Changes in the biochemical parameters of blood serum of laying hens by varying the feeding diet

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Abstract. The article presents experimental data on the influence of the feeding diet on the biochemical blood parameters of laying hens. As feed additives in the poultry diet were used: feed vitamin-mineral concentrate (FVMC) in the amount of 0.3% of the basic diet (BD) (1st experimental group), probiotic additive “Bacell-M” in the amount of 0.2% of BD (2nd experimental group), complex feed concentrate (CFC), containing FVMC and probiotic additive “Bacell-M”, in the amount of 0.3% of BD (3rd experimental group). The control group received only BD. It was found that the introduction of the feed additives into the diet of laying hens stimulated the metabolic processes in the poultry body, which was manifested by an increase in a number of biochemical constants. So, the level of total protein in the 1st experimental group increased by 34%, in the 2nd and 3rd experimental groups it increased in 1.43 and 1.42 times in comparison with the control group. The urea concentration exceeded the analogical values of the control poultry by 27% (1st experimental group), by 23.2% (2nd experimental group) and by 24.3% (3rd experimental group), respectively. The use of feed additives influenced the state of carbohydrate and lipid metabolism of hens, which was manifested by an increase in glucose concentration in the groups by 6.3%, 13.1% and 21.5% and by an increase of triglycerides by 13.2%, in 1.7 and in 2.0 times respectively. The inclusion of additives in the feeding schemes contributed to the correction of mineral metabolism, providing more complete absorption of calcium by the poultry, the increase of which in the experimental groups was by 16.2%, 1.68% and in 2.21 times, and also prevented the development of hepatocyte cytolysis, as indicated by a decrease in the activity of hepatoindicatory enzymes: ALT decreased by 9.8%, 29.5% and in 1.85 times, AST decreased by 7.9%, 12.7% and 18.9%, respectively. The maximum positive effect in the indicators of the biochemical status of laying hens was observed when CFC was used in the diet, which was due to the synergy of FVMC and the Bacell-M probiotic additive.

1. Introduction

Chicken eggs are important and common type of poultry products. It is quite difficult to cover the whole spectrum of their use in food: eggs are used in the production of bakery, flour confectionery and confectionery, ice cream, meat semi-finished products and so on. It is necessary to add that chicken eggs are rich in amino acid balanced protein, carotenoids, vitamins, polyunsaturated fatty acids and micro
and macro elements involved in the regulatory processes of the body. The digestibility of eggs by the human body is at the level of 97–98%, while their use in food has virtually no age restrictions.

However, the production of quality eggs is only possible while maintaining the health of laying hens. Currently, scientists in several countries pay a great attention to the issues of prevention and health recovery of laying hens without the use of antibiotics, since the improper or excessive use leads to their accumulation in the received products.

An alternative to the use of antibiotics are plant and animal feed additives containing organic acids [1], amino acids [2], vitamins [3-5], mineral substances [6, 7] and other biologically active substances (BAS) [8] as well as probiotics [9-13], stimulating protective functions of the poultry organism.

The feed additive L-theanine, used in an amount of 200 mg / kg of the basic diet (BD) of broiler chickens, has a protective immune modulating function, the number of pro-inflammatory cytokines in hens decreases, and the antioxidant ability of the body increases [14,15], (resveratrol can be successfully used in laying hens diets to prevent fatty liver hemorrhagic syndrome [16].

The use of Boswellia serrata resins in the form of a feed additive in the diets of broiler chickens for 6 weeks affects the accumulation of polyunsaturated fatty acids in the muscle tissue of the poultry, at the same time the atherogenic and thrombogenic parameters, as well as hypercholesterolemic / hypercholesterolemic coefficient improves [17].

The use of sugar beet pulp and other cork products of sugar production up to 7% in diets of laying hens not only increases their productivity, improves blood biochemical parameters and egg quality, but also provides a decrease in the content of malondialdehyde (MDA) while increasing glutathione peroxidase, which, in turn, allows to increase the shelf life of eggs [18, 19].

To increase the productivity of laying hens, feed additives containing fennel seeds and red pepper are used, and to reduce the MDA content in the blood serum of laying hens and in eggs, black cumin and red pepper are used [20].

When keeping laying hens in conditions of low temperatures, the use of Tribulus terrestris extract in diets is effective, which allowing not only to increase productivity, but also to improve the quality of the shell [21]. There are well known studies on the use of Yucca extract [22], rosemary and cinnamon essential oils [23], ginger [24], soybean extract [25], which effect is aimed at increasing productivity and normalizing biochemical blood parameters. The influence of feed additives containing organic acids on the microflora of the intestines of broiler chickens has been proved.

In connection with the foregoing, the relevant area of our research was the possibility of studying the influence of the feeding diet containing various feed additives on the biochemical blood parameters of laying hens.

2. Materials and methods
As the feed additives used in the diet of laying hens, were used: feed vitamin-mineral concentrate “Tetra +” (FVMC), containing a complex of biologically active substances such as beta-carotene, vitamins C and E, plant phospholipids and selenium in organic form; “Bacell-M” probiotic feed additive (Bacell-M), containing a complex of probiotic cultures Bacillus subtilis, Lactobacillus paracasei, Enterococcus faecium and a complex feed concentrate (CFC), containing FVMC and Bacell-M in an effective ratio.

A scientific production experiment to study the influence of the feeding diet on the biochemical parameters of poultry blood was carried out on the laying hens of the Adler silver breed of meat and egg line at the age of 20 weeks.

According to the research scheme (table 1), the poultry was divided into 4 groups of 100 hens. The first group in addition to the basic diet (BD) received Bacell-M in an amount of 0.2% by the feed weight, the second group received FVMC in an amount of 0.3% by the feed weight, and the third group received 0.3% of CFC by the feed weight. The control group received only the feed of the basic diet.

**Table 1.** Feeding scheme of young laying hens.

| Groups | Feeding diet |
|--------|-------------|

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The duration of the experimental period was 30 days.

On the 30th day of the experiment, blood was taken from the axillary vein of poultry from each group (n=5) to assess the main indicators of metabolism and liver activity. Biochemical studies of blood serum were carried out on an automatic analyzer – Vitalab Selectra Junior (Netherlands) using reagents from ELITEch Clinical Systems (France) and Analyticon biotechnologies AG (Germany).

The obtained data were subjected to statistical analysis, the results of the Student’s t-test and confidence intervals were presented.

3. Results and discussion

Since the changes in protein metabolism are one of the important stages in studying the adaptogenic response of the body, the protein status of the poultry during the experiment was estimated by the level of total protein, urea, and creatinine in blood serum (table 2).

| Group of laying hens   | Indicator                      |
|------------------------|--------------------------------|
|                         | Total protein, g / l | Urea, mmol / l | Creatinine, μM / l |
| Control group (BD)     | 38.25 ±2.45          | 1.85 ±0.13     | 15.10 ±0.97        |
| Experimental groups:   |                  |                |                    |
| 1 – BD+Bacell-M        | 51.25 ±3.29*        | 2.35 ±0.18*    | 14.00 ±0.99        |
| 2 – BD+FVMC           | 54.73 ±3.51**       | 2.28 ±0.14     | 10.43 ±0.67*       |
| 3 – BD+CFC           | 54.30 ±3.48*        | 2.30 ±0.15*    | 8.08 ±0.52**       |

** p>0.01; * p<0.05.

The results of studies showed that in all experimental groups of laying hens an increase in the concentration of total protein was determined by the end of the experiment. The differences between the first experimental group and the control group amounted to 34.0% (p<0.05) in favor of the experimental poultry. While the maximum difference with the control analogues (in 1.43 and in 1.42 times) was recorded in the 2nd and 3rd experimental groups of laying hens (figure 1).

According to the level of urea, a significant increase in this metabolite was recorded in comparison with the control: in the 1st experimental group by 27% (p < 0.05); in the 2nd experimental group – by 23.2%; in the 3rd experimental group – by 24.3% (p <0.05) (figure 2).

The changes in the protein metabolism of poultry due to the influence of the components of additives were revealed, the effectiveness of which was manifested in the activation of protein metabolism according to the better inclusion of amino acids in protein synthesis.

In addition, the use of feed additives provided a decrease in creatinine concentration with a maximum difference in 1.87 times in hens treated with CFC (figure 3). Separate use of feed additives led to a decrease in this factor of protein metabolism by 7.3% (Bacell-M) and by 30.9% (FVMC). An increase of creatinine in blood serum may be an objective indicator of the activation of the adrenohypophysial system, which indicates the stress stimulation of the body. The antioxidants presented in FVMC showed a protective effect at the cellular level, which allows optimizing the protein status of the poultry, including increasing the adaptive capacity of the body.
Figure 1. Influence of the feeding diet on the change in the total protein concentration in the blood serum of laying hens of the experimental groups in comparison with the control group.

Figure 2. Influence of the feeding diet on the change in the urea concentration in the blood serum of laying hens of the experimental groups in comparison with the control group.

Figure 3. Influence of the feeding diet on the change in creatinine in the blood serum of laying hens of the experimental groups in comparison with the control group.

The table 3 presents data on the influence of the feeding diet of laying hens on lipid metabolism. The data of the table 3 shows that in the poultry of the control group a moderate hypocholesterolemia is observed. The use of the probiotic in the diet (1st experimental group) contributed to a slight increase
in blood cholesterol of hens (differences with the control analogues amounted to 16.9% in favor of experimental laying hens). However, the limits of the species norm for cholesterol levels in this group for 30 days of experiment could not be reached.

**Table 3.** Influence of the feeding diet of laying hens on lipid metabolism.

| Group of laying hens | Indicator | Triglycerides, mmol / l | Cholesterol, mmol / l |
|----------------------|-----------|-------------------------|-----------------------|
| Control group (BD)   |           | 0.53 ±0.03              | 2.18 ±0.14            |
| Experimental groups: |           |                         |                       |
| 1 – BD+ Bacell-M      |           | 0.60 ±0.04              | 2.55 ±0.16*           |
| 2 – BD+FVMC          |           | 0.91 ±0.06**            | 3.08 ±0.13*           |
| 3 – BD+CFC           |           | 1.07 ±0.07*             | 4.10 ±0.26**          |

**p>0.01; *p<0.05.**

At the same time, the introduction of FVMC to the feeding diet of laying hens had a stabilizing effect on cholesterol metabolism. In the 2nd and 3rd experimental groups, cholesterol concentration was registered within the reference norm values, exceeding this indicator of the control poultry in 1.4 and 1.9 times, respectively (figure 4).

**Figure 4.** Influence of the feeding diet on the change in cholesterol in the blood serum of laying hens of the experimental groups in comparison with the control group.

The level of triglycerides increased significantly by 13.2% (1st experimental group), in 1.7 times (2nd experimental group) and in 2.0 times (3rd experimental group) relative to control indicators (figure 5).

The use of feed additives influenced on the state of carbohydrate metabolism, in which the blood glucose level of the experimental poultry receiving Bacell-M increased by 6.3% (p<0.05); in the group receiving FVMC it increased by 13.1% (p<0.05), and in the group receiving CFC it increased by 21.5% (p>0.01) (table 4). Analyzing the results, it can be noted that FVMC have a dominant effect on carbohydrate metabolism, while Bacell-M potentiates the action of FVMC.

The influence of the feeding diet of laying hens on the activity of liver enzymes (AST and ALT) is presented in the table 5.

Enzymological changes in blood, the nature of which is presented in table 5, have not a diagnostic but a metabolic meaning and characterize the biochemical status of the body, since the enzymes react as quickly as possible by changing their activity even to minor metabolic disorders and can be an indicator of pathological conditions of the body. Studies of poultry blood serum revealed a general decrease in the activity of aminotransferases under the influence of additives.
Figure 5. Influence of the feeding diet on the change in the triglycerides concentration in the blood serum of laying hens of the experimental groups in comparison with the control group.

Table 4. Influence of the feeding diet of laying hens on glucose in the blood serum.

| Group of laying hens         | Glucose concentration, mmol / l | The change in glucose concentration in relation to the control group, % |
|------------------------------|---------------------------------|---------------------------------------------------------------------|
| Control group (BD)           | 12.18 ±0.62                     | –                                                                   |
| Experimental groups:         |                                 |                                                                     |
| 1 – BD+Bacell-M              | 12.95 ±0.79*                    | +6.3                                                                |
| 2 – BD+FVMC                 | 13.78 ±0.85*                     | +13.1                                                               |
| 3 – BD+CFC                  | 14.80 ±0.95**                    | +21.5                                                               |

**p>0.01; *p<0.05.

Table 5. Influence of the feeding diet of laying hens on the activity of liver enzymes.

| Group of laying hens         | Indicator          | AST, Units / l | ALT, Units / l |
|------------------------------|--------------------|----------------|----------------|
| Control group (BD)           |                    | 183.75 ±11.79  | 15.25 ±0.98    |
| Experimental groups:         |                    |                |                |
| 1 – BD+Bacell-M              |                    | 169.25 ±10.86* | 13.75 ±0.95*   |
| 2 – BD+FVMC                 |                    | 160.50 ±11.07* | 10.75 ±0.69**  |
| 3 – BD+CFC                  |                    | 154.50 ±9.91** | 8.25 ±0.53**   |

**p>0.01; *p<0.05.

The most significant effect of changes in the activity of hepatoinicator enzymes was registered in laying hens receiving CFC (3rd experimental group), which was manifested by a decrease of ALT level in blood in 1.85 times and AST level by 15.9% with a high degree of confidence (p<0.01) relative to the control group (figures 6 and 7).

However, it should be noted that the decrease in enzyme activity in all experimental groups occurred within the species norm for poultry. Nevertheless, a similar effect should be considered as a positive factor, manifested when using feed additives, and indicating a more stable state of the cellular structures of the liver of poultry under their influence.
Figure 6. Influence of the feeding diet on the change of ALT concentration in the blood serum of laying hens of the experimental groups in comparison with the control group.

Figure 7. Influence of the feeding diet on the change of AST concentration in the blood serum of laying hens of the experimental groups in comparison with the control group.

Analyzing the indicators of mineral metabolism (table 6), it was found that the use of Bacell-M led to an increase in the total calcium concentration by 16.2%, inorganic phosphorus – by 27.9% relative to the control poultry.

Table 6. Influence of the feeding diet of laying hens on the concentration of calcium and phosphorus in the blood serum.

| Group of laying hens | Total calcium, mmol / l | Inorganic phosphorus, mmol / l | Ca : P ratio |
|----------------------|-------------------------|-------------------------------|--------------|
| Control group (BD)   | 2.28 ±0.15              | 1.68 ±0.11                    | 1.4:1        |
| Experimental groups: |                         |                               |              |
| 1 – BD+Bacell-M      | 2.65 ±0.17*             | 2.15 ±0.14**                  | 1.2:1        |
| 2 – BD+FVMC          | 3.83 ±0.25*             | 2.23 ±0.14**                  | 1.7:1        |
| 3 – BD+CFC           | 5.05 ±0.32**            | 2.68 ±0.11**                  | 1.9:1        |

*p>0.01; *p<0.05.

The use of FVMC to laying hens contributed to an increase in the concentration of total calcium in 1.68 times and an increase in inorganic phosphorus by 32.7%. However, the most pronounced effect on the level of macronutrients was noted when using CFC due to an increase in the calcium concentration in the blood serum of laying hens by 5.05 ± 0.32 mmol / l (in 2.2 times relative to the control group).
terms of phosphorus level, the difference in intergroup indicators was in 1.6 times higher (2.68 ± 0.11 mmol/l versus 1.68 ± 0.11 mmol/l).

4. Conclusion
The results of studies on the influence of the feeding diet containing feed additives (Bacell-M – 0.3% to BD, FVMC–0.2% to BD and CFC – 0.3% to BD) confirm their positive effect on metabolic status and biochemical homeostasis of laying hens blood. The use of these additives reduces the activity of hepatoinicator enzymes – AST and ALT, helping to stabilize the functional state of liver cells. At the same time, the maximum positive effect of the poultry biochemical status is observed when using CFC in the diet, which was due to the synergy of FVMC and Bacell-M.

Thus, it can be assumed that the introduction of CFC to the BD of laying hens will maximize their productivity. In addition, it is advisable to identify in the diet, which was due to the synergy of FVMC and Bacell

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