Formation of element status at chickens when using enzyme, probiotic and antibiotic agents in food

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Abstract. The paper considers the biological effect of probiotic, enzyme and antibiotic agents on metabolism, efficiency and the element status of chickens. Joint inclusion of soybean-bifidum (0.7 ml/kg of feed), Tselloviridin G20x and Biovit-80 resulted in the increase of the egg weight by 9.8 and 9.0%, respectively in comparison with other groups. The group receiving Sporobacterin (10 ml/kg of feed) with feed was characterized by high fat availability (68%). The greatest conversion of feed gross energy was observed in groups in case of joint inclusion of Tselloviridin with probiotic and antibiotic agents (2.8 and 3.0%) and crude protein (6.2 and 7.0%). The spectral microelement analysis showed that the additional inclusion of biologically active agents caused their redistribution towards the increase of the need for main micronutrients for metabolism. All groups were characterized by a reliable increase of essential and conditional-essential elements such as Ca, K, Mg, Na, P, Cr, Cu, Fe, I, With, Mn, Se, Zn against the background of decrease such toxic elements as As, Al, Ag, Sr, Pb, Cd. The received results confirm the modeling role of probiotics, enzymes and antibiotics in the exchange of micro- and macronutrients.

1. Introduction

Intensification of livestock production and poultry farming is based on the use of various biologically active agents necessary to compensate the costs of feeding and prevention of diseases. They include probiotic, enzyme and antibiotic agents. Since their discovery in the 1920s and until now the antibiotics are used in feeding at subtherapeutic level to improve growth, efficiency of feed and prevent infections [1]. However, there are still ambiguous opinions on the transfer of resistance genes to antibiotics from animals to pathogens of a person [2-6]. Besides, uncontrollable use of antibiotics may lead to alimentary diseases connected with the violation of the element status of an organism. In recent years there has been many studies devoted to the development of alternative agents to antibiotics to prevent diseases and increase the productivity of birds, which was recorded in 2015 in VFD (Veterinary Feed Directive).

Such potential alternatives to probiotics as direct fed microbial (DFM) [7] are used to stimulate digestion, activate digestive enzymes, improve egg laying and egg weight [8-9] against no information on
the stimulating effect of probiotics on the organism of broiler chickens, laying birds and turkeys [10-11].

Enzymes [12-13], which effect also depends on type, source, quantity, side effects, type of diet [14-15], also have a similar effect on digestion.

Thus, the use of biologically active agents in diets of agricultural birds is favorable for the synthesis of antimicrobial compounds and stimulation of immune reactions of an organism [16]. At the same time, any stimulation of metabolism processes causes the imbalance of animal microecological status [17-18], in particular microelements, many of which serve as cofactors of many biochemical reactions.

In this regard, the study of specific influence of probiotic, enzyme and antibiotic agents on the exchange of certain elements, shall ensure effective correction of diets concerning a complex of macro- and microelements in the long-term perspective.

2. Materials and methods

2.1. Animals and feed

The study was conducted on laying chickens (21 weeks old) in laboratory conditions of biological tests and trials of the Federal Scientific Center of Biological Systems and Agrotechnologies of the Russian Academy of Sciences and the Testing Laboratory (Federal Scientific Center of Biological Systems and Agrotechnologies of the Russian Academy of Sciences, certificate of accreditation RA. RU.21PF59 of 02.12.15). Duration of experiment – 7 weeks.

Experimental part of the study was carried out according to the protocols of the Geneva Convention and the principles of appropriate laboratory practice (National standard of the Russian Federation GOST P 53434-2009), good laboratory practice of pre-clinical studies in the Russian Federation (GOST 3 51000.4-96), while the experimental study on animals was conducted according to instructions of the Russian Regulations, 1987 (Order No. 755 of 12.08.1977, USSR Ministry of Health) and the Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996).

The birds were kept in specialized cages KUN-05 4050 cm² in area (90 × 45 × 45 cm). The birds were marked with plastic foot tags.

2.2. Observation, estimated indicators

During the study the laying chickens were kept in similar conditions. The birds were fed 2 times a day with a diet created taking into account the recommendation [19]. The basal diet (BD) included barley and wheat mixed rations (available energy – 9.8 MJ/kg of dry solids and 172.3 – crude proteins). The ration of chickens of the I experimental group included BD with enzyme agent Tselloviridin G20x (60 g/t of feed), II experimental group – BD and Tselloviridin G20x and probiotic agent Bifidumbacterin (Soybean-bifidum, 0.7 ml/kg of feed); III group – BD and Biovit-80 (10 g/kg of feed); IV group – BD and Tselloviridin G20x and Biovit-80; V group – BD and Bifidumbacterin, VI experimental group – BD and Sporobacterin (10 ml/kg of feed).

Tselloviridin G20x is produced by the Federal State Unitary Enterprise Berdinsky Plant of Biological Products: cellulose – 2000 pcs/g, beta-1,3-glucanase – 3101 pcs/g, arabinoylase – 74510 pcs/g, etc.

The Soybean-bifidum (state registration of the Ministry of Health of the Russian Federation No. 77.99.11.3. U.5249.10.04 and No. 77.99.11.3.U.5246.10.04 with inclusion into the Federal Register of Biologically Active Dietary Supplements) includes Bifidobacterium longum bacteria, content of microbial cells – at least 10⁸ per 1 g.

Biovit-80 (registration No. GDH 2/05/0079-95) includes Chlorotetracycline – 8%, from 8 to 20 mg/kg of vitamin B12.

Probiotic Sporobacterin (Bacillus subtilis, strain 534) contains 10⁹ microbial bodies in 1 ml of the product (state registration of the Ministry of Health of the Russian Federation No. 000792/01-2001 of 01.11.2001), dosage according to data [8].

The biomaterial for the study was received after decapitation of chickens under nembutal anesthesia at the age of 28 weeks. This followed postmortem poultry cutting with further average test from
muscular and bone tissues, fur, internals with subsequent grinding and combustion (Multiwave 3000, Anton Paar, Austria). The trace element analysis was carried out by atomic emission spectrometry (Optima 2000 V, Per556 kin Elmer, USA) and mass spectrometry (Elan 9000, Perkin Elmer, USA) according to manufacturer’s recommendations.

The studies were conducted in the laboratory of the Center of Biotic Medicine in Moscow (ISO 9001:2000, Certificate number 4017) via ICP-AES and ICP-MS (MUK4.1.1482-03).

2.3. Statistical processing

The statistical analysis was performed using ANOVA techniques (Statistica 10.0 software package, StatSoft Inc., USA) and Microsoft Excel. Statistical processing included the calculation of the average value (M) and standard errors of the mean (±SEM). The statistical significance of compared indicators was determined by the Student’s t-test. The level of significant difference was set as $P<0.05$.

3. Results

The inclusion of used agents into a diet of laying chickens had no reliable impact on the quantity of consumed feed and body weight.

The greatest digestibility of crude protein was noted in the II (84.27%) and IV (85.37%) experimental groups, and the highest fat (68%) in the group receiving Sporobacterin with feed. The digestibility of feed carbohydrates in the II and VI experimental groups reached 85.91 and 86.5% respectively. The chickens of these groups exceeded the control group in terms of the amount of laid eggs by 13.1 and 12.0% respectively against the background of insignificant decrease of the average egg weight – 56.0 and 57.0 g in relation to the control group (58.2 g).

The joined use of agents in diets of the II and IV groups increased the egg weight in relation to the control group by 9.8 and 9.0% respectively. In other groups the egg laying intensity was less expressed.

The inclusion of antibiotic and probiotic agents into the ration of laying chickens against the background of enzyme-containing diets changed the conversion of feed protein and its gross energy (Table 1).

| Table 1. Conversion ratio of feed energy and protein into products, % |
|---------------------------------------------------|------------------|
| Group     | Conversion ratio     |
|          | gross energy | crude protein |
| Control  | 23.3          | 25.7          |
| I experimental | 24.0      | 31.1          |
| II experimental | 26.1      | 30.8          |
| III experimental | 24.3      | 29.0          |
| IV experimental | 26.3      | 31.9          |
| V experimental     | 22.9          | 27.8          |
| VI experimental    | 20.5          | 32.7          |

In particular, the greatest conversion of gross energy was noted in the II and IV experimental groups (2.8 and 3.0% compared to the control group) and the greatest conversion of crude protein – IV and VI experimental groups (6.2 and 7.0% compared to the control group). In other groups the effect was rather low.

According to trace element analysis and the established significant difference ($P \leq 0.05$) with the control group the element profiles demonstrating variability of the element status depending on nutrient availability of an organism were created.

The content of manganese (21.6%), cobalt (14.6%), copper (16.3%), iron (24.5%), nickel (23.9%), calcium (28.0%), phosphorus (22.8%), lead (44.1%), vanadium (15.5%), strontium (20.4%) and aluminum (55.9%) decreased with the inclusion of enzyme agent Tselloveridin against the background of the increase of silver by 44.9% in relation to the control group.
The joint feeding of Tselloveridin and Bifidobacterin increased the content of manganese (20.3%), cobalt (18.4%) and silver (2.3 times) and decreased iron (17.3%) and nickel (15.6%) in the body tissues of experimental birds.

\[
I = \frac{\uparrow Ag \downarrow Mn, Co, Cu, Fe, Ni, Pb, V, Sr, Al, Ca, P}{Fe, Ni}
\]

The antibiotic had the expressed impact on the mineral status of an organism, which was expressed by the increase of selenium (10.8%), cobalt (26.8%), chromium (26.4%), silver (2.0 times), potassium (25.4%), magnesium (23.6%) and sodium (16.9%). Biovit-80 as part of a diet reduced the availability of copper, iron, nickel, lead and cadmium by 18.4; 23.5; 20.2; 41.5 and 24.2% respectively.

\[
II = \frac{\uparrow Mn, Co, Ag}{\downarrow Fe, Ni}
\]

Joint feeding of birds with antibiotic and probiotic agents stimulated the exchange of cobalt (29.7%), chromium (24.2%), cadmium (2.6 times), silver (by 2.8 times) and magnesium (10.9%). The depression of exchange was typical for copper – 31.7%, iron – 20.9%, iodine – 18.9%, arsenic – 26.2%, lead – 58.8%, aluminum – 47.3%.

\[
IV = \frac{\uparrow Co, Cr, Cd, Ag, Mg}{\downarrow Cu, Fe, J, As, Pb, Al}
\]

The inclusion of probiotic agent Soybean-bifidum increased the content of chromium and aluminum by 25.2 and 74.3% respectively (P <0.05).

\[
V = \frac{\uparrow Cr, Al}{\downarrow Cu, Fe, Ni, As, Pb, Sr, Cd, Ca}
\]

The opposite influence of the agent was noted concerning copper, iron, nickel, arsenic, lead, strontium, cadmium and calcium (P <0.05). The decrease of these elements in the control group made 25.4; 26.5; 12.5; 21.6; 49.9; 20.0; 28.7 and 30.4% respectively.

The addition of probiotic Sporobacterin in the feed of experimental birds of the VI group was expressed in reliable (P <0.05) accumulation of cobalt (23.2%), iodine (by 2.1 times), vanadium (13.0%) and arsenic (2.3 times), toxic aluminum (3.3 times) and decrease of selenium by 13.0%, iron – 10.8%, phosphorus – 8.7%, cadmium – 25.5%, lead – 53.6%.

\[
VI = \frac{\uparrow As, Co, I, Al, V}{\downarrow Se, Fe, Cd, P, Pb}
\]

The obtained data showed that the use of biologically active agents affects homeostasis and the mineral status of an organism in all studied groups.

4. Discussion

It shall be noted that from the perspective of the functional loading on digestive tract as a device that adjusts the intake metabolites with genetically programmed requirements of an organism the inclusion of biologically active agents forms individual mineral metabolism in the organism. Microelements are markers of adaptation to the habitat. The study specified the role of probiotics in mineral metabolism, as well as productive qualities without reserve functions of an organism [20].

In particular, the probiotics based on Bacillus sp. (B. coagulans, B. subtilis, B. licheniformis и B. amylophiluateformi) [21] most actively participate in the metabolic process. It is known that the gut microflora is the main consumer of chemical elements, and the additional inclusion of biologically active agents leads to redistribution of substances towards the increase of the need for basic micronutrient for
metabolism. A significant amount of reduced substance in gut contents may contribute to the development of microflora. It is possible due to joint use of enzyme agents with antibiotics, which strengthens the positive action of the former ones.

Similar effect of enzyme agents on productivity and efficiency of birds is well studied. However, the created mechanisms of action of biologically active agents do not include the influence on the element status of an organism. Direct action of enzyme and probiotic agents in mono- and polyvariant system is mainly caused by the variability of qualitative and quantitative structure of microorganisms in the digestive tract. These shifts may lead to balanced microbiota, which is more stable and able to stimulate the transformation of energy and nutrients for building tissues [22].

The study made it possible to establish that the coefficient of digestibility of crude protein was the highest in the group of birds jointly receiving enzyme-probiotic and enzyme-antibiotic agents – II and IV experimental groups. Similar studies were conducted in works [23], which describe the antibiotic mechanism of action on metabolism in the organism of animals. It was established that the inclusion of enzyme agents changed the mineral status of chicken organisms. The study also showed that the content of such essential elements as Mn (20.3%), Co (18.4%) and Ag (2.3 times) did not decrease in only in group II receiving enzyme agents together with probiotic, but at the same time Fe (17.3%) and Ni (15.6%) were fixed and released from an organism thus changing microbiocenosis of digestive tract.

The problem of interaction of enzymes and mineral substances of feeds was studied by some authors [24], which showed that chemical elements can strengthen and suppress their effect on the activity of enzymes. The results received during laboratory tests showed that the joint feeding of enzyme and probiotic agents increases the conversion of gross energy by 2.8% and crude protein by 5.8%. It also decreased the content of toxic elements: Pb (44.1%), Sr (20.4%) and Al (55.9%).

The studies showed [25] that long application of feeds containing antibiotics decreases riboflavin, folic acid, niacin, which participate in the vitamin synthesis. The addition of biologically active agents shall consider the fact that a certain set of chemicals is necessary for functional activity of each cell. Their concentration shall fall within a narrow range of values since certain threshold values of chemical elements in an organism activate the specific compensatory mechanisms aimed at normalization of level of this element. These actions could trigger various effects of using biologically active agents.

The study also showed that the introduction of antibiotic and probiotic agents into the ration reduces the accumulation of such toxic elements as As, Al, Ag, Sr, Pb, Cd in the organism of chickens. It shall be noted that the productive qualities of birds depend on various reasons, including the availability of macro- and micronutrients.

5. Conclusion

Thus, the received results prove the modeling role of probiotics, enzymes and antibiotics in the metabolism of micro- and macronutrients. Probiotic agents in pilot studies were exerting protective action on toxic elements, reducing their content and ensuring bioactive properties thus regulating the impact on intensity of metabolic processes, enhancing functional activity of organs and systems of an organism, increasing the autarcosis level of birds. The potential of using enzymes as an alternative to antibiotics to improve the productivity of poultry is quite high. In this regard to ensure the efficiency of the above agents there is a need for continuous monitoring of the element status of an organism for further correction of macro- and microelements.

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