivisions and the 60th anniversary from the foundation of the Institute of Zoologists (2021); Международная Научно-Практическая Конференция, Посвященная 30-летию Комратского Государственного Университета, Комрат, Р. Молдова (2021); The Conference for Veterinary Medicine organised by Faculty of Veterinary Medicine the International Scientific Congress „Life Sciences, a Challenge for the Future”, Iaşi, România (2020).

Publications on the thesis topic. The main results of the thesis have been published in **15** scientific papers, among which: **2** in journals in international databases (one of which with IF 0,442), **1** article in a journal of the National Register of journals, category B, **5** articles in collections of international conferences (abroad and in Moldova) and national with international participation, **7** theses of scientific communications presented at national and international forums.

Work volume and structure: introduction, 6 (six) chapters, general conclusions and recommendations, 137 pages of basic text, bibliography of 230 titles, 33 tables and 10 figures. The results are published in 15 scientific papers.

Keywords: Egg-laying hens, ZooBioR product, clinical-hematologic status, protein, carbohydrate, lipid and mineral metabolism, liver, trypsin-antitrypsin system, productive indexes.

CHAPTER SUMMARIES

The **Introduction** describes the relevance of studying the impact of the local biological active product ZooBioR on the physiological and metabolic status and bioproductive potential of laying hens, highlighting the importance of using innovative CBA of natural origin for the health and welfare of birds, as well as for the harmlessness of the products obtained from them. The aim, objectives and methodology of the research are specified, the summary of the thesis compartments is presented, as well as the approval of the scientific results.

In chapter 1, **General considerations on the impact of growth promoters on the animal organism. Strengthening the health, physiological-metabolic status and productive potential of birds with biologically active remedies**, the present state of knowledge on the action of biologically active compounds (BACs) on animals is related. The main categories of growth promoters applied to animals, their impact on the physiological and metabolic status, productive

potential are catalogued, highlighting as priority such properties as: adaptive, anti-stress and bioproductive, as well as harmlessness and quality of the products obtained after the use of biologically active remedies of plant origin in animals.

In chapter 2, **Research material and methods**, the stages and methods applied in the 2 studies (series) carried out in the zootechnical unit are described - „Acustic Tehnologie” LTD Floreni village, Anenii Noi district. The researches of the first series were carried out on 70 hens of the Braun-Nic hybrid, divided into 5 batches of 14 birds each, according to Table 1.

Table 1. Scheme of ZooBioR administration to laying hens in the 1st series

| Batches of birds | Number of birds | Administration pathway | Dose, mg active substance/kg feed | Administration Scheme |
|------------------|-----------------|------------------------|-----------------------------------|-----------------------|
| Control | 14 | - | - | |
| Experimental 1 | 14 | per os with food | 5,0 | daily |
| Experimental 2 | 14 | | 10,0 | |
| Experimental 3 | 14 | | 15,0 | |
| Experimental 4 | 14 | | 20,0 | |

During the experiment, the birds were examined and body temperature and respiratory rate were determined for 5 hens in each batch. For laboratory investigations, blood samples were taken at three stages: at the beginning of the experiment from 5 hens at random; about 1 month after the experiment began and at the end of the experiment from 5 birds in each batch. In the second series, which had a scientific-practical implementation, the action of the dose of 10 mg active substance/kg ZooBioR feed, identified as optimal in the first series, was verified (Table 2).

Table 2. Administration scheme of ZooBioR to laying hens at the optimal dose

| Batches of birds | Number of birds | Administration pathway | Dose, mg active substance/kg feed | Administration Scheme |
|------------------|-----------------|------------------------|-----------------------------------|-----------------------|
| Control | 56 | - | - | - |
| Experimental 1 | 56 | per os with food | 10,0 | daily |

To assess the health status, the birds were examined, and body temperature and respiratory movements in one minute were determined for 5 hens in each batch. To determine the hematological and biochemical status, blood samples were taken in three stages: at the beginning of the experiment from 5 hens at random; during the study from 5 birds from each batch - about 1 month after the beginning of the study, and then at the end of the study.

At all research stages, blood samples from hens were collected from the axial and jugular veins, while respecting antisepsis and asepsis requirements. *The hematological markers* determined (by means of Myndrey 500 hematological analyzer) in experimental series I and II were: hemoglobin concentration - HgB; erythrocyte count - RBC; hematocrit - HCT; mean erythrocyte volume - MCV; mean erythrocyte hemoglobin - MCH; mean erythrocyte hemoglobin

concentration - MCHC; leukocyte count - WBC; platelets. The components of the leukocyte formula: non-segmented neutrophils, segmented neutrophils, eosinophils, basophils, lymphocytes, monocytes were determined by microscopic examination of the blood smear stained according to Romanovschi.

In order to determine the hens' metabolic health and to elucidate the action of the ZooBioR product on this state, marker indices (Autohumalyzer 900 S-Human biochemical analyzer) of protein and nitrogen metabolism (total protein, albumin, urea, uric acid, creatinine) were quantified in the blood serum; Carbohydrate metabolism (glucose); lipid metabolism (total lipids; cholesterol (Ch), triglycerides (TG), β -lipoproteins; mineral metabolism: (Ca, P, Ca/P ratio and Fe, as well as indices with impact on mineral metabolism (ceruloplasmin (CP), transferrin); liver samples (ALT and AST transaminases, alkaline phosphatase (ALP) and its isoenzymes, pseudocholinesterase (PCE), total bilirubin and its fractions); trypsin-antitrypsin system (trypsin, α 1-antitrypsin and α 2-macroglobulins).

To assess the *liver functional state*, in the liver tissue were determined total proteins; albumins; alkaline phosphatase - ALP; alanine aminotransferase - ALT; aspartate aminotransferase - AST; gluco-6-phosphate dehydrogenase - G-6-PDH; lactate dehydrogenase - LDH; pseudocholinesterase - PCE; gamma glutamyltransferase - G-GTP.

In both studies, for *egg production* complex investigation, the following were determined: numerical egg production, laying intensity, taking into account: first day of research; first (1) phase of investigation: start - 10 days of experiment; in study I, the whole period of investigation: start - 128th day of study; in study II, different periods, as well as the whole experimental period.

The productive marker parameters investigated in birds were: average hen weight at the beginning of the study; during the study and at the end of the study; total gain/period segment/hen; total gain/experimental period/hen; average daily gain/period segment, g; numerical egg production and laying intensity/first and last day/study; laying intensity/period segment/study; laying intensity period/study; viability (integrity) of hens; daily number of eggs; egg mass, individual weighing; percentage differences. Morphometric parameters of eggs were determined 3 consecutive times: at the end of the laying period; 2 weeks and about 4 weeks after egg collection, respectively, determining egg weight; large and small egg diameter; yolk width and height; albumen height; albumen weight; shell weight; shell thickness at the tip, middle and round end.

The obtained experimental results were statistically processed by calculating the parameters of the variate series, arithmetic mean (M), mean error (m). Statistical evaluation of the biochemical markers was carried out using the parametric criterion t-student with accepted veracity - less than 0.05, using Microsoft Office programs (Microsoft Excel).

In chapter 3, **ZooBioR product influence on clinical-hematologic status on intermediate metabolism in hens during the early laying phase**, the clinical and hematologic markers were assessed, proving the impact of tested remedy ZooBioR on them. During 2 periods of monitoring and examination - about 4 and about 8 months - no deviations from the normal physiological state of the birds involved in the research, as well as no pathologies or disorders of the gastrointestinal tract were recorded during the administration of the tested product. During the research, while fixating, determination of body temperature, respiratory rate, body mass, etc., the birds were calmer than the ones in the control group.

Table 3. Dynamics of clinical values in laying hens treated with ZooBioR (M±m)

| Markers | Bird flocks | | | | |
|--------------------------------|-------------|-------------|--------------|--------------|-------------|
| | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| Body temperature, °C | | | | | |
| Study beginning | 40,98±0,19 | 41,04±0,28 | 40,94±0,19 | 41,00±0,24 | 40,98±0,30 |
| 1st research | 41,12±0,12 | 41,08±0,19 | 41,02±0,17 | 40,96±0,08 | 40,82±0,14 |
| 2nd research | 41,22±0,11 | 41,02±0,09 | 40,96±0,23 | 41,00±0,06 | 41,16±0,10 |
| 3rd research | 41,56±0,06 | 41,38±0,18 | 41,30±0,15 | 41,50±0,16 | 41,46±0,17 |
| Respiration rate, moves/minute | | | | | |
| Study beginning | 30,00±2,12 | 31,20±2,51 | 30,00±2,12 | 31,20±2,51 | 30,00±2,12 |
| 1st research | 26,40±1,64 | 21,60±1,64 | 21,60±1,64 | 19,20±1,34** | 22,80±1,34 |
| 2nd research | 24,00±2,12 | 18,00±2,12 | 18,00±2,12 | 16,80±1,34* | 18,00±2,12 |
| 3rd research | 26,40±1,64 | 20,40±1,87* | 19,20±1,34** | 20,40±1,87* | 20,40±1,87* |

Note: study debut - on 05.08.2019; 1st survey - on 31.08.2019; 2nd survey - on 5.10.2019; 3rd survey (end of study) - on 1.12.2019.

Body temperature was uniform at the beginning of the study, and by the 1st study showed a slight increasing tendency in CF, EF 1 and 2 and a decreasing tendency in EF 3 and 4. By the 2nd investigation this tendency was maintained at EF, EF 3 and 4, while at EF 1 and 2 (low doses of ZooBioR) this index decreased slightly. Towards the end of the study, body temperature decreased by 0.18-0.26 °C for the low doses (EF 1 and 2), while the higher doses induced a difference of only 0.06-0.10 °C compared to EF (Table 3).

In the first research series, erythrocytes (RBC) in chicken blood were 1.3 times ($p<0.001$) lower ($p<0.001$) in CF at the first experimental stage, a tendency that persisted in EF, with RBC being slightly lower (up to 4.8%) than in CF. At the last research stage, absolute erythrocyte values were up to 8.0% lower in EF compared to CF (Table 4). A similar pattern was characteristic for HB in chicken blood during the study. The mean cell volume (MCV) at the 1st investigation term was lower in all EF compared to CF, where MCV was 12.9% lower compared to background ($p<0.001$). At the end of the investigation, MCV in EF 2 and 3 decreased slightly compared to CF, and only in EF 4 (maximum dose of ZooBioR) MCV values increased slightly compared to control and the other three EFs. Mean erythrocyte hemoglobin (MCH) at the 1st investigation showed an increase. In CF the increase of this index was 11.4% compared to the background ($p<0.01$). In EF, MCH was 1.0-

5.1% higher than in CF, and at the end of the study the investigated index was at the same level in all groups.

Table 4. Hematological parameters evolution in laying hens under the influence of ZooBioR

| Markers | Study debut | Bird flocks | | | | |
|-------------------------------|-------------|----------------|-------------|-------------|-------------|--------------|
| | | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| RBC, 10 ¹² /l | 3,20±0,09 | | | | | |
| 1st sampling | | 2,48±0,09*** | 2,44±0,04 | 2,45±0,13 | 2,41±0,05 | 2,36±0,05 |
| 2nd sampling | | 2,88±0,15 | 2,74±0,09 | 2,80±0,09 | 2,65±0,08 | 2,83±0,05 |
| HB, g/l | 114,60±1,68 | | | | | |
| 1st sampling | | 98,60±1,89*** | 98,20±3,60 | 100,60±5,07 | 100,00±3,12 | 95,20±2,49 |
| 2nd sampling | | 99,00±3,22 | 100,00±3,48 | 102,60±4,66 | 94,40±2,37 | 103,60±2,49 |
| HCT, % | 44,82±0,68 | | | | | |
| 1st sampling | | 29,96±0,90*** | 29,40±1,09 | 29,92±2,00 | 29,06±0,75 | 28,34±0,38 |
| 2nd sampling | | 30,28±1,25 | 29,76±0,88 | 30,34±0,99 | 29,12±0,97 | 31,42±0,96 |
| MCV, fl | 140,12±2,60 | | | | | |
| 1st sampling | | 122,02±1,67*** | 120,36±2,02 | 122,94±3,21 | 121,32±2,76 | 120,58±2,19 |
| 2nd sampling | | 124,04±0,86 | 123,24±1,81 | 120,64±2,24 | 121,84±2,85 | 126,18±1,03* |
| MCH, pg | 35,82±0,59 | | | | | |
| 1st sampling | | 39,92±0,78** | 40,30±0,77 | 41,10±0,77 | 41,94±1,52 | 40,44±0,47 |
| 2nd sampling | | 40,58±0,82 | 40,38±0,70 | 40,70±0,78 | 39,52±1,00 | 41,08±0,75 |
| MCHC, g/dl | 25,52±0,06 | | | | | |
| 1st sampling | | 32,92±0,33*** | 33,44±0,43 | 33,44±0,46 | 36,02±2,22 | 35,06±1,91 |
| 2nd sampling | | 32,70±0,50 | 32,78±0,76 | 33,70±0,48 | 32,42±0,44 | 32,52±0,28 |
| Platelets, 10 ⁹ /l | 43,00±8,61 | | | | | |
| 1st sampling | | 27,00±4,39 | 16,00±1,17* | 20,20±4,38 | 20,00±1,97 | 14,20±1,47* |
| 2nd sampling | | 45,20±5,02* | 49,00±6,54 | 42,20±3,23 | 51,80±6,51 | 43,40±2,46 |

Note: * – p<0,05; *** – p<0,001.

The average MCHC value at the first sampling was 29.0% higher in CF compared to the debut study (p<0.001). In EF birds, MCHC values were up to 9.4% higher compared to the debut study, a result that can be attributed to the beneficial action of ZooBioR. Platelets at the 1st stage of the study were 1.6 times lower in CF compared to the debut study. In EF they were 1.4-1.9 times lower than in CF, which can be categorized as a physiological characteristic.

Leukocyte (WBC) evolution at the first investigation in all flocks shows a normal bird health state, as well as a different level of involvement of the test product in this physiological process, depending on the dose used. At the end of the study for WBC in EF 1 and 2 (low doses of ZooBioR) an increase of 2.7-9.2% was characteristic, while in EF 3 and 4, the opposite - a decrease of 7.6-7.9% compared to CF. For the lymphocyte levels in blood, in general the same pattern as for leukocytes was observed (Table 5).

Table 5. Evolution of leukocytes and basic components of the leukocyte formula in laying hens treated with ZooBioR (M±m)

| Markers | Study debut | Bird flocks | | | | |
|-------------------------|-------------|--------------|-------------|------------|------------|--------------|
| | | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| WBC, 10 ⁹ /l | 12,58±1,03 | | | | | |
| 1st sampling | | 17,10±0,59** | 16,78±0,69 | 16,18±0,90 | 16,72±0,58 | 19,80±0,48** |
| 2nd sampling | | 14,18±0,85* | 15,48±0,86 | 14,56±0,79 | 13,06±0,44 | 13,10±0,48 |
| Lymphocytes, % | 35,80±2,77 | | | | | |
| 1st sampling | | 55,40±4,64** | 52,60±3,98 | 52,00±3,32 | 53,60±1,99 | 50,00±3,22 |
| 2nd sampling | | 42,80±8,00 | 39,40±10,92 | 34,40±0,84 | 43,20±6,23 | 37,40±3,11 |
| Granulocytes, % | 50,00±3,24 | | | | | |
| 1st sampling | | 31,20±4,34** | 33,20±5,14 | 32,80±3,34 | 32,20±2,68 | 35,20±3,78 |
| 2nd sampling | | 43,20±6,55 | 44,00±10,36 | 45,60±7,26 | 41,60±6,14 | 47,20±3,56 |
| Eosinophils, % | 0,80±0,10 | | | | | |
| 1st sampling | | 0,80±0,10 | 0,60±0,27 | 0,80±0,10 | 0,60±0,27 | 0,60±0,27 |
| 2nd sampling | | 0,20±0,20 | 0,00±0,00 | 0,20±0,20 | 0,40±0,40 | 0,20±0,20 |
| Monocytes, % | 13,40±2,20 | | | | | |
| 1st sampling | | 12,60±1,35 | 13,60±1,35 | 14,40±1,89 | 13,60±0,96 | 14,80±0,92 |
| 2nd sampling | | 13,80±3,31 | 16,60±3,70 | 12,60±2,33 | 14,80±2,81 | 15,60±1,82 |

Note: * - p<0,05; ** - p<0,01.

At the 2nd sampling, the lymphocytosis was characterized by a marked decrease: in CF it was 22.7%, and in EF 1, EF 2 and EF 4 - 7.9-19.6% compared to the debut study. A diametrically opposite evolution regarding WBC and lymphocytes was characteristic for granulocytes. In the first study, this index was lower in CF, by 37.6% compared to the debut study (p<0.05). In EF the granulocyte level was up to 12.8% higher than in CF, a beneficial effect of the tested product ZooBioR. Eosinophils - without palpable differences between experimental groups. The relative number of monocytes in the CF decreased insignificantly compared to the debut study, and in the EF under the action of the tested product this index did not decrease, but was up to 17.5% higher than in the CF, a persistent manifestation also at the end of the study in EF 1, 3 and 4, and only in EF 2 monocytes were 8.7% lower than in the CF.

During scientific and practical implementation study, RBCs showed an insignificant decrease. At the first stage of the study MCV was higher in EF compared to CF, and at the end of the study it decreased by about 6% compared to the control, which was also observed for mean erythrocyte hemoglobin (MCH). An increase with aging of the birds was also characteristic for MCHC, with slightly decreased levels in EF compared to CF, proving the harmlessness of ZooBioR. Leukocytes at the first stage of the study did not differ between flocks, an increase of 17.1% compared to CF being established at the end of the study.

Protein metabolism markers are of particular significance in the evaluation of processes in the animal body, including testing of biologically active remedies in poultry (Putin, Macari și

Rotaru 2020, p. 48-56; Pavlicenco 2019; Voinițchi ș. a. 2018; Khazaei et al. 202; Macari V ș. a. 2021).

Total protein in hens in all flocks without large fluctuations, with a decrease of 1.4% in the first study compared to the CF (Table 6).

Table 6. Mean values of protein metabolism indices in blood serum of young laying hens treated with ZooBioR

| Markers | Study debut | Bird flocks | | | | |
|--------------------|-------------|--------------|--------------|--------------|--------------|---------------|
| | | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| Total protein, g/l | | | | | | |
| 1st sampling | 67,04± | 66,11±1,11 | 65,82±1,42 | 67,54±0,88 | 66,25±1,21 | 67,69±1,31 |
| 2nd sampling | 1,64 | 66,11±0,61 | 66,83±1,09 | 65,53±1,09 | 67,62±2,09 | 65,46±1,07 |
| Albumins, g/l | | | | | | |
| 1st sampling | 22,59± | 24,91±0,70 | 23,78±0,44 | 25,17±0,61 | 23,72±0,57 | 23,84±0,76 |
| 2nd sampling | 0,80 | 26,28±1,14 | 25,06±0,70 | 25,35±0,88 | 24,97±0,84 | 25,00±0,72 |
| Urea, mM/l | | | | | | |
| 1st sampling | 7,74± | 7,64±0,06 | 7,71±0,09 | 7,76±0,12 | 7,65±0,05 | 7,75±0,05 |
| 2nd sampling | 0,15 | 8,46±0,20** | 8,85±0,11 | 9,14±0,43 | 8,88±0,11 | 8,71±0,14 |
| Uric acid, μmol/l | | | | | | |
| 1st sampling | 339,61± | 335,03±17,68 | 331,37±12,28 | 320,38±8,25 | 316,72±15,47 | 311,23±8,56 |
| 2nd sampling | 20,0 | 358,83±30,34 | 455,86±29,11 | 476,92±43,37 | 527,26±52,8* | 470,51±26,06* |
| Creatinine, μmol/l | | | | | | |
| 1st sampling | 191,91± | 218,92±11,8 | 219,85±12,69 | 214,73±22,65 | 196,56±5,91 | 222,18 ±6,97 |
| 2nd sampling | 13,4 | 218,10±15,7 | 211,93±18,65 | 224,05±26,20 | 260,38±31,97 | 239,88±20,94 |

Note: * – $p < 0,05$.

The albumin content at the first monitoring stage increased by 10.3% in CF and by 5.0-11.4% in EF compared to the debut study ($p < 0.05$, for LE 2). At the last experimental stage albuminemia in EF birds decreased by 3.5-5.0% compared to CF, the explanation is that the decrease in serum albumin occurs due to its intensive use for the supply of plastic material for the processes in the animal body. The uremia at the first stage of research was characterized by uniformity, which also attests the harmlessness of the tested product on birds, especially at the protein metabolism level, as well as at the liver level, indicating a good health status of the hens.

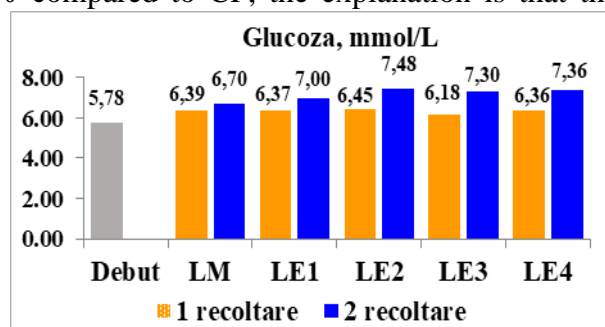


Figure 1. Dynamics of average glucose values in laying hens treated with ZooBioR

At the end of the study, the urea content of the CF evolved positively compared to the first study ($p < 0.01$), an increase in this index being also characteristic for the hens treated with ZooBioR. In the first study, uric acid levels in EF were up to 7.1% lower than in CF. At the end of the study, in hens from all EF, this index was 27.0-46.9% higher than in CF ($p < 0.05$ for LE 3 and 4). In the EF, creatinine was 15.8% higher compared to the debut study. ZooBioR administration

beneficially favored the serum creatinine value in EF 2, 3 and 4 at the end of the experiment, when the investigated index was higher than CF by up to 19.4%.

At the 1st investigation, glucose levels increased compared to the debut by 10.6% ($p < 0.05$) in CF and by 6.9-11.6% without reaching statistical significance in EF. Towards the end of the study, serum glucose levels in CF birds increased by 15.9% ($p < 0.05$), an increase also reported in all EF. The more pronounced glucose amplification in EF could be explained as a result of intensified physiological-metabolic processes in birds' bodies, which were reflected in higher values of the respective index in EF than in CF, the statistically significant increase, constituting 4.5-11.6% (Figure 1). Such a manifestation of glucose - by increasing its level in the blood serum - was established also when administering other CBAs to animals, being categorized as beneficial (Balanescu ş. a. 2014; Balanescu ş. a. 2019; Szaboova et al. 2012; Pavlicenco 2019; Мартынова и Корниенко 2019). In the scientific-practical implementation study, the protein content increased with aging. Thus, towards the end of the study, a progressive 1.4- ($p < 0.05$) fold increase in proteinemia occurred in CF birds, confirming the previous increase in this index in experimental birds. In EF birds, the protein content in the counter decreased by 15.3%. The serum creatinine during the study showed an increase at the 1st time point, more pronounced in EF (14.3%) than in **phon**. At the last experimental stage, creatinine values were higher in EF, by about 8% compared to CF, positive results which are a reflection of the body's muscle mass.

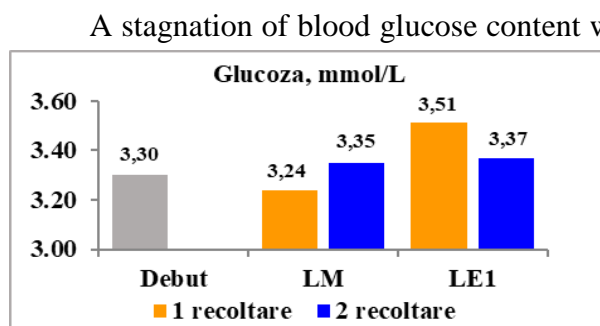


Figure 2. Dynamics of average glucose values in hens treated with the optimal dose of ZooBioR

in birds to meet energy requirements under the action of CBA (Putin, Macari și Rotaru 2020, p. 52-56; Khazaei et al. 2021). Marker indices of lipid metabolism in blood serum are also investigated to assess the effects of CBA on the animal body (Putin, Macari și Rotaru 2020, p. 52-56; 59-63; Khazaei et al. 2021). ZooBioR administered with the young hens' feed exerted a positive action on lipid metabolism, translated by up to 26.9% lower lipidemia values in EF compared to CF values, with statistically significant changes for EF 3 and 4 (Table 7). At the end of the study the serum lipid level in CF was insignificantly decreased, under the action of the tested product the serum lipid lowering level, compared to CF, constituted 2.0-26.3% ($p < 0.01$, for LE 1), confirming the CBA role in lipid metabolism normalization in livestock [Macari V. ş.a., 2014; 2021; Pavlicenco N., 2019; Putin, V., Macari, V., Rotaru, A., 2020, p. 64-65].

Table 7. Some lipid metabolism parameters in young laying hens treated with ZooBioR (M±m)

| Markers | Study debut | Bird flocks | | | | |
|----------------------|-------------|-------------|-------------|------------|--------------|------------|
| | | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| Total lipids, g/l | 3,73±0,19 | | | | | |
| 1st sampling | | 6,02±0,4*** | 5,17±0,28 | 5,67±0,55 | 4,63±0,15** | 4,40±0,38* |
| 2nd sampling | | 5,90±0,34 | 4,35±0,3** | 5,78±0,69 | 5,22±0,25 | 5,13±0,46 |
| Cholesterol, mmol/l | 4,28±0,08 | | | | | |
| 1st sampling | | 4,39±0,11 | 4,39±0,09 | 4,41±0,09 | 4,25±0,13 | 4,21±0,04 |
| 2nd sampling | | 4,53±0,10 | 4,80±0,14 | 5,17±0,20* | 5,44±0,28* | 5,14±0,18* |
| Triglyceride, mmol/l | 1,90±0,05 | | | | | |
| 1st sampling | | 2,15±0,05** | 2,03±0,07 | 2,01±0,06 | 1,82±0,04*** | 1,99±0,09 |
| 2nd sampling | | 2,23±0,17 | 2,69±0,05* | 2,66±0,20 | 2,88±0,09** | 2,70±0,25 |
| β-lipoprotein, u/c | 73,96±4,96 | | | | | |
| 1st sampling | | 101,6±2,9** | 88,68±6,25 | 82,94±7,25 | 109,42±8,52 | 84,54±7,62 |
| 2nd sampling | | 105,14±7,17 | 91,76±10,46 | 94,90±4,38 | 103,08±4,51 | 101,56±4,8 |

Note: * - p<0,05; ** p<0,01; *** - p<0,001.

Serum cholesterol at the 1st sampling was slightly increased in both CF and EF 1 and 2, while in EF 3 and 4, on the opposite, it was slightly decreased. At the 2nd sampling the cholesterol at EF was higher (6.0-20.1%) compared to CF, a characteristic manifestation with CBA administration (Pavlicenco 2019). Triglyceride content at the 1st sampling was higher (by 13.2%) compared to the CF group (p<0.01), a characteristic manifestation also for three experimental groups (EF 1, 2 and 4). Compared to CF, in EF birds, the monitored index recorded lower levels (by 5.6-15.3%), reaching statistical significance in EF 3 (p<0.001). Towards the end of the experimental series, triglyceride levels in bloodstream of birds in all EF increased by 19.3-29.2% compared to CF, with significant differences (Macari V. et al., 2021). There was an increase in β-lipoproteins in blood, in both CF and EF, with statistical significance between flocks. Administration of ZooBioR in three EF (EF 1, 2 and 4) reduced the increased parameter, by 12.1-19.9% compared to the debut. At the end of the study the β-lipoprotein levels decreased insignificantly in CF, and in EF also decreased, not reaching the serum level of CF, without statistical significance.

In the scientific-practical study, towards the first monitoring stage, there was a 21.6% decrease in blood serum lipids in CF hens (p<0.01) and a 25.0% decrease in EF hens (p<0.001) compared to the debut. With aging of the hens - towards the end of the study, the respective index in CF increased 1.5-times (p<0.001) and 1.4-times (p<0.01) compared to the first study (Table 8).

Table 8. Some lipid metabolism parameter values in laying hens treated with ZooBioR in the implementation study (M±m)

| Markers | Study debut | Bird flocks | |
|----------------------|-------------|--------------|-------------|
| | | Control | EF 1 |
| Total lipids, g/l | 7,87±0,36 | | |
| 1st sampling | | 6,17±0,11** | 5,90±0,11 |
| 2nd sampling | | 9,31±0,57*** | 8,02±0,61** |
| Cholesterol, mmol/l | 4,88±0,14 | | |
| 1st sampling | | 4,70±0,07 | 4,52±0,14 |
| 2nd sampling | | 4,75±0,06 | 4,58±0,11 |
| Triglyceride, mmol/l | 2,01±0,03 | | |
| 1st sampling | | 1,94±0,03 | 1,91±0,05 |
| 2nd sampling | | 2,03±0,02* | 1,97±0,03 |
| β-lipoproteins, u/c | 24,34±5,54 | | |
| 1st sampling | | 30,41±3,87 | 22,89±2,91 |
| 2nd sampling | | 26,74±2,59 | 15,05±1,99 |

Note: * – p<0,05; ** p<0,01; *** – p<0,001.

Cholesterolemia at the first investigation showed a decrease in CF, a characteristic tendency also for EF, where towards the end of the study, the level of this index decreased slightly compared to the control. The same pattern was characteristic for triglycerides, and towards the end of the study their level in the blood increased slightly, both in CF (by 4.6%, p<0.05) and in EF (+3.1%). A decrease in β-lipoproteins by about 25% compared to CF was recorded at the 1st sampling, which towards the end of the study decreased by 1.8-times in EF (p<0.01). A decrease in β-lipoprotein levels was also reported in broilers, pigs under the action of BioR remedy (Macari 2003; Putin, Macari and Rotaru, 2020, p. 59-68), It was noted that ZooBioR tested on chickens beneficially influences Ca metabolism. The decrease in P may probably be due to its more intense utilization for the physiological and metabolic processes that take place in the hens' bodies, especially in egg formation, and particularly in shell formation. The Ca/P ratio fully reflects the levels of some indices of mineral metabolism detected in the blood serum of hens (Table 9). Feed supplementation with ZooBioR product leads to the maintenance of an adequate mineral metabolism, due to its involvement in the biological role of Ca and P during periods of increased metabolic stress.

The ZooBioR remedy administered to hens during the early laying phase also showed beneficial properties on iron levels, represented both by providing birds with this available chemical element and by its use for vital needs, emphasizing the adaptogenic properties of the tested product, under intensive conditions of laying hens exploitation.

Table 9. Some elements of mineral metabolism concentration in young hens in early laying stage under the action of ZooBioR (M±m)

| Markers | Study debut | Bird flocks | | | | |
|--------------------|-------------|--------------|------------|------------|------------|------------|
| | | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| Calcium, mmol/l | 2,52±0,09 | | | | | |
| 1st sampling | | 3,44±0,08*** | 3,42±0,19 | 3,36±0,06 | 3,10±0,16 | 3,16±0,09 |
| 2nd sampling | | 3,74±0,16 | 3,71±0,09 | 3,99±0,20 | 4,12±0,13 | 3,96±0,15 |
| Phosphorus, mmol/l | 1,90±0,31 | | | | | |
| 1st sampling | | 0,74±0,05** | 0,91±0,14 | 0,77±0,03 | 0,92±0,03* | 1,28±0,20* |
| 2nd sampling | | 1,98±0,30** | 1,49±0,07 | 1,47±0,10 | 1,74±0,05 | 2,25±0,11 |
| Ca/P ratio | 1,33 | | | | | |
| 1st sampling | | 4,65 | 3,76 | 4,36 | 3,37 | 2,47 |
| 2nd sampling | | 1,89 | 2,50 | 2,71 | 2,37 | 1,76 |
| Iron, µM/l | 24,83±1,41 | | | | | |
| 1st sampling | | 21,63±0,83 | 21,63±0,83 | 21,78±1,03 | 22,17±0,37 | 21,71±0,59 |
| 2nd sampling | | 20,83±0,83 | 23,08±0,90 | 21,95±0,64 | 19,97±0,73 | 18,65±1,34 |

Note: * - $p<0,05$; ** - $p<0,01$; **** - $p<0,001$.

In the second study, the administration of ZooBioR induced, towards the end, a decreased Ca level in serum up to the debut level, reflecting the beneficial effect of the tested product. The P concentration in birds serum in both groups decreased towards the 1st study ($p<0.05$), and continued to decrease towards the last study stage, when the P concentration in EF was more decreased compared to the control. ZooBioR induced the intensification of Fe utilization for the birds' physiological needs. With the advancement in the technological cycle, the Mg content in EF was less decreased compared to CF, the increase compared to CF being about 14% ($p<0.05$).

In chapter 4, **Evaluation of liver metabolism parameters and trypsin-antitrypsin system in laying hens treated with ZooBioR**, some marker indices of the functional state of the liver are reported, as well as the impact of the tested product on the trypsin-antitrypsin system. In particular, the liver functional tests revealed the ability of the tested product to stop the decline in alkaline phosphatase (ALP) - by up to 22.5% compared to CF. By the 2nd stage of the study, the liver marker tested at 3 EF showed a 1.2-1.4-times decrease, which can be considered beneficial and attests ZooBioR's antistress and hepatoprotective action (Table 10). ZooBioR induced a statistically relevant increase in serum ALP-termostable (liver fraction) by up to 44.2% over the control. The decrease in the levels of this enzyme persisted, with the exception of EF 3, a similar manifestation also recorded in other animals of zootechnical interest treated with BAC (Mațencu 2019; Putin, Macari, Rotaru, 2020, p. 72-75). ZooBioR caused a decrease in the ALP-termolabile activity (bone fraction) in hens, with the exception of EF 4 (maximum dose), which can be explained by the involvement of this enzyme fraction in the process of eggshell formation. At the end of the research, the enzyme activity decreased progressively - by 1.1-1.7-times both in intact hens and in those treated with the tested product.

Table 10. Values of alkaline phosphatase and its fractions in serum of young laying hens

| Markers | Study debut | Bird flocks | | | | |
|-----------------------|--------------|---------------|---------------|--------------|---------------|---------------|
| | | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| ALP, u/l | 729,72±64,11 | | | | | |
| 1st sampling | | 579,56±111,21 | 710,01±88,69 | 604,02±88,66 | 571,41±78,65 | 673,33±110,62 |
| 2nd sampling | | 527,92±108,11 | 377,77±20,3** | 455,22±65,71 | 580,92±125,71 | 383,20±29,45* |
| ALP-thermostable, u/l | 519,09±87,60 | | | | | |
| 1st sampling | | 350,59±66,80 | 505,50±54,00 | 401,55±80,49 | 366,90±45,56 | 425,33±73,79 |
| 2nd sampling | | 347,87±87,12 | 235,09±32,55 | 307,11±48,10 | 364,18±127,89 | 242,56±14,04 |
| ALP thermolabile, u/l | 210,63±25,25 | | | | | |
| 1st sampling | | 228,97±47,74 | 204,51±47,52 | 202,47±32,82 | 204,51±37,09 | 248,00±47,42 |
| 2nd sampling | | 235,77±52,80 | 142,68±24,64 | 148,12±21,54 | 216,74±67,55 | 140,64±26,03 |

Note: * - $p < 0,05$; ** - $p < 0,01$.

In the scientific-practical implementation study, the total ALP levels decreased insignificantly, then by the end of the study this enzyme in EF had an activity of about 21% higher than in CF, which denotes the positive impact of ZooBioR on the liver by fortifying the functional state of this gland (Table 11).

Table 11. Alkaline phosphatase and its fractions values in serum of laying hens in the scientific-practical implementation study

| Markers | Study debut | Bird flocks | |
|--|--------------|--------------|----------------|
| | | CF | EF 1 |
| Total alkaline phosphatase, u/l | 214,78±27,39 | | |
| 1st sampling | | 207,98±31,30 | 198,17±22,47 |
| 2nd sampling | | 242,33±32,50 | 292,91±14,30** |
| Thermostable alkaline phosphatase, u/l | 137,40±26,11 | | |
| 1st sampling | | 137,02±18,27 | 141,17±14,43 |
| 2nd sampling | | 160,42±21,72 | 198,17±13,40* |
| Thermolabile alkaline phosphatase, u/l | 77,38±10,92 | | |
| 1st sampling | | 70,96±13,42 | 57,00±12,72 |
| 2nd sampling | | 81,91±15,60 | 94,74±11,53 |

Note: * - $p < 0,05$; ** - $p < 0,01$.

Thermostable ALP at the first sampling was practically maintained at baseline, increased by 17% in CF towards the end, and by about 40% under ZooBioR compared to the previous step ($p < 0,05$). Also, at the end of the study, in EF, compared to CF, the activity of this enzyme was about 23% more intense, reflecting a better liver activity, which was also determined in other livestock animals under the action of BAC (Putin, Macari, Rotaru, 2020, p. 70-75; Mațencu, 2019). The functional level of thermolabile ALP (bone fraction) was characterized by a decrease, under a more progressive ZooBioR action compared to the debut, of about 26%, a manifestation considered physiological and explained by the metabolic adaptation of chicks to the "new physiological regime" of laying eggs. By the end of the study, this fraction of AP had increased: by about 15% in CF and by about 66% in EF compared to previous levels.

The serum ALT enzyme in birds towards the 1st sampling inconclusively increased, and by the end of the study decreased compared to CF, by 4.6-20.9% ($p < 0.05$, for EF 1) (Table 12). At the first experimental stage, AST levels were higher in birds subjected to the action of ZooBioR, increasing by 12.2-34.9% ($p < 0.001$, for EF 3). By the end of the study, AST levels were 17.5% higher in EF 2 compared to CF and by 12.7-33.3% compared to the other EF, a manifestation considered characteristic for CBA action [Pavlicenco N., 2019; Balanescu S., Voinitschi E. et al. 2019]. A possible mechanism of action of the tested product could be the improvement of the proteosynthetic function of the liver, reduction of hepatocyte alteration, as well as improvement of protein metabolism in general.

Table 12. Values of transaminases and total bilirubin and its fractions in serum in hens

| Markers | Study debut | Bird flocks | | | | |
|-------------------------------|-------------|-------------|-------------|------------|---------------|------------|
| | | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| ALT, u/l | 13,41±1,41 | | | | | |
| 1st sampling | | 16,97±2,35 | 14,67±1,78 | 15,30±1,68 | 19,49±0,88 | 15,29±2,39 |
| 2nd sampling | | 18,02±1,14 | 14,25±1,09* | 17,18±1,60 | 16,34±1,42 | 14,88±1,83 |
| AST, u/l | 45,68±2,86 | | | | | |
| 1st sampling | | 45,47±3,18 | 52,59±1,79 | 52,38±2,57 | 61,33±2,51*** | 53,43±2,77 |
| 2nd sampling | | 59,92±3,15* | 52,80±2,84 | 70,40±8,36 | 62,44±7,11 | 61,81±4,32 |
| Total bilirubin µmol/l | 60,93±1,58 | | | | | |
| 1st sampling | | 63,88±3,21 | 69,37±2,97 | 56,28±1,32 | 60,93±4,15 | 56,99±2,71 |
| 2nd sampling | | 59,09±3,25 | 60,32±4,36 | 67,56±7,68 | 71,05±5,48 | 65,42±3,34 |
| Direct bilirubin, µmol/l | 31,42±1,12 | | | | | |
| 1st sampling | | 34,66±2,35 | 42,79±4,40 | 31,03±1,70 | 37,89±3,89 | 33,63±3,52 |
| 2nd sampling | | 32,53±2,09 | 33,23±3,49 | 38,69±4,57 | 45,00±6,70 | 38,62±3,90 |
| Indirect bilirubin, µmol/l | 29,51±0,60 | | | | | |
| 1st sampling | | 29,22±1,85 | 26,58±2,79 | 25,26±1,67 | 23,03±2,24 | 23,36±2,61 |
| 2nd sampling | | 26,56±1,55 | 27,09±1,27 | 28,87±3,55 | 26,05±1,88 | 26,80±1,94 |

Note: * – $p < 0,05$; * – $p < 0,001$; 1st sampling - about 1 month after the study debut; 2nd sampling - at the end of the study, 129 days after the study debut.

ZooBioR's beneficial action on bilirubin was highlighted in EF 2, 3 and 4, during high metabolic stress periods, such as early laying period, when it was able to maintain the initial level or to reduce its concentration in blood by 10.8-11.9% compared to the control flock. A late decrease in serum bilirubin by 7.5% in CF and 13.0% in EF 1 compared to previous levels was also identified at the end of the study. The EF hens showed a slight increase in serum bilirubin compared to the CF, generally dependent on the dose of the product tested. A similar increase of bilirubin in the blood circulation was also established when other BACs were administered to livestock (Maţencu, 2019). At the end of the study direct (conjugated) bilirubin increased in EF birds by about 38% compared to CF, which can be explained by the intensification of metabolic processes in the body, especially in the liver. Indirect bilirubin (free, unconjugated), showed stability

in serum in young, intact hens in the 1st study, being at the background level, results that sum up several factors, reflected in good health status. Under the ZooBioR action, the level of indirect bilirubin decreased up to about 21% compared to CF, an undeniably positive manifestation showing an intensification of physiological and metabolic processes in liver, as well as a better functionality and condition of erythrocytes. At the last research stage, indirect bilirubin in EF 1, 2 and 4 showed a weak increase compared to CF.

Upon ZooBioR implementation, ALT activity was about 22% higher at the first investigation and 12.9% higher than CF at the end of the study. The same decrease persisted for AST, indicating the adaptive and prolonged effect of the tested product. Indirect bilirubin in the first study was not different from the CF, whereas at the end of the study it was lower (by 17.6%) compared to the control flock, a result with double significance: the tested product does not negatively influence the liver status, but on the contrary has a beneficial impact on this organ.

Markers of the trypsin-antitrypsin system (STA) - trypsin, α 1-antitrypsin and α 2-macroglobulins, reflect the dynamic equilibrium of the processes occurring in liver, pancreas, intestines, etc. (Putin, Macari, Rotaru, 2020, p. 85-90). Trypsin activity towards the first stage of research showed an increase in all birds involved in the study: in CF the increase was 24.1% compared to phonon; in experimental birds, only in EF 2, the increase was smaller, while being more decreased compared to CF and EF 1, 3 and 4 (Table 13). Towards the end of the study, trypsin increased significantly in CF compared to previous levels (by 34.7%, $p < 0.01$), and in three EF, the tendency was negative. ZooBioR medication produced a pronounced anti-stress effect, reflected in the conclusive decrease in trypsin in all EF (by 26.5-35.6%) compared to CF. Lower than background levels of α 1-antitrypsin were recorded at the first stage of research. This manifestation can be considered as a physiologic age peculiarity, which was more pronounced in EF, where α 1-antitrypsin levels decreased by up to 50.0% compared to CF ($p < 0.001$, EF 1 and $p < 0.05$, EF 2).

Table 13. Serum trypsin-antitrypsin system values in ZooBioR-treated hens

| Markers | Study debut | Bird flocks | | | | |
|---------------------------------|-------------|-------------|---------------|--------------|------------|------------|
| | | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| Trypsin, mkmol/l | 0,79±0,08 | 0,98±0,06 | 0,97±0,11 | 0,93±0,11 | 1,00±0,09 | 1,02±0,09 |
| | | 1,32±0,04** | 0,85±0,07*** | 0,97±0,07** | 0,96±0,15* | 0,97±0,15 |
| α 1-antitrypsin, mkmol/l | 41,78±2,55 | 34,33±2,15 | 17,18±1,54*** | 21,40±3,23* | 30,30±3,70 | 29,55±3,44 |
| | | 24,58±2,35* | 25,18±3,33 | 26,08±1,33 | 21,03±3,09 | 25,63±3,36 |
| α 2-MG, g/l, | 2,34±0,22 | 2,83±0,36 | 2,22±0,67 | 2,65±0,27 | 1,96±0,24 | 2,84±0,24 |
| | | 2,28±0,09 | 2,19±0,11 | 3,79±0,20*** | 3,21±0,35* | 2,40±0,45 |

Note: * - $p < 0,05$; ** - $p < 0,01$; * - $p < 0,001$.

At the end of the study there were no noticeable differences between flocks, which reflects the uniform, good condition of birds involved in the research. α 2-Macroglobulins (α 2-MG) at the first experimental stage were characterized by an increase of 20.9% compared to the background in CG, while in EF there was no obvious increase in this index. At the end of the study, α 2-MG levels in serum decreased both in CF and in EF 1 and 4, while in EF 2 and 3 they were significantly increased (by 40.8-66.2%) compared to CF, confirming the anti-stress and adaptive properties of the tested product.

In the implementation of the scientific and practical study, trypsin levels were lower at the first sampling in CF and decreased in EF compared to CF. At the end of the study, trypsin in EF was about 17% higher than in CF, which can be considered as positive, but at the same time at the baseline level. The activity of α 1-antitrypsin at the first stage of the study was similar to trypsin, characterized towards the end of the study by the same levels, showing a good health status of the birds. α 2-MG in blood was manifested by its decreased levels in CF and increased levels in EF compared to CF, which can be considered as beneficial.

In chapter 5, **Changes in some indices of liver and muscle tissue metabolism in laying hens treated with ZooBioR**, the changes in liver and muscle tissues, taken from the birds involved in the research, are described by objectively quantifying the metabolic indices of these two tissues. The evaluation of changes in liver and muscle tissue metabolism allows to highlight the state of metabolic health, as well as to evaluate the impact of BAC on animals (Rotaru, 2016; Macari, et al., 2014; 2021; Crivoi et al., 2022). In our study, under the action of ZooBioR the total protein in liver tissue did not univocally change: at EF 1 and 4 the tested product induced a slight increase, and at EF 2 a decrease in the investigated constant compared to the control flock (Table 14).

Table 14. The values of protein metabolism in the hepatic tissue in laying hens

| Markers | Bird flocks | | | | |
|---------------------|-------------|------------|------------|------------|------------|
| | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| Total proteins, g/l | 43,64±1,39 | 44,41±2,37 | 42,04±1,69 | 43,34±2,26 | 44,71±0,82 |
| Albumins, g/l | 17,23±0,36 | 17,23±0,32 | 16,55±0,52 | 16,73±1,04 | 16,64±0,38 |
| ALT, u/g.prot. | 0,88±0,09 | 0,91±0,09 | 0,83±0,11 | 0,69±0,07 | 0,59±0,02* |
| AST, u/g.prot. | 0,99±0,11 | 1,13±0,07 | 1,01±0,12 | 0,87±0,07 | 0,81±0,05 |
| G-GTP, u/g.tissue | 6,08±0,47 | 7,54±0,43 | 7,88±0,78 | 4,94±1,02 | 8,07±0,63* |

Note: * - $p < 0,05$.

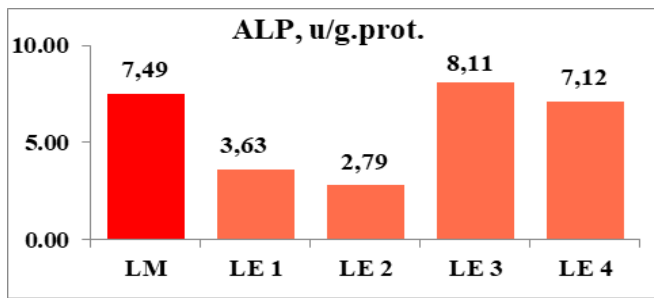


Figure 4. ALP dynamics in liver tissue under the action of ZooBioR

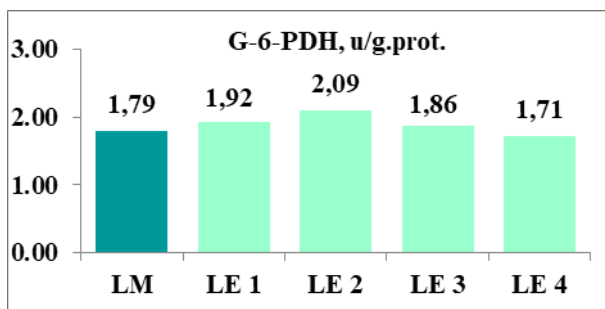


Figure 5. Changes in G-6-PDH levels in liver tissue under the action of ZooBioR

($p < 0.05$, for EF 4), a manifestation that can be interpreted as a result of the intensified protein metabolism in the body.

Lower doses of ZooBioR (EF 1 and 2) resulted in a minor increase in LDH levels, while higher doses (EF 3 and 4) resulted in lower LDH levels (up to 21%) compared to the control flock, which are considered beneficial in two ways: a) the tested remedy is harmless for the health of birds at the molecular level; b) ZooBioR has a beneficial influence on the functional state of the liver (Figure 3).

The alkaline phosphatase (ALP) (Figure 4) in liver tissue towards the end of the study decreased significantly and progressively (2.1-2.7-times) compared to CF, clearly showing the hepatoprotective effect of ZooBioR administered especially at low doses.

G-6-PDH activity in liver tissue of hens at the end of the study (Figure 5) was increased by up to 16.8% in EF 1 and 2 (doses of 5 and 10 mg active substance/kg feed), while EF 4, which was supplemented with the maximum dose of ZooBioR, had lower G-6-PDH activity than CF.

In the scientific and practical study under the action of ZooBioR in the liver tissue insignificant - total protein value decreased, while albumin increased compared to CF. AST

A decrease compared to the control flock of hepatic albumin was recorded in EF treated with high doses of ZooBioR, the hepatoprotective effect of the tested product being identified by a conclusive decrease of up to 33% of ALT in EF 2, 3 and 4 compared to the CF level ($P < 0.05$, for EF 4), a manifestation considered in our opinion as a

positive one, the similar trend of ALT decrease being also reported in the action of other CBAs (Pavlicenco, 2019). ZooBioR administered to EF 1 and 2 induced an increase up to about 14% in liver tissue of AST enzyme compared to CF. At the same time, AST activity in EF 3 and 4 was lower compared to CF, the decrease being about 12-18%. G-GTP in liver tissue was higher in EF 1, 2 and 4 compared to CF, by 24.0-32.7%,

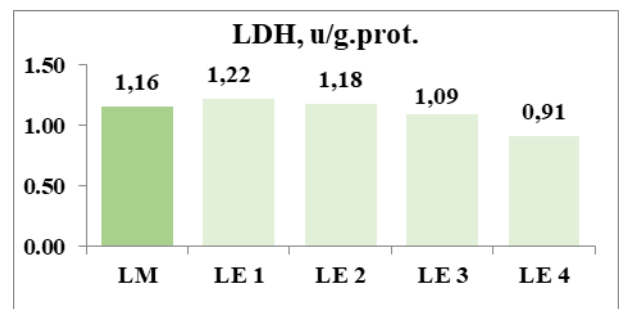


Figure 3. Changes in LDH levels in liver tissue under the action of ZooBioR

enzyme activity was 16.4% higher, while ALT activity decreased by 36.4% ($p<0.05$) compared to the control flock. An increase of about 10% compared to CF was registered regarding G-GTP enzyme. ZooBioR product induced a decrease of about 12% in LDH, a conclusive 2.1-times ($p<0.01$) increase in total ALP, a 2.4-times ($p<0.001$) increase in the total ALP, a 2.4-times ($p<0.001$) increase in the total ALP, and a 1.6-times increase in the liver fraction (ALP-thermolabile) compared to CF.

The tested product ZooBioR exerted a potent action on protein metabolism in muscle tissue expressed by: increased total protein in muscle at EF 2, 3 and 4 ($p<0.05$, for EF 4), and increased albumin by up to about 20% compared to CF (Table 15).

Table 15. Marker indices of protein and carbohydrate metabolism in muscle tissue of hens treated with ZooBioR

| Markers | Bird flocks | | | | |
|---------------------|-------------|-------------|-------------|--------------|-------------|
| | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| Total protein, g/l | 35,92±1,38 | 34,70±1,31 | 38,49±3,38 | 39,85±1,55 | 41,46±1,96* |
| Albumins, g/l | 12,05±0,56 | 12,77±0,63 | 13,38±0,72 | 14,47±0,26** | 14,17±0,37* |
| ALT, u/g.protein | 0,37±0,06 | 0,44±0,03 | 0,35±0,02 | 0,31±0,01 | 0,39±0,04 |
| AST, u/g.protein | 0,56±0,14 | 0,85±0,08 | 0,58±0,05 | 0,82±0,07 | 0,69±0,05 |
| G-GTP, u/g. tissue | 8,82±0,87 | 14,10±1,52* | 13,11±1,36* | 11,17±0,95 | 9,45±1,39 |
| LDH, u/g.protein | 5,96±0,57 | 7,69±0,09* | 6,18±0,70 | 6,28±0,56 | 7,27±0,92 |
| G-6-PDH, nM/s.l | 1,13±0,15 | 1,36±0,12 | 0,96±0,10 | 0,70±0,11* | 0,77±0,05 |
| CK-NAC, u/g.protein | 1,23±0,09 | 2,34±0,28** | 1,59±0,27 | 1,08±0,03 | 1,18±0,09 |

Note: * – $p<0,05$; ** – $p<0,01$.

ALT enzyme manifestations were not so evident and uniform in the EF and CF groups, whereas AST increased by up to about 52% compared to the control flock. The action of ZooBioR on G-GTP enzyme was most pronounced in the birds that received lower doses of it (EF 1 and 2), when the increase of the tested enzyme was 48.6-59.9% compared to control ($p<0.05$). ZooBioR induced the increase in muscle LDH by up to 29.0%, in EF 1 the difference was also conclusive (29.0%, $p<0.05$) compared to the control, which denotes the beneficial effect of the tested remedy on carbohydrate metabolism in muscles. In EF 1 (minimum dose of ZooBioR) G-6-PDH in muscle increased by 20.4% compared to CF, while in EF 2, 3 and 4 it decreased, more pronounced in EF 3 (by 61.9%, $p<0.05$), which reveals the beneficial impact of the tested product on energy processes in muscle tissue. The CK-NAC enzyme increased 1.3-1.9 times under the low doses of ZooBioR ($p<0.05$, for EF1), while in EF 3 and 4 (high doses of ZooBioR) it decreased by up to 12% compared to the control.

In chapter 6, **Effects of ZooBioR remedy on productivity and the efficacy of the optimal dosing scheme and regime for the tested product in young laying hens**, the effects of tested product on bioproductive indices are described, which have a particular significance in objective confirmation of clinical-hematologic and biochemical constants obtained in the investigation

process, as well as in the motivation of the dose and administration scheme of ZooBioR to young laying hens. The first investigated indicator - body mass, recorded almost the same levels in the hens included in the study at the beginning of the study (age 129 days). In dynamics, the body mass increased, and at the last stage of the study (119 days) the body mass was 35.02-69.35 g higher in EF than in CF. On preventive treatment basis, a positive dynamics of the average day/period weight gain index was observed, which was 1.6-2.2 times higher in EF than in CF. In study II, at the end of the study, the body mass showed a positive dynamics and was practically not different in EF than in CF.

Of the complex range of zootechnical indicators, an important place is held by egg production, regarding which the tested ZooBioR product showed a positive effect. The egg production during the monitored period of 1-118 days was 9.6-21.2% higher in EF hens compared to CF (1338.0 pcs/egg/period) and was dependent of the ZooBioR dose. In EF 1 and 2 (minimum and low doses) birds produced 274-283.0 eggs more than CF during the study period. And bird viability (percentage of birds alive) at the end of the study was 7, 15-14.3% higher in EF compared to CF (Table 16). In study II, egg production under the action of ZooBioR showed clear superiority over the control. During the experimental period, the marker parameters of egg production were as follows: *feed consumed per 10 eggs produced* - in CF hens - 1.34 kg, which was 4.48-13.43% lower in EF than CF; *feed consumed per 1 kg egg mass* - in CF hens - 2.53 kg, which was 7.91-16.21% lower than EF. Production of egg mass in hens subjected to ZooBioR was 757.41 kg, which is 26.0 kg more than the control, while feed consumed per 10 eggs in CF was 1.22 kg, while in EF 1.20 kg, which is 0.02 kg less than the control.

Table 16. Dynamics of egg production in hens receiving feed supplemented with ZooBioR

| Markers | Bird flocks | | | | |
|---|-------------|--------|--------|--------|--------|
| | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| No. hens/start, head | 14 | 14 | 14 | 14 | 14 |
| Numerical egg production/first study day, pcs | 10,0 | 10,0 | 9,0 | 9,0 | 10,0 |
| Egg laying intensity/first study day, % | 71,4 | 71,4 | 64,3 | 64,3 | 71,4 |
| Numerical egg production/1-10 day of study, pcs | 109,0 | 120,0 | 123,0 | 114,0 | 113,0 |
| Numerical egg production/10th day of study, pcs | 11 | 14 | 14 | 11 | 13 |
| Egg laying intensity/ 10th day of study, % | 78,57 | 100,0 | 100,0 | 78,57 | 92,85 |
| Numerical egg production/1-118 study day, pcs | 1338,0 | 1612,0 | 1621,0 | 1467,0 | 1489,0 |
| No. hens/finish, head | 12 | 14 | 14 | 13 | 13 |
| Numerical egg production/ last day of study, (118th from laying cycle start), pcs | 11 | 14 | 14 | 13 | 13 |
| Egg laying intensity/last study day, (118th from laying cycle debut) % | 91,67 | 100,0 | 100,0 | 100,0 | 100,0 |
| Viability, % | 85,71 | 100,0 | 100,0 | 92,86 | 92,86 |
| Mortality, % | 14,29 | - | - | 7,14 | 7,14 |

Positive results were also recorded when determining feed intake per 1 kg egg mass, which in EF was 2.00 kg and in CF 2.06 kg (Table 17).

Table 17. Economic indicators in the process of using ZooBioR in hens

| Markers | Bird flocks | | | | |
|--|-------------|--------|--------|--------|--------|
| | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| No. hens/start, head | 14 | 14 | 14 | 14 | 14 |
| The average egg mass at study debut., g | 45,30 | 45,00 | 45,60 | 46,00 | 45,30 |
| The average egg mass on the 27th day of study, g | 49,50 | 49,71 | 52,36 | 52,50 | 51,54 |
| The average egg mass on the 63rd day of study, g | 56,09 | 57,71 | 57,31 | 57,21 | 59,38 |
| Egg mass at the end of study, g | 61,64 | 60,79 | 63,29 | 61,42 | 63,00 |
| Average weight of an egg/period, g | 53,13 | 53,30 | 54,64 | 54,28 | 54,80 |
| Numerical egg production/1-118 study day, pcs | 1338,0 | 1612,0 | 1621,0 | 1467,0 | 1489,0 |
| Average egg mass production / period, kg | 71,09 | 85,92 | 88,57 | 79,73 | 81,60 |
| Amount of feed consumed by birds/period, kg | 180,0 | 190,0 | 188,0 | 185,0 | 190,0 |
| Feed consumed per 10 produced eggs, kg | 1,34 | 1,18 | 1,16 | 1,26 | 1,28 |
| Feed consumed per 1 kg egg mass, kg | 2,53 | 2,21 | 2,12 | 2,32 | 2,33 |

Egg morphometric parameters are relevant in determining the eggs' quality, which in our research were assessed at the end of the study, three consecutive times: at 1-2 days and at about 2 and 4 weeks after sampling. A progressive decrease of 6.2% (3.8 g) in egg weight in CF and 3.7-7.9% (2.3-5.0 g) in EF eggs by the 30th day of monitoring was established (Table 18).

Table 18. Eggs morphometric parameters, about 1 month after collection, (M±m)

| Markers | Bird flocks | | | | |
|-------------------------------|-------------|------------|------------|------------|------------|
| | CF | EF 1 | EF 2 | EF 3 | EF 4 |
| Egg mass, g | 57,80±1,05 | 58,40±1,41 | 59,30±1,66 | 59,10±2,24 | 58,00±1,45 |
| Large diameter, cm | 5,51±0,05 | 5,59±0,07 | 5,56±0,07 | 5,56±0,09 | 5,50±0,05 |
| Small diameter, cm | 4,34±0,03 | 4,35±0,04 | 4,36±0,03 | 4,41±0,06 | 4,35±0,04 |
| Yolk diameter, cm | 4,00±0,05 | 4,09±0,05 | 4,04±0,05 | 4,12±0,06 | 4,09±0,04 |
| Yolk height, cm | 1,81±0,05 | 1,85±0,05 | 1,97±0,05* | 1,81±0,14 | 1,66±0,12 |
| Yolk weight, g | 16,70±0,55 | 17,10±0,37 | 16,50±0,39 | 16,00±0,54 | 16,90±0,69 |
| Albumen height, cm | 0,89±0,03 | 1,03±0,04* | 0,84±0,03 | 0,92±0,04 | 0,98±0,05 |
| Albumen weight, g | 32,90±0,81 | 33,60±1,09 | 34,20±1,14 | 34,70±1,58 | 33,40±1,01 |
| Shell weight, g | 8,30±0,32 | 7,70±0,22 | 8,60±0,32 | 8,30±0,27 | 7,80±0,21 |
| Shell thickness/top, mm | 0,36±0,01 | 0,34±0,01 | 0,36±0,01 | 0,36±0,02 | 0,33±0,01 |
| Shell thickness/middle, mm | 0,42±0,01 | 0,42±0,01 | 0,42±0,00 | 0,42±0,01 | 0,41±0,01 |
| Shell thickness/round end, mm | 0,42±0,01 | 0,42±0,01 | 0,44±0,01 | 0,43±0,00 | 0,43±0,00 |

Note: * - p<0,05; ** - p<0,01.

The yolk in EF 1 and 4 was 0.2-0.4 g higher than the control, and in EF 2 and 3 it was 0.2-0.7 g lighter than the CF, which allows us to assume that the eggs from these batches are of higher quality at 1 month after the end of the research. The albumen height was lower in EF 2 by 5.6% compared to the control and by 8.7-18.4% compared to EF 1, 3 and 4; the albumen was heavier in EF 2 and 3, results of real use in determining the optimal dose of tested product. The egg white in

CF was higher and lighter in weight, indicating a higher volume probably generated by the gases resulting from the albumen alteration processes. ZooBioR administered at 10 mg active substance/kg/feed (EF 2) stopped this albumen degradation process, which was confirmed by the lower height and higher weight of the egg white, demonstrating also a higher albumen density. Egg shell weight at 1 month of storage in EF 2 had a difference of 0.3 g, (+3.6%) compared to CF and 0.3-0.9 g (+3.6-11.7%) compared to the other three EF. A slight increase in shell at the egg round end was observed in EF 2, 3 and 4 by 0.01-0.02 mm, or 2.4-4.8% compared to CF, probably due to the fortification of mineral metabolism by the tested product.

GENERAL CONCLUSIONS

1. It has been established that ZooBioR product administered to laying hens had no negative impact on the marker components of the hemogram, while on the opposite, it had a beneficial effect on hematopoiesis, reflected in MCV decrease, hemoglobin, MCH and MCHC hemogram indicators increase, as well as platelet count increase at the end of the research.
2. The clinical investigation results confirm the role of the autochthonous product ZooBioR in fortifying the health of hens, a positive effect outlined in a positive balance of both the clinical and hematological status and in maintaining the granulocyte/lymphocyte population in balance, data that demonstrate the anti-stress effect of the tested product. At the same time, the results obtained emphasize both the harmlessness of the tested remedy and the positive impact of ZooBioR at the cellular level, reflected by a higher non-specific resistance and a more pronounced metabolic display in the investigated laying hens.
3. The obtained results in the first study show a decrease in blood total lipids, and on the opposite an increase in Ch by 9.3-28.0% and TG by 19.3-29.2% compared to the control group, which provides useful energy for normal and adequate vital processes, including the ovogenesis process. In both studies, at the end of the investigations, the tested remedy induced a decrease of β -lipoproteins in blood: in the first study by 2.0-12.7% and in the second study by 43.7%, which explains the role of this lipid fraction in blood transportation of Ch and TG actively involved in metabolic processes.
4. The serum protein content (study I) remains constant throughout the experiment, whereas the serum albumin content fluctuates according to the stage of birds' exploitation and the dose of tested remedy. Thus, the blood albumin content during the study shows a clear increase, which in intact hens, at the end of the study, was 16.3% in the first series of experiments and 33.25% in the second series of experiments compared with the control group, which can be explained by the massive involvement of this protein in basal metabolism.

5. It was highlighted that in both studies, the bioactive product ZooBioR, especially in the scientific-practical study (study I), induces at the end of the experiment an increase in urea content by 3.0-8.0%, in uric acid by 27.0-46.9% ($p < 0.05$, for EF 3 and 4), creatinine in EF 2, 3 and 4 - by 2.7-19.4%, as well as in study II - by 8.3% compared to control values, pointing out the intensification of proteosynthetic processes in liver, and obviously anabolic processes aimed at strengthening and maintaining the productive potential. In confirmation of the above, the serum glucose level is also higher, at the end of the first study by 4.5-11.6%, a substance needed to provide energy for metabolic processes, whereas in the implementation study this is not so evident.
6. It has been estimated that the ZooBioR remedy administered to chickens has beneficial properties on mineral metabolism, highlighted both by providing the body with available chemical elements and by their utilization for vital needs, highlighting the adaptogenic properties of the newly tested product.
7. It has been established that ZooBioR product fortifies liver metabolic processes, a conclusion based on the improvement of serum levels of ALT, AST, PCE, alkaline phosphatase and its fractions, total bilirubin and its fractions in periods of high metabolic stress, such as intensive egg laying, a beneficial impact reflected in the liver functional fortification and the improvement of erythropoiesis.
8. ZooBioR administered to laying hens brings to the focus the liver state (total protein, albumin, ALT, AST, G-GT, LDH, ALP, G-6-PDH in liver tissue), as it doesn't have a negative effect on this organ, but on the opposite improves its functional state, through hepatoprotective and proteosynthetic properties, as well as on liver carbohydrate metabolism. The tested product has anti-stress, trypsin-antitrypsin system-correcting and immunostimulatory properties, based on higher levels of $\alpha 2$ -macroglobulin, a multifunctional protein that has also been shown to modulate biological responses in the body.
9. The ZooBioR remedy beneficially influenced muscle tissue metabolic processes. There was a clear tendency of total protein increase, albumin and the enzymes AST, G-GTP and LDH in muscle tissue in treated birds, which leads to the conclusion that the tested product enhances protein metabolism, especially in muscle tissue, results that are in direct accordance with the quality and safety of poultry meat.
10. ZooBioR product administered to hens shows beneficial properties on production indicators: egg production was 12.2-24.6% higher compared to CF values; egg mass production in EF was 12.2-24.6% higher compared to CF; the parameter - feed consumed per 10 eggs produced - in birds, in CF was 1.34 kg, a value lower by 4.48-13.43% compared to similar values in EF; feed consumed per 1 kg egg mass - in hens in CF was 2.53 kg, a lower indicator by 7.91-

16.21% compared to the values mentioned in EF. Similar but less pronounced tendencies were also reported in study II.

RECOMMENDATIONS

- 1.** For a better management of laying hens, especially those raised and exploited under intensive conditions, it is useful the implementation by practicing medical staff the clinical investigation of birds (during the exploitation), with monitoring of body temperature and respiratory rate per minute, but also the evaluation of the physiological-metabolic status, through laboratory analysis of biological substrates.
- 2.** It is recommended to include the ZooBioR remedy in laying hens' feed (daily, during the first 4 months of operation), in the proportion of 10 mg active substance/kg feed as to increase resistance, health, productive potential, and to improve egg quality.

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2. Articles in scientific journals

2.1. in journals from the Web of Science and Scopus databases

1. BONDAR A., MACARI V., RUDIC V., **PISTOL Gh.**, PUTIN V., ROTARU A., CHIRIAC T., SOLCAN GH., SOLCAN C. Effects of ZooBioR2 product as feed supplement in layinghens on the morphofunctional state of intestinal mucosa. In: *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia*. 2022, nr. 4(74), pp. 626-632. 0,72 c.a. ISSN 0102-0935. (IF 0.442).
<https://doi.org/10.1590/1678-4162-12592>.

2.2. articles in recognized foreign journals

2. BONDAR A., MACARI V., RUDIC V., **PISTOL Gh.**, PUTIN V., ROTARU A., CHIRIAC T., SOLCAN C. Efectele extractului de *Spirulina platensis* (ZOOBIOR2) asupra mucoasei intestinale la găinile ouătoare. In: *Romanian Journal of Veterinary Medicine & Pharmacology*. 2023, vol., № 39 (1), pp. 18-24. 0,38 c.a. ISSN 2558-9318. Categoria B+; BDI CABI și SIS.
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Category B

3. **PISTOL Gh.**, MACARI V., PUTIN V., ROTARU A. Efectele suplimentării hranei găinilor tinere cu produsul ZooBioR asupra statusului clinico-hematologic. În: *Știința agricolă*. 2021, nr. 1, pp. 129-136. 0,87 c.a. DOI: 10.5281/zenodo.5080033. ISSN 2587-3202.
https://ibn.idsi.md/sites/default/files/imag_file/129-136_7.pdf.

3. Articles in scientific journals

3.1. in the scientific papers of international scientific conferences (abroad)

4. MACARI V., **PISTOL Gh.**, GUDUMAC V., ROTARU A., PUTIN V., PINTEA V., ROTARI L., PAVLICENCO N. Changes of protein metabolism indices in hepatic tissue in young hens under the influence of ZooBioR spirulin remedy. In: *Materials of International, scientific and practical conference Azerbaijan at a newstage of development – Food and nutrition security in the period of global alizationand post-pandemic period: modern situation, challenges, and prospectives”* (Bacu-Lankaran st., October, 8-9, 2021). Lankaran: LSU, 2021, pp. 317-321. 0,42 c.a.
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3.2. in international scientific conference papers (Republic of Moldova)

6. MACARI V., **PISTOL Gh.**, PUTIN V. Efectele produsului ZooBioR– remediu autohton utilizat în diferite doze – asupra sănătății și productivității găinilor ouătoare în prima fază de ouat. In: *Știință, educație, cultură: materialele conf. șt. - practice intern., 12 feb. 2021*, Universitatea de Stat din Comrat, vol. I, pp. 187-191. 0,47 c.a. file:///D:/Downloads/mezhdunarodnaya-nauchno-prakticheskaya-konferenciya-nauka-obrazovanie-kultura-posvyashchennaya-30-oj-godovshchine-kgu-tom--1%20(3).pdf.
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3.3. in the scientific papers of national scientific conferences with international participation

8. MACARI V., **PISTOL Gh.**, PUTIN V., RUDIC V., CHIRIAC T., CHISELIȚA O., ROTARU A., DJUR S., ROTARI L., PAVLICENCO N., CHIȘLARI Iu. În: Aspecte ale influenței remediei ZooBioR asupra sănătății, metabolismului lipidic și unor indici bioproductivi la găini în prima fază de ouat. În: *Conferința științifico-practică cu participare internațională, dedicată celei de-a 65-a aniversări de la fondarea Institutului*. Maximocva, 2021, pp 661-669. ISBN 978-9975-56-911-8. 0,47 c.a.
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4.2. in international scientific conferences papers (Republic of Moldova)

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- în prima fază tehnologică de ouat. În: *Simpozion Științific Național cu participare Internațională: Biothnologii Moderne – Soluții pentru provocările lumii contemporane*. Institutul de Microbiologie și Biotehnologie. 20-21 mai (online). Chișinău, 2021, p. 149. ISBN 978-9975-3498-7-1. 0,05 c.a. https://ibn.idsi.md/sites/default/files/imag_file/149_6.pdf.
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13. **PISTOL Gh.** Aspecte ale metabolismului mineral la găini, tratate cu produsul ZooBioR utilizat într-un studiu de implementare. În: *Conf. națională cu participare internațională „Integrare prin cercetare și inovare”* 10-11 noiembrie 2022, Chișinău: CEP USM, 2022, pp. 93-95. ISBN 978-9975-152-48-8. https://ibn.idsi.md/sites/default/files/imag_file/93-95_30.pdf.
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15. **PISTOL Gh.**, MACARI V., ROTARI L., ROTARU A., PUTIN V., PAVLICENCO N. Efectele produsului ZooBioR asupra performanței productive, parametrilor morfometrici ai ouălor și ai sănătății găinilor ouătoare tinere. În: *Conferința științifică națională, consacrată jubileului de 95 ani din ziua nașterii academicianului Boris Melnic*, 13 februarie 2023. Chișinău: CEP, USM, 2023, p. 108-112. ISBN 978-9975-62-496-1. https://ibn.idsi.md/sites/default/files/imag_file/108-112_37.pdf.

ANNOTATION

PISTOL Ghorghie "Strengthening the health and bioproductivity of laying hens as a result of the use of the autochthonous product ZooBioR", PhD thesis in veterinary medical sciences, **Scientific specialty:** 431.01 Veterinary therapeutics, pharmacology and toxicology. Chisinau, 2025.

Thesis structure: introduction, 6 (six) chapters, general conclusions and recommendations, 137 pages of basic text, bibliography of 230 titles, 33 tables and 10 figures. The results are published in 15 scientific papers.

Keywords: Chickens, ZooBioR product, clinical and haematological status, protein, carbohydrate, lipid and mineral metabolisms, liver, trypsin-antitrypsin system, productive indices.

Purpose. To investigate the action of ZooBioR on health, clinical haematological status, basal metabolism, liver function, egg production in hens.

Objectives: Tolerance of ZooBioR by laying hens; To determine the action of ZooBioR on clinical and haematological parameters, metabolism and functional state of the liver in hens; to evaluate the action of ZooBioR on egg production and morphometric parameters; to investigate protein and carbohydrate metabolism in liver and muscle tissue; to investigate the trypsin-antitrypsin system in hens; to determine the contribution of the investigated indicators to the dose of ZooBioR in hens during the first laying period.

Scientific novelty and originality: For the first time, two studies were carried out: a scientific-practical study and an implementation study, consisting in the evaluation of the impact of the ZooBioR preparation on the clinical-hematological status, biochemical research on blood serum, liver tissue and muscle, egg production and egg morphometric investigations, determining the optimal dose of use of this product in hens, completed with the implementation of innovative procedures.

Main results: For the first time, the role of ZooBioR administered to chickens on clinical haematological status, protein, carbohydrate, lipid and mineral metabolism, liver functional status and trypsin-antitrypsin system was established. For the first time, biochemical changes in liver and muscle tissue in chickens were elucidated, as well as the implementation of this new procedure to enhance health and productivity in chickens.

Theoretical significance: A major problem has been solved in the field of poultry farming, which has allowed to note the results of the impact of ZooBioR on health, physiological-metabolic status, the development of a procedure for strengthening the health and productivity of hens.

Application value: The efficacy of using ZooBioR to fortify health, metabolism, liver functional status and bioproductive potential in chickens has been argued.

Implementation of the scientific results: The results of the study have been implemented in the poultry practice and used in the training process of students and master students of the Technical University of Moldova.

ADNOTARE

PISTOL Gheorghe „Fortificarea sănătății și bioproductivității găinilor-ouătoare în rezultatul utilizării produsului autohton ZooBioR”, teză de doctor în științe medical veterinare, **Specialitatea științifică:** 431.01 Terapie, farmacologie și toxicologie veterinară. Chișinău, 2025.

Structura tezei: introducere, 6 (șase) capitole, concluzii generale și recomandări, 137 pagini de text de bază, bibliografie din 230 de titluri, 33 tabele și 10 de figuri. Rezultatele obținute sunt publicate în 15 lucrări științifice.

Cuvinte-cheie: Găini-ouătoare, produsul ZooBioR, statusul clinico-hematologic, metabolismul proteic, glucidic, lipidic și mineral, ficat, sistemul tripsină-antitripsină, indici productivi.

Scopul cercetării: Elucidarea caracterului acțiunii produsului ZooBioR asupra creșterii și exploatarei găinilor ouătoare în vederea ameliorării sănătății și fortificării potențialului bioproductiv, cât și asupra statusului fiziologo-metabolic (sânge, ser sanguin, mușchi, ficat, ouă), și elaborarea procedurii de fortificare a sănătății și potențialului bioproductiv la aceste păsări.

Obiectivele lucrării: Aprecierea toleranței produsului ZooBioR și a acțiunii lui asupra parametrilor clinico-hematologici, metabolismului și stării funcționale a ficatului la găinile-ouătoare; Evaluarea acțiunii ZooBioRului asupra producției de ouă și parametrilor morfometrici ai acestora; Investigarea în țesutul hepatic și muscular a metabolismului proteic și glucidic și a sistemului tripsină antitripsină la găinile-ouătoare; Stabilirea dozei de administrare a ZooBioRului la găini în prima perioadă de ouat.

Noutatea și originalitatea științifică: În premieră a fost realizat un studiu științifico-practic și unul de implementare în care s-a evaluat impactul preparatului ZooBioR asupra statusului clinico-hematologic și realizate cercetări biochimice a serului sangvin, țesutului hepatic și muscular, determinată producția de ouă și indicii morfometrici ai ouălor, precum și conturată doza optimă de utilizare a acestui produs găinilor, finalizat cu implementarea procedurilor inovative.

Semnificația teoretică: A fost soluționată în domeniul aviculturii, o problemă majoră, ce a permis notarea rezultatelor de impact a ZooBioR asupra sănătății, statusului fiziologo-metabolic, elaborarea unui procedeu de fortificare a sănătății și productivității găinilor.

Valoarea aplicativă: S-a argumentat eficacitatea utilizării produsului ZooBioR pentru fortificarea sănătății, metabolismului, stării funcționale a ficatului și potențialului bioproductiv la găini.

Implementarea rezultatelor științifice: Rezultatele studiului realizat au fost implementate în practica avicolă și folosite în procesul de instruire a studenților și masteranzilor din cadrul Universității Tehnice a Moldovei

PISTOL GHEORGHE

**STRENGTHENING HEALTH AND BIOPRODUCTIVITY OF
LAYING HENS AS A RESULT OF USE OF THE
AUTOCHTONOUS PRODUCT ZOBIOR**

165.01 – HUMAN AND ANIMAL PHYSIOLOGY

431.01. VETERINARY THERAPEUTICS, PHARMACOLOGY AND TOXICOLOGY

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