Peculiarities of the metabolism in broiler chickens under the introduction of multienzyme complex “Rovabio” in the diet

A Yu Nikitin1, S V Lebedev1,2, V V Grechkina1,3, A I Vishnyakov2

1 Federal Scientific Centre of Biological Systems and Agrotechnologies of Russian Academy of Sciences, 29, 9 Yanvarya str., Orenburg, 460000, Russia
2 Orenburg State University, 13, Pobedy ave., Orenburg, 460018, Russia
3 Orenburg State Agrarian University, 18, Chelyuskintsev str., Orenburg, 460018, Russia

E-mail: lsv74@list.ru, +7 (912) 345-87-38

Abstract. The article presents data on studying effects of the enzyme preparation “Rovabio” in rye-containing diets for broilers of the cross “Smena-7” at the age of 14 to 42 days. It was revealed that the inclusion of the enzyme preparation “Rovabio” in the dose of 50 g/t in the combination with trace elements CoCO3 (0.57 mg/kg of feed), Cr2(SO4)3*6H2O (0.38 mg/kg of feed) with the replacement of 15% of wheat to 15% of rye into the diet demonstrated a positive impact of the used dosages of the trace elements on digestibility of organic matter (6.49%), crude protein (4.59%), the protein synthesis and stimulated hematopoiesis in birds. The multienzyme complex increases the integrity of chickens to 96.7% and profitability of poultry meat production by 2.0% due to endo-1,4-ß-xylanase and endo-1,3(4)-ß-glucanase.

1. Introduction
Livestock production is currently a hot topic worldwide, it is directly related to the quality of human nutrition. And in a relatively short time, the poultry industry could provide the market with high-quality meat products. Feeding with high quality and inexpensive animal feeds is always one of the most important tasks in the poultry industry. The application of biologically active feed additives to optimize the metabolism with the inclusion of non-conventional feed such as rye in the diet becomes more relevant at poultry farms [1-2].

The problem of the rye application in the poultry industry lies in its anti-nutrition from 7.5 to 9.1% of pentosan and from 0.5% to 3.0% of β-glucan that lower digestibility and cause disorders in the gastrointestinal tract of birds. Nowadays, enzyme preparations are added to feeds to reduce the impact [3-4].

At the same time, the application of biologically active substances in the diet against the background of the stimulation of the metabolism can lead to an imbalance in many microelements. Given the various rate of absorption of microelements and their biological effects, there is a necessity to monitor the trace element status of an organism in animals with high potential of productivity. This claim gets supported by accumulated by modern science information testifying to the stimulating effects of trace elements on biologically active substances applied in the poultry industry [5-7].
Thus, further improving the approaches to the feeding optimization should take into account the accumulated information on the biological activity of enzyme preparations in view of monitoring of the trace element status in broiler chickens [8–10].

The purpose of this study was to examine effects of the enzyme preparation “Rovabio” against the background of the diet correction by microelements Co and Cr introduced to the main diet on the body and the level of productivity of broilers when replacing 15% of wheat to 15% of rye.

2. Materials and Methods

2.1. Animals and feed

The studies were carried out at Federal Scientific Center “Biological Systems and Agrotechnologies of the RAS” on broiler chickens of the final cross “Smena-7”.

The experimental part of the work was carried out in accordance with the protocols of the Geneva Convention and the principles of good laboratory practice (the national standard of the Russian Federation GOST R 53434-2009) with standard procedures for manipulating bioobjects. The experiments were conducted in accordance with the requirements of humane treatment to animals. Poultry housing and procedures during the experiments conformed to the requirements of the instructions and recommendations provided by national rules (order of the Ministry of the health of the USSR 755 from 12.08.1977) and “Guide for the care and use of laboratory animals”.

Bird feeding was conducted two times a day with all-in-one feed completed in accordance with the recommendations by the NRC (1994) (Table 1).

| Component            | 14-28 days | 29-42 days |
|----------------------|------------|------------|
| Wheat grain          | 320        | 182        |
| Barley               | 10         | 10         |
| Sunflower cake       | 184        | 180        |
| Soybean meal         | 200        | 75         |
| Fish flour           | 40         | 75         |
| Vegetable oil        | 58         | 43         |
| Corn grain           | 163        | 400        |
| Wheat bran           | 10         | 10         |
| Limestone            | 10         | 10         |
| Salt                 | 3          | 3          |
| Premix*              | 2          | 2          |

*premix (LLC Koudijs MKorma, Russia) includes vitamins A, D,E, K₁, B₁, B₂, B₃, B₅, B₆, B₁₂, B₇c, and H; microelements Fe, Mn, Cu, Zn, I, Se, and Co (the rate of introduction in the ration – 2 %)

Animals of each group were contained at the vivarium of the Federal Scientific Center “Biological Systems and Agrotechnologies of the RAS” in separate cages KUN-05 with the useful area of 4050 cm² (90 × 45 × 45 cm) with automatic water supply. The birds were marked with plastic toe labels.

Calculating the needs of a broiler body for chemical elements cobalt and chromium was done by the proven methodology [11]. Based on the calculations of the microelement content in feed and tissues, dosages of the chemical element introduction were determined: Cr – 0.38 mg/kg, Co – 0.57 mg/kg of feed.

The method of pairs-analogs helped form four groups (n = 120) of 7-day broiler weighing 160-170 g: one control group and three experimental groups. During the preparatory period (from 7th to 14th d), the birds of all experimental groups received the main ration (MR) with the replacement of 15% wheat to 15% rye. In the experimental period (from 15th to 42nd day), the control group had MR replaced 15% wheat to 15% rye; group I received MR + enzyme preparation “Rovabio” (50 g/t) + CoCO₃ (0.57 mg/kg of feed; group II – MR + Rovabio (50 g/t) + Cr₂(SO₄)₃*6H₂O (0.38 μg/g); (III) group III – MR + Rovabio (50 g/t) + mineral complex of Co and Cr.
The temperature was held with a thermostat for indoors PTP-B to maintain the preset temperature with strict temperature regulation from +15 to +25 °C (error not more than 1 °C). The lighting mode was 12 h of light followed by 12 h of darkness. Humidity was 60%. Air quality was assessed by several components: the content of oxygen – 18%, carbonic acid – 0.15%, ammonia – 7 mg/m³, and hydrogen sulfide – 2 mg/m³.

There was applied the enzyme preparation “Rovabio”, which is a multienzyme complex produced by strain Penicillium funiculosum and it includes the following enzymes: endo-1.4-β-xylanase with the activity of not less than 22 000 visko IU/g, endo-1,3(4)-β-glucanase with the activity of not less than 2 000 AGL IU/g, as well as wheat flour (up to 100%) as the carrier. This beige powder is not soluble in water. It is compatible with all the ingredients of food, drugs and other components of the additive.

2.2. Observation and autopsy

Monitoring the growth of chicks was held at the beginning, on the 21st, and on the 42nd days of the experiment before feeding. A total and average daily gain in the experimental animals was recorded. Daily feed intake was recorded for calculating the consumption and the feed conversion ratio.

Biomaterial for the study was obtained after broiler decapitation (five in each experimental group and in the control one) on the 21st and 42nd days with the subsequent formation of the average sample in accordance with [12].

2.3. Morphological and biochemical composition of blood

Blood sampling took place in the morning on an empty stomach before the slaughter on the 21st and 42nd days from the axillary vein, for the morphological analysis – to vacuum tubes with anticoagulant (EDTA), to evaluate the biochemical indices – to vacuum tubes with the coagulation activator (thrombin).

Morphological parameters were measured with the automatic hematological analyzer URIT-2900 Vet Plus (URIT Medical Electronic Co., Ltd., China). Biochemical analyses of serum were carried out with the automatic analyzer CS-T240 (Dirui Industrial Co., Ltd., China) using commercial kits for veterinary DiAvTest (Russia) and Randox Laboratories Limited (the United Kingdom).

2.4. Statistical processing

The results of the study were processed using the program Microsoft Excel and the technique ANOVA, using the Student's t-test. The level of the significant difference was P < 0.05.

3. Results

In the experimental period, the least amounts of feed were consumed by birds of groups II and III (the difference with the control was 7.4% and 5%, respectively), which reflected in better digestibility of nutrients in these groups (Table 2).

| Index                | Control              | Experimental 1 | Experimental 2 | Experimental 3 |
|----------------------|----------------------|----------------|----------------|----------------|
| Organic matter, %    | 78.65±1.62           | 81.12±1.3*     | 83.17±1.71*    | 85.14±2.13*    |
| Crude protein, %     | 83.43±1.00           | 86.21±0.88**   | 86.97±1.27**   | 88.02±1.73*    |
| Crude fat, %         | 65.16±1.04           | 66.18±0.79*    | 68.54±1.04*    | 69.13±1.47*    |
| Carbohydrates, avg. %| 78.42±1.99           | 79.65±1.61**   | 80.03±2.02*    | 80.19±2.43*    |

* – p < 0.05;
** – p < 0.01 when the control group compared to the experimental ones

The largest amounts of feed were utilized by the control and experimental group I (3883.8 and 3910.4 respectively). The best result was obtained in experimental group III, in particular, the coefficient of
digestibility of organic matter and crude protein was 3.54% and 4.59% higher in comparison to the birds of the control group.

The supremacy of the use of feed nutrients affected the dynamics of broiler live weight (Table 3).

Table 3. Dynamics of broiler live weight, g.

| Age, days | Control | Experimental 1 | Experimental 2 | Experimental 3 |
|-----------|---------|----------------|----------------|---------------|
| 7         | 165.2±9.0 | 169.0±9.7     | 169.1±8.7     | 169.7±9.1    |
| 14        | 312.1±15.0 | 317.2±21.3    | 318.1±13.4    | 319.3±11.3   |
| 21        | 551.2±14.6 | 568.1±28.1    | 569.4±22.8    | 571.2±23.8   |
| 28        | 911.1±39.6 | 915.3±66.4    | 942.0±61.2    | 961.5±74.0   |
| 35        | 1255.4±65.8 | 1354.1±53.2   | 1428.7±86.6   | 1524.2±58.4  |
| 42        | 1814.2±26.5 | 1952.1±34.8   | 2098.2±20.3   | 2163.4±34.1* |

* *– p<0.05 when the control group compared to the experimental ones

In particular, the live weight of broiler chicks of the third experimental group excelled the index of the control group by 26.2% (p < 0.05) at the end of the experimental period. They were characterized with the best daily gains, as well as adequate indices of the morphological blood composition (Table 4).

Table 4. Morphological blood parameters of broiler-chickens.

| Index                  | Control | Experimental 1 | Experimental 2 | Experimental 3 |
|------------------------|---------|----------------|----------------|---------------|
| Hemoglobin, g/l        | 103.2±9.44 | 108.5±7.54     | 109.4±8.16     | 110.7±7.15    |
| Red blood cells, 10^{12}/l | 1.95±0.14 | 2.03±0.11     | 2.09±0.09     | 2.14±0.13     |
| Globular value, 10^{12}/l | 1.49±0.03 | 1.59±0.05     | 1.62±0.07     | 1.71±0.09*    |

* *– p<0.05 when the control group compared to the experimental ones

The study found that the level of hemoglobin in the broilers of the experimental groups was within the physiological norm. The number of red blood cells in the experimental groups was higher by 0.08, 0.14, and 0.19×10^{12} (p < 0.05) than that of the control group, which proves the involvement of cobalt in enzymatic processes, the iron uptake, the hemoglobin synthesis, and the stimulation of erythropoiesis.

The highest hemoglobin saturation in erythrocytes was recorded in chickens of experimental group III – by 0.22 × 10^{12} (p < 0.05), experimental group II – by 0.13 × 10^{12}, experimental I – by 0.10 × 10^{12} compared to the control.

The broiler chickens of the experimental groups showed a faster protein synthesis, its level was higher by 1.4%, 1.78% (p < 0.05), and 1.9% (p < 0.05) in comparison to the control (Table 5).
Table 5. Biochemical parameters of blood in broilers.

| Index                        | Group                  |
|------------------------------|------------------------|
|                              | Control | Experimental 1 | Experimental 2 | Experimental 3 |
| Total protein, g/l           | 47.4±2.03 | 52.1±0.33       | 55.2±2.03       | 56.4±0.33       |
| Total bilirubin, μmol/l      | 3.1±0.11  | 2.9±0.05**      | 2.7±0.04*       | 2.8±0.03        |
| ALAT, u/l                    | 6.3±0.88  | 5.6±1.05        | 5.7±0.61        | 5.2±0.81        |
| ASAT, u/l                    | 249.2±2.02| 247.2±0.54*     | 248.1±18.62     | 241.1±8.15*     |
| Creatinine, μmol/l           | 14.2±2.89 | 19.7±0.33       | 20.3±5.55       | 19.2±0.33       |
| Calcium, mmol/l              | 2.2±0.19  | 2.8±0.12        | 2.7±0.21*       | 3.1±0.11**      |
| Phosphorus, mmol/l           | 2.1±0.13  | 2.4±0.22        | 2.5±0.31        | 2.9±0.15        |

* – p<0.05;  ** – p<0.01 when the control group compared to the experimental ones

In the serum, the calcium and phosphorus content was highest in the experimental group with the additional inclusion of cobalt and chromium by 1.9 mmol/l and 0.8 mmol/l (p < 0.05) in the diet, respectively.

Metabolic shifts expressed in differences of conversion of energy and protein (Figure 1).

![Figure 1. The difference in energy and feed protein conversion rate between the control group and experimental groups I, II, and III, %](image)

According to the studies, the conversion of protein and fat in experimental group III was 12.1% and 18.2% higher than that of the control. Other experimental groups receiving the enzyme preparation and microelements had a positive conversion.

So, monogastric animals have the cobalt absorption that occurs in the small intestine and is from 15 to 20% of cobalt and 2% of chromium due to the formation of stable, hard-to-absorb hydrates [13] in the intestine. The additional inclusion of Co and Cr in the combination with the enzyme preparation “Rovabio” in the diet had positive effects on birds expressed in the stimulation of hematopoiesis and the protein exchange.

4. Discussion

Quite unique biological effects of trace elements are revealed in the stimulation of the microflora activity in the intestine (cobalt – the synthesis of vitamin B12 [14], chromium – the amino acid and insulin synthesis [15-16]), which allows suggesting a corrective impact of these microelements on the enzyme preparation included in the rye-containing ration for broiler chickens.

The obtained results showed a promising outlook of applying the used scheme of feeding broiler chickens [17]. In particular, the level of organic matter usage by chickens of the control group was lower by 2.47, 4.52, and 6.49% (p < 0.05), respectively, than that of the birds of the experimental groups. The maximal coefficient of crude fat digestibility was characterized for experimental group III, which was...
3.97% (p < 0.05) higher than that of the control. It proves the positive influence of cobalt and chromium on the metabolism of broiler chicks [18-19].

It should be noted that the live weight and gain are integral scores of body weight gaining in broiler chickens, which serve as indicators of their overall development both as economic and physiological precocity [20]. The research results confirmed the growth-stimulating action of the enzyme preparation “Rovabio” in combination with chromium and cobalt.

Some authors claim that in order to obtain high average daily gains, it is necessary to use the enzyme preparations decomposing nutrients of macromolecular nature (starch, proteins, lipids, components of cellulose) to digestible forms. The results prove the role of enzymatic preparations as essential agents for the stimulation of metabolic processes in broilers when using low nutrient diets, the correction of trace elements eliminates the depression in mineral exchange [21].

It is known the blood along with lymph constitute the interior body medium as, going through all tissues and cells, it provides their livelihoods serving as an intermediary between the environment and cells. It reflects the general condition of an organism and its physiological processes proceeding from conditions of life [22]. Feeding chickens with additional microelements of cobalt and chromium—significantly strengthened erythropoiesis [23], the hemoglobin saturation in erythrocytes [24].

In our research, the decline of bilirubin by 9.68% (p < 0.05) compared to the control group demonstrated the absence of toxic effects on the organism as a whole [25]. Also, another important indicator for the growth and development of poultry – phosphorus, which is required for calcium absorption, – was within the physiological norm.

Domestic and foreign authors [26] studied the effects of a mineral complex on biochemical blood indices of chickens and based on the results of scientific and commercial experience revealed the role of mineral substances in the productive quality of birds.

As researches showed [27-29] all enzymatic preparations become the most effective in feed for poultry during the early period when birds have a period of intense growth and the level of nutrients in the body increases.

In general, the presented results of the study indicate the prospects of applying “Rovabio” as a bioactive substance together with minerals Co and Cr, which helps increase the enzyme activity to reduce antinutrient properties of rye in the diet, has a beneficial effect on the metabolism and efficient use of feed nutrients while there was stated a decline in feed consumption, the growth-stimulating effect and stimulation of morphobiochemical indices of blood.

5. Conclusion

Thus, the correction of the rye-containing ration for broilers with the multienzyme preparation “Rovabio” in dosage of 50 g/t and with the mineral complex of Co and Cr in a dose of 0.57 mg/kg and 0.38 mg/kg of feed improves the integrity of chickens to 96.7% and profitability of poultry meat production by 2.0%. The study found that these doses have a beneficial impact on morphobiochemical parameters of the organism, the growth and the development of chickens and contribute to better use of feed.

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Ethical approval: All applied international, national, and institutional recommendations on treatment and usage of animals were followed.

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