Effects of food supplemented with ZooBioR product in young chickens on the functional state of the liver

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Abstract
Cyanobacteria Spirulina platensis is widely used as a biotransformer of bioelements and as a producer of biologically active substances with a wide spectrum of use. The current study is aimed at objectively examining the impact of the product ZooBioR (obtained from Spirulina platensis) on health, and especially on the marker parameters of the functional state of the liver in hens, in the first technological period of laying. The experiment was performed on 5 groups of birds (of 14 heads/group). In 4 groups out of 5, the food was supplemented with the remedy ZooBioR in different doses (5.0; 10.0; 15.0; 20.0 mg active substance/kg of fodder). It has been established that the tested product improves the health of laying hens, in the first technological period of laying, significantly contributes to improving the metabolic processes in the body, especially the functional state of the liver
Key words: *Young laying hens; ZooBioR Remedy; Liver; ALT and AST transaminases; Bilirubin and its fractions.*

Introduction

In recent years, in the Republic of Moldova, as well as in other countries worldwide, there is a growing interest in aviculture, which has certain advantages over other branches of modern animal husbandry due to its intensification and functionality (Macari V., Putin V., Gudumac V., 2009; Macari V. et al., 2014; Van Il., Marin Gh., 2016; Zoltan P. et al., 2011; Фисинин В. И., 2012). Birds` breeding and exploitation, especially using intensive breeding systems, is difficult, very even often compromised by the technological stress, of different origin and intensity. These factors indisputably influence in a negative way the birds` productivity and health. Also, according to literature data, in the process of breeding and intensive exploitation of birds, the most requested and the most affected organ is the liver (decrease of ALT transaminase and increase of AST, decreasing tendency in the first stage of research of total bilirubin and its fractions, as well as an increasing tendency at the end of research of total bilirubin and its fractions, alkaline phosphatase and its fractions decreased activity in the blood serum during research), the positive impact being oriented towards the optimization of metabolism in situations of higher metabolic loads, leading as well to higher egg production. (Macari V. et al., 2014; Putin V., 2012 ; Pavlicenco N., 2019; Rotaru A., 2016; Кольберг Н. А., Садовников Н. В., 2010).

Therefore, it is necessary a profound literature review of the various biologically active remedies action on the functional state of the liver, up to date studies regarding the long-term action of harmful external and internal factors on the animal body, on the digestive system, and especially on the liver (Macari V. et al., 2014; Maţencu D., 2019). Besides that, in recent years, there has been an increase in interest in growth stimulants, especially natural ones. Out of a great range of growth stimulators of different origin and categories, with varoius properties, the ones of natural origin, especially of plant origin, are considered to be the best ones, as they are harmless, and have good usage potential (Macari V. et al., 2014; Maţencu D., 2019; Pavlicenco N., 2019; Rotaru A., 2016; Moostan KM, 2011; Nickolova M., Penkov D., 2010; Offor CE, Aja PM, 2014).

As for these reasons, we have decided to evaluate the impact of the ZooBioR spirulina product, administered with food to young laying hens, on the liver functional state, studying the tolerance of this medicinal product, and shaping of the optimal dose of this remedy.

Material and method

The experiment was carried out at the avicol factory within the ***"Acustic Tehnologic"*** LLC, Floreni village, Republic of Moldova. The objective of the research was focused both, on the study of the new medicinal product - ZooBioR, as well as on the influence of this remedy on young laying hens. The product ***ZooBioR***, tested by us, is a complex natural remedy containing biologically active compounds derived from the cyanobacterium *Spirulina (Arthrospira) platensis*. ***ZooBioR - 2plus*** contains: amino acids, including free immunoactive ones and as component parts of peptides and proteins; polysaccharides; sulphated polysaccharides; phospholipids and the trace elements **zinc** and **selenium**.

The research was carried out on a number of 70 hens belonging to the Braun-Nic hybrid, divided into 5 groups, 14 heads per each. The birds included in the research were analogous in terms of age, physiological condition, origin, body weight, being accommodated in the same hall, with the same environmental conditions, and veterinary care. During the experiment the birds were monitored and examined for health assessment.

At the same time, the object of the research was the local product ***ZooBioR - 2plus***, administered to birds, in different doses, according to the experimental scheme, from table 1.

Birds` groups	No of birds	Administration route	Dose, mg active substance/kg of fodder	Administration regimen
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	

In order to assess the state of health, at the beginning of the experiment, and later on, the birds were examined, and in 5 laying hens, from each group, the body temperature and respiratory movements had been determined in one minute.

For laboratory investigations, blood samples were taken in three stages, in standard test tubes: at the beginning of the experiment, until the administration of the ZooBioR remedy, from 5 random hens; during the study, from 5 birds in each group – at about 1 month after the beginning of the study, as well as at the end of the experiment, which coincided with the 129th day of research.

The functional status of liver in birds was assessed by determining the activity of ALT and AST transaminases, alkaline phosphatase and its fractions, as well as bilirubin level and its fractions. The analyses were performed in blood serum on PowerWave HT plate spectrophotometric reader, BioTek, USA. The statistical results of the clinical and hematological indices were made using the parametric criterion Student`s t-distribution with a veracity of less than 0.05 ($P < 0.05$).

Results and discussions

During the experiment, for a period of more than 4 months, the ZooBioR product, tested on young laying hens, in the first technological laying period, in avicola factory conditions, did not cause side effects or other deviations in the development, productivity or health of birds. Regarding the morphopathological aspect, following the control sacrifices (5 hens from each group), no significant changes were found in the carcass and organs of the thoraco-abdominal cavity or the

brain. The study reveals that, in general terms, the tested product improves the birds` health status, presenting anti-stress and adaptive properties, through lower values of body temperature and respiratory movements as well. Besides other parameters investigated in this research, the most accurate and conclusive marker of liver function state is the activity of ALT and AST transaminases, which are presented in the statistics in table 2.

Table 2.

Transaminases and total bilirubin and its fractions values in blood serum in laying hens

Signification	Onset	Birds` Groups				
		CG	EG 1	EG 2	EG 3	EG 4
ALT, u/l						
1 sampling	13,41±1,4	16,97±2,35	14,67±1,78	15,30±1,68	19,49±0,88	15,29±2,39
2 sampling		18,02±1,14	14,25±1,09*	17,18±1,60	16,34±1,42	14,88±1,83
AST, u/l						
1 sampling	45,68±2,9	45,47±3,18	52,59±1,79	52,38±2,57	61,33±2,51***	53,43±2,77
2 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±1,6					
1 sampling		63,88±3,21	69,37±2,97	56,28±1,32	60,93±4,15	56,99±2,71
2 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±1,1					
1 sampling		34,66±2,35	42,79±4,40	31,03±1,70	37,89±3,89	33,63±3,52
2 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					
1 sampling		29,22±1,85	26,58±2,79	25,26±1,67	23,03±2,24	23,36±2,61
2 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 – approx. 1 month from the study onset; 2nd sampling – the end of the study, at the 129th day from the onset.

The results obtained (table 2) reveal that in the blood serum, at the first research term, the AST transaminase activity shows a marked growth tendency, which in the intact birds from the control group is 16.97 ± 2.35 u/l, representing an increase of 26.5%, compared to the background values. At this stage, the studied product exerts a dose-dependent action on the activity of the AST enzyme. Thus, the activity of this biochemical parameter decreased in three experimental groups (EG 1, 2 and 4), by 9.8-13.6% compared to the control group. At the same time, the parameter investigated in hens from EG 3, on the contrary, increased by 14.8% compared to the control values. The dynamics of ALT enzyme activity, at the end of the study, shows an upward tendenct of its value in the birds from the CG (+ 6.2%) compared to the previous level, while this upward tendency in EG 2 is 12.3%. The study shows that the ZooBioR product contributes to the decrease of AST transaminase activity, its value decreasing by 4.7-20.9% compared to the control group (p <0.05, for EG 1), good results that show the positive action of the tested product on the functional state of the liver. Similar results were obtained by other authors who administered to animals different biologically active remedies (Balanescu S., Voiniţchi E. et al., 2014; 2019; Macari V., Putin V., Gudumac V., 2009; Caradailî D., Manastîrli T., Roşca I., 2018).

The results estimating the activity of ALT (table 2) reveal that, at the first research, there were no changes in the control group, while the value investigated in the experimental groups showed a clear increase tendency of 15.2-34.9% in relation to the control group ($p < 0.001$, for EG 3). Similar results were found in quails, also used for egg production, and treated intramuscularly with the BioR remedy (Macari V. et al., 2014; Pavlicenco N., 2019), as well as in broilers, which benefited from other bioactive remedies. (Balanescu S., Voinițchi E. et al., 2019; Falcă C., Mocofan E., Morar D., 2009). In this context, the justification of the obtained results is revealed at the end of the experiment, when in the birds from the CG was found an increase of 31.8%, of ALT enzyme activity, compared to previous values ($p < 0.05$), repeating the dynamics of this enzyme, previously reported in the birds from the experimental groups. Therefore, the increased activity of this enzyme (AST) in the blood serum could be physiological, specific to the laying cycle of hens. At this last experimental stage, the serum activity of the ALT enzyme in 3 EG, except for EG 1 (minimum dose of ZooBioR), showed higher levels, of 3.2-17.5% in relation to the reference values. Based on the results obtained, a possible action mechanism of the test product could be the improvement of the proteosynthetic function of the liver, the reduction of hepatocyte alteration, as well as the improvement of protein metabolism in general.

In order to assess the evolution of liver metabolic processes in intact hens, as well as under the action of the studied remedy, we considered as important to assess the total bilirubin content and its fractions in the blood serum (table 2). Based on our study, we found out that at the first research term, serum bilirubin, in the birds from the reference group, showed a growth tendency of 4.8%, such a tendency being also reported in EG 1, of 8.6% compared to the group of control. The administration of the tested bioremedy, with food, to the birds from EG 2, 3 and 4, in periods of high metabolic load, such as the first laying period, managed either to maintain at the initial level, or to reduce the concentration of total bilirubin in blood by 10.8-11.9% compared to the control group. The decrease could be due to the involvement of the ZooBioR product in the metabolic processes that take place in the body of hens, and especially in the liver. Those specified are certified at the end of the study when there is a delayed tendency of serum bilirubin decrease in the CG by 7.5%, and in EG 1 by 13.0% compared to previous values. At the same time, in hens, whose food had been supplemented with the tested remedy, there was a slight serum bilirubin increase tendency, in general depending on the dose of the administered product, of 2.1-20.2% compared to the reference data. Similar results were obtained by other authors who administered to pregnant dogs the BioR remedy (Caradailî D., Manastîrli T., Roşca I., 2018), as well as to the rabbits, also the BioR remedy (Maţencu D., 2019).

The content of direct bilirubin (conjugated, bound) in serum, in intact hens, at the 1st research, has an increasing tendency of 10.3%, as compared to the background values, a phenomenon that can be attributed to the intensification of the physiological-metabolic processes that take place in the body of birds in the first intensive laying period. At this research term, the tested product did not unequivocally influence the investigated parameter, inducing in the birds from EG 1 and 3 an incresing tendency, of 9.3-23.5% compared to the reference indices. The study shows that the usage of the studied phytoproduct, at a dose of 10.0 mg active substance/kg fodder determines the maintenance of the investigated biochemical indicator at background levels, while the average value of direct bilirubin in EG 2 being 31.03±1.70 µmol/l, a decrease of 10.5% compared to the control group, and of 7.7-27.5% compared to the other three experimental groups ($p < 0.05$, compared to EG 1), results that will be taken into consideration as to determine the optimal dose of the product.

The study reveals that, at the last stage of research, the value of the parameter investigated in birds, from the CG, decreased, reaching an average of 32.53±2.09 µmol/l, the decrease being of

6.1%. Higher decreased values had been also reported in EG 1, birds in which the food was supplemented with ZooBioR in the lowest dose, the decrease being of 22.3% compared to previous values. At this last stage, the tested remedy induced, in all bird from all EGs, higher values of direct serum bilirubin, of 2.2-38.3% compared to the control group. These results can be explained by the intensification of the body metabolic processes, especially in the liver. Similar results regarding the increase of the serum level of direct bilirubin in rabbits, during the reproductive cycle, physiological, but stressful periods for the animals, have been obtained following the use of another biologically active remedy - BioR (Maţencu D., 2019).

An important biochemical criterion in assessing the functional state of the liver - indirect bilirubin (free, unconjugated) presented in the first research a serum stability in young intact hens, located at the background level, results that sum up several factors, reflected in birds` good health state. Indirect bilirubin shows a marked decrease tendency in hens from the groups supplemented with ZooBioR. While in the control group, the value of indirect bilirubin was 29.22 µmol/l, in birds from the EG it was 26.58-23.03 µmol/l, the decrease being 9.0-21.2%, an undeniably positive phenomenon, which probably denotes the intensification of the physiological-metabolic processes in the liver, as well as the improvement of erythrocytes function. Indirect bilirubin in the birds from the CG, at the end of the study, showed a decreasing tendency, of 9.1% compared to previous values, a delayed manifestation, which occurred in birds from EGs, during the first research. Meanwhile, in the hens from the EGs there was a slight upward tendency, of 1.9-14.7% compared to previous values, obviously in those groups. At the same time, much lower increased values had been reported in the birds from EG 1, 2 and 4 compared to the control group, the increase being of 0.9-8.7%. Similar direct bilirubin dynamics had been reported in animals by other authors, following the administration of other biologically active remedies. This phenomenon could be explained by the improvement of the liver function duet o these remedies (Maţencu D., 2019).

The evaluation results of the alkaline phosphatase and its fractions dynamics, in blood serum, in young laying hens, are shown in table 3.

Tabel 3.

Alkaline phosphatase and its fractions vaues, in blood serum, in young laying hens

Signification	Onset	Birds` Groups				
		CG	EG 1	EG 2	EG 3	EG 4
Total alkaline phosphatase, u/l	729,72± 64,11					
1 sampling		579,56±111,21	710,01±88,69	604,02±88,66	571,41±78,65	673,33±110,62
2 sampling		527,92±108,11	377,77±20,33**	455,22±65,71	580,92±125,71	383,20±29,45*
Termostable alkaline phosphatase, u/l	519,09± 87,60					
1 sampling		350,59±66,80	505,50±54,00	401,55±80,49	366,90±45,56	425,33±73,79
2 sampling		347,87±87,12	235,09±32,55	307,11±48,10	364,18±127,89	242,56±14,04
Termolabile alkaline phosphatase, u/l	210,63± 25,25					
1 sampling		228,97±47,74	204,51±47,52	202,47±32,82	204,51±37,09	248,00±47,42
2 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

The general analysis of the results (tabel 3) allows to highlight in birds a unique tendency of the total alkaline phosphatase decrease at the first research, this parameter reaching in the CG, the 579.56±111.21 u/l value, the decrease being of 20,6%, compared to the background values. In the case of EG 1, 2 and 4, the ZooBioR product stopped the decline of this enzyme by 4.2-22.5%, compared to the control group. Similar tendencies of ALP increase, in broilers, have been obtained by other authors who administered with food selenium (Falcă C., Mocofan E., Morar D., 2009).

We have noticed that, in the second research, the analyzed enzyme undergoes relevant changes, in the birds from the CG, decreasing by 8.9%, compared to the first research, a marked reduction tendency reported as well in EG 1, 2 and 4, its value decreasing by 1.3-1.9 times, compared to the previous values ($p < 0.05$, EG 4; $p < 0.01$, EG 1). We would like to highlight the fact that the parameter in the birds from the EG 3, proved to be higher, the increase being of 10.0% compared to the control group. Meanwhile, in the EGs, with the exception of EG 3, the total alkaline phosphatase (ALP) value shows a marked tendency of decrease compared the values of the control group, a decrease of 1.2-1.4 times, a phenomenon that could be considered positive, showing the anti-stress and hepatoprotective action of the ZooBioR product.

Studies have shown that the serum activity of thermostable ALP (liver fraction), in the 1st research, in birds from the CG, has a marked decreasing tendency, of 32.5%, compared to the initial level. It has been established that the usage of the tested product induces a statistically significant increase in the level of this enzyme, in the blood serum, which exceeded by 4.7-44.2% the control values. The results of the studies indicate that the serum concentration of the investigated enzyme, at the end of the experiment, remains practically at the same level (+ 0.80%), which invokes a unique health state of the intact birds throughout the whole experiment. The decreasing tendency of the thermostable ALP enzyme persists in birds from EG 1, 2 and 4, in which the investigated enzyme value is also lower than the control group values, the decrease being 11.7-32.4%. Similar results, regarding the possibility of decreasing the serum level of this enzyme, in rabbits, especially in the 45th postpartum day, as well as in broilers, at the end of the experiment, have been obtained by other authors, following the usage of certain bioactive remedies (Maţencu D., 2019; Putin V., 2014).

The data obtained (table 2) show that the activity of the thermolabile ALP enzyme (bone fraction) in intact birds, in the first research stage, has a growth tendency (+ 8.7%), an increase that persists in birds in EG 4, whose food was supplemented with the maximum dose of ZooBioR, the increase being of 17.7%, compared to the background values. In the other three EGs, the tested bioactive compound reduces the activity of the studied parameter, in which the functional capacity is 2.9-3.9% lower than the background, results that can be considered positive. At the end of the research, in birds from the reference group and EG 3, in serum had been attested a weak positive dynamics of the investigated enzyme activity, of 3.0% and 6.0%, respectively, compared to the previous values. At the same time, the investigated parameter in EG 1, 2 and 4 shows a marked decreasing tendency, of 1.4-1.8 times compared to the previous values, in the respective groups.

At the same time, in birds from all groups whose food was supplemented with the tested product, the investigated parameter has a marked decreasing tendency compared to the control values, by 1.1-1.7 times, a phenomenon that can probably be explained by the intensification of metabolism in general, and mineral metabolism in particular, including the processes related to eggshell formation. In the analyzed context, we would like to specify that animal production, including egg production, are accurate indicators of animal health. The study reveals that the egg laying intensity, at the beginning of the study, was 64.3-71.4% in all 5 groups of hens. It is important that, on the last day of study, this zootechnical parameter in the CG, constituted an average of 91.67%, compared to 100% in the EGs, resulting an increase of 8.3%.

The obtained results attest show that the ZooBioR product administered for a long period of time to laying hens was well tolerated. In addition, the changes of the enzymes` activity, namely the hepatic ones, probably represent one of the body's adaptation reactions, aimed at optimizing metabolism in situations of high metabolic load, such as intensive hens` exploitation, especially during the first technological period of laying.

Conclusions

1. The ZooBioR product, obtained from Spirulina platensis, administered with food, to young laying hens, for a period of approximately 4 months, was well tolerated and did not induce adverse reactions.

2. During the oral administration of the ZooBioR remedy to young laying hens, bred under physiological conditions of avicol factory, has been established a decreasing tendency of the ALT enzyme and an increasing tendency of the AST enzyme. ZooBioR has also induced, in the first research, a tendency of decrease of the total bilirubin and its fractions in blood serum, and an increase tendency of these parameters, at the end of the experiment, dynamics that reveal the functional state of the liver.

3. The study of the ZooBioR action on the activity of the ALP enzyme and its fractions revealed the property) of this local product to stop the decline of the ALP enzyme and its thermostable fraction, at the first experimental stage, of high metabolic load, while at the end of the experiment, a decreasing tendency of these parameters persisted, especially in hens treated with lower doses of the tested remedy.

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