The effect of feeding Biotin and zeolite on the performance of rumen metabolism in steers during the growing period

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Abstract. This article presents the results of a physiological experiment on 3 bulls-analogs with rumen fistulae to study the metabolic processes in the main part of the stomach when introducing a microbiological protein Supplement of biotrine and zeolite of natural origin into the main feeding diet. The balance of nitrogen and the main macro-and microelements was studied, and the hematological parameters of the blood of bulls during the growing period were studied. Based on the results obtained, it is recommended to use biotrin in the feeding diets of raised young cattle both separately and in different ratios with zeolite, which creates favorable conditions for fermentation and assimilation of feed nutrients.

Keywords: biotrine; zeolite; gobies; fistula; scar; diet; scar metabolism; digestibility; nitrogen balance; minerals; blood Hematology.

1. Introduction
The lack of protein and biologically active substances in animal feeding diets forces the use of non-traditional sources of feed and additives with specified characteristics to improve the quality of feed consumed [4-11].

In this regard, the goal was to study the effect of biotrin and zeolite in the diet of bull calves on the dynamics of scar digestion, to determine the digestibility of feed nutrients [12-15], to calculate the balance of nitrogen, calcium and phosphorus, as well as to study the morphological and biochemical parameters of blood.

2. Methods and materials
To conduct a physiological experiment, three bulls-analogs of the black-and-white breed at the age of 9 months had operations to impose fistulas on the scar using the method [1]. The experiment was performed using the period method [2]. The duration of each period is 15 days: 10 – preliminary and 5 – accounting. In the scar content, the concentration of hydrogen ions (pH), the cellulolytic activity of bacteria, the amount of volatile fatty acids, and ammonia nitrogen were determined.

Rations for experimental animals were made in accordance with detailed feeding standards [3]. On average, energy and nutrients were consumed per head per day for the periods of the experiment: 68-69 MJ, 1097.5-1110.4 g of raw and 650-658, 5 g of digestible protein, 541.5-549.0 g of sugar. The sugar-protein ratio for the periods was 0.81-0.84.
The concentration of nutrients in 1 kg of dry matter of the diet was in the first period: exchange energy – 8.54 MJ; raw protein – 13.28, including digestible – 8.06; fiber – 26.7 %; in the second – 8.5; 13.8; 8.1; 27.6; in the third-8.43; 13.44; 8.04; 27.5; in the fourth-7.85; 13.41; 8.05; 27.5; in the fifth– 8.6; 13.80; 8.22; 28.0 %.

Features of feeding of experimental animals in physiological experience is: 1 period – the main diet (MD), balanced according to the norms; in the 2 – OR + Biotin 0.5 g per 1 kg of live weight; in the 3 – OR + 0.5 g of zeolite per 1 kg of live weight; in 4 – OR + Biotin 0.5 g + 0.5 g of zeolite per 1 kg of live weight; a 5-period – OR + Biotin 0.7 g + 0.3 g zeolite per 1 kg of live weight (table 1).

Table 1. Structure of the main feeding ration in bulls during the experiment period.

| Feed          | Structure of the main diet by nutrition, % |
|---------------|-------------------------------------------|
|               | I period | II period | III period | IV period | V period |
| Hay           | 13.8     | 13.8      | 13.8       | 13.8      | 13.8     |
| Haylage       | 27.0     | 26.0      | 28.0       | 29.0      | 29.2     |
| Silage        | 30.0     | 30.6      | 28.4       | 27.8      | 28.0     |
| Feed molasses | 5.8      | 5.8       | 5.8        | 5.8       | 5.8      |
| Concentrated  | 23.0     | 23.8      | 24.0       | 23.6      | 23.2     |

3. Results and discussion

Concentration of hydrogen ions (pH). The concentration of hydrogen ions (pH) in the rumen content changed slightly in the first period as the feed was consumed in bull calves. So, 1 hour after the start of feeding by 0.09 units; after 2 hours by 1.48 units. and after 4 hours, the pH was restored to the level of 6.98 units.

In animals at 2-5 periods, the amount of feed consumed and their nutritional content were at the same level. However, the use of biotrine and zeolite both separately and in the ratios (0.5:0.5) and (0.7:0.3) g per 1 kg of live weight, and especially in periods 4 and 5, had a positive effect on the scar digestion of bulls (table 2).

So, in the morning before feeding, the concentration of hydrogen ions (pH) was without significant differences in all periods, and as the feed was consumed, they gradually began to decrease in the acidic side. In 1 hour after feeding on 0.12-0.30 units, in 2 hours on 1.32-1.36 units. (after giving the seed), after 3 hours at 1.27-1.17 units, and 4 hours after the first dacha, as micro-organisms grow and feed is evacuated, the alkaline medium was established to a pH of 7.08 units. In other words, the observed changes in the pH of the rumen content in bulls at 2-5 periods are closely related to the acceleration of enzymatic processes, which is especially noticeable in animals in the second period (1.32 units), who consumed biotrine 0.5 g per 1 kg of live weight and in the third (1.36 units), who received 0.5 g per 1 kg of live weight with zeolite concentrates. When fed Biotin and zeolite in a ratio of 0.5 : 0.5 g per 1 kg of live weight, the concentration, the pH decreased by 1.27 units, that is, 3.7%, and a 5 period 5.1% increased than in the first as by increasing rate of feeding Biotin in combination with zeolite concentration pH scar content increased.

Consequently, the pH in the liquid part of the rumen contents was affected not only by the structure of feeding diets, but also by the ratio of vegetable and microbiological synthesis proteins, as well as by the natural mineral substance – zeolite, which has unique ion exchange, adsorption and catalytic properties.

Volatile fatty acids (VFA). In the morning before feeding the animals in the 1st period, the concentration of VFA was in the range of 7.43 mmol / ml, 1 hour after giving hay, it increased by 10.4%, 2 hours after giving silage by 20.2% and concentrates by 32.3%. After 4 hours after the start of feeding, the concentration of LVK in General increased by 34.6%, which indicates the enzymatic activity of rumen microorganisms, which depends on the set of feeds in the diet. Biotin and inclusion of zeolite in diets 2-5 periods is not the same effect on activities of microorganisms according to the depth of conversion of available carbohydrates, in particular, of fiber consumed feed to the end products of fermentation – volatile fatty acids. Especially noticeable increase in their concentration in bulls in 2-5 periods: if an hour after the beginning of feeding, the concentration of LVK in the 1st period was 8.20
mmol/ml, then in 2-5 periods it exceeded by 22.4; 26.7; 18.7; 21.7%, and after 2 hours, respectively, by 21.8; 48.4; 16.2; 40.3% and 4 hours after feeding by 25.8; 31.5; 41.5 and 48.6% compared to the first period, which is accompanied by a significant increase in the cellulolytic activity of the microflora.

**Table 2. Dynamics of rumen metabolism in bulls**

| Period | In the morning before feeding | Periods of study, hours (after feeding) |
|--------|------------------------------|----------------------------------------|
|        |                              | 1   | 2   | 3   | 4   |
|        | Concentration of hydrogen ions (pH) |     |     |     |     |
| 1      | 6.93±0.05                    | 6.84±0.04 | 5.45±0.05 | 6.88±0.04 | 6.98±0.02 |
| 2      | 6.95±0.05                    | 6.75±0.03 | 5.63±0.04 | 6.98±0.04 | 7.05±0.03 |
| 3      | 6.98±0.05                    | 6.82±0.04 | 5.62±0.05 | 6.94±0.05 | 7.02±0.04 |
| 4      | 6.92±0.05                    | 6.80±0.04 | 5.65±0.04 | 6.95±0.04 | 7.06±0.04 |
| 5      | 6.90±0.05                    | 6.65±0.05 | 5.73±0.05 | 6.90±0.05 | 7.08±0.05 |
|        | Volatile fatty acids, ml EQ/100 ml |     |     |     |     |
| 1      | 7.43±0.28                    | 6.20±0.11 | 8.93±0.12 | 9.83±0.08 | 10.00±0.04 |
| 2      | 8.00±0.11                    | 10.04±0.23 | 10.88±0.08 | 12.52±0.09 | 12.58±0.05 |
| 3      | 8.88±0.05                    | 10.39±0.17 | 13.25±0.12 | 14.20±0.13 | 13.16±0.28 |
| 4      | 7.68±0.07                    | 9.63±0.08 | 10.38±0.11 | 13.26±0.04 | 14.51±0.19 |
| 5      | 7.72±0.06                    | 9.98±0.10 | 12.53±0.09 | 14.46±0.12 | 14.86±0.14 |
|        | Cellulolytic activity, %      |     |     |     |     |
| 1      | 18.62±0.96                   | 22.46±1.32 | 26.48±1.40 | 27.42±0.88 | 27.12±2.0 |
| 2      | 20.88±1.02                   | 25.42±1.05 | 28.12±1.23 | 30.43±1.13 | 29.38±1.17 |
| 3      | 20.32±1.04                   | 26.38±1.04 | 29.45±1.25 | 32.23±2.01 | 30.28±1.11 |
| 4      | 20.42±0.99                   | 27.40±0.78 | 30.26±1.16 | 34.46±2.16 | 29.42±1.21 |
| 5      | 21.13±1.12                   | 28.35±1.25 | 34.11±1.19 | 33.58±2.13 | 30.25±1.26 |
|        | Number of protozoa, thousand 1 ml |     |     |     |     |
| 1      | 350.10±67.0                  | 383.18±56.0 | 398.53±32.0 | 453.19±72.0 | 480.28±81.0 |
| 2      | 369.64±52.0                  | 386.22±54.0 | 446.22±71.0 | 511.50±71.0 | 547.80±72.0 |
| 3      | 452.36±36.0                  | 528.31±67.0 | 598.46±77.0 | 628.60±63.0 | 677.88±67.0 |
| 4      | 432.11±48.0                  | 452.28±61.0 | 532.19±81.0 | 558.12±67.0 | 618.13±76.0 |
| 5      | 396.62±67.0                  | 407.63±58.0 | 460.39±82.0 | 503.19±70.0 | 528.23±79.0 |

Note: * P<0.05; **P<0.01; ***P<0.001.

**Cellulolytic activity.** The cellulolytic activity of rumen microorganisms during the growing period in all groups was quite high. Observations after (1, 2, 3, 4 hours) after feeding showed an increase in its activity and, especially after 3 hours of taking coarse feed and 2 hours of concentrates, at this time the peak of activity in them was higher by 11.00 - 22.47%. If we compare the activity of microorganisms that destroy fiber, it increased by 1.47 times in the first period, and in the rest, receiving biotrine and zeolite in different ratios by 1.59 - 1.70 times.

Thus, the use of biotrine and zeolite in the diets of bulls during the growing period creates an optimum of nutritional, biologically active substances that have catalytic, prolonging properties that enhance the activity of microorganisms and promote their growth.

**The number of protozoa.** The number of microorganisms in the morning before feeding in the first period was 350.1 thousand/ml, and in 2-5 periods within 369.6 – 452.4 thousand/ml. An hour after giving feed, their number in the first period increased from 382.2 thousand/ml to 528.3 thousand/ml in the third, that is, by 16.58; 75.95; 20.17 and 10.91 thousand / ml more than at the beginning of feeding. Further, the number of protozoan microorganisms increased in the control by 1.37 times, and in 2-5 periods, respectively, by 1.48; 1.50; 1.43 and 1.33. The use of biotrin in the diets of bulls, both separately and in
different ratios with the natural mineral, accelerated the growth of microflora and their activity, which created conditions for better digestibility and assimilation of feed nutrients.

**Concentration of nitrogenous metabolites in the content of the rumen.** Indicators of nitrogenous metabolites in the rumen content in period 1 animals were within the limits of physiological norms. However, the use of biotrin as a protein supplement had a positive effect on the dynamics of nitrogen metabolism in the body of experimental bulls. Thus, after giving concentrates with biotrin, there was an increase in total nitrogen in 2-5 periods – by 11.1; 13.6; 19.1; 20.1 mg%, which is higher by 22.5; 26.1; 36.1 and 38.8%. The same trend was observed in the content of protein nitrogen, the highest concentration of it was detected in animals of 2-5 periods 1.38-1.70 times more than in 1 period. A high concentration of non-protein nitrogen was observed in bulls in period 3 – by 5.7%; in period 4 by 20.4; and ammonia nitrogen in periods 2-5 was higher by 7.0-20.4%.

The introduction of a certain amount of biotrin and zeolite in the diet of bull calves, positively affected the fermentation processes in the body of animals 2-5 period. At the same time, the dynamics of ammonia formation increased, which contributed to the activation of microorganisms and, in general, to increase the synthesis of protein in one's own body.

**The coefficient of digestibility of nutrients.** The results on the digestibility of feed nutrients showed that the addition of biotrine to the diet of steers, as well as biotrine with zeolite, helped to increase the digestibility of organic matter and its components. The highest digestibility coefficients were observed in bulls in the 4th and 5th periods. Compared to period 1 animals, they digested organic matter better by 4.8 and 5.0%; protein by 5.4 and 6.0%; fat by 9.6 and 10.2%; fiber by 4.6 and 3.7%; and BEV by 4.0 and 7.1%. It should be noted that the lowest indicators for the digestibility of the main nutrients were animals of period 2 and 3 (except for period 1), who received biotrine and zeolite separately. Compared to period 1, their digestibility of organic matter was higher by 0.4-1.4%, protein by 3.3-4.0%, fat by 3.7-5.5%, fiber by 3.4-4.0% and BEV by 2.6-4.4%.

**Exchange and use of nitrogen, calcium, phosphorus, and trace elements.** The nitrogen balance in all groups was positive and was at a fairly high level. The inclusion of 2-5 periods of biotrine and zeolite in the diet of bulls separately and in the ratio (0.5:0.5) g and (0.7:0.3) g per 1 kg of live weight, had a positive effect on the exchange of the nitrogen part of the diet and its assimilation by the animal body (table 3).

With the same consumption of nitrogen feed animals 2-5 period, it is retained in the body by 4.77; 9.21; 10.75 and 13.34 g more than in 1 period. The amount of nitrogen used is 2.48-7.39% more than the amount taken and 2.32-7.77% more than the amount digested. It should be noted that in the 2 and 3 period in the process of metabolism, its excretion, compared to 1 period 4 period was at the same level, and in period 5 at 1.25% less, which increased the nitrogen digestibility of feed compared with other periods. At the same time, in the 2-5 period, the body of bulls retained nitrogen by 4.42-13.16 g more than in 1 period. It should be noted that the processes of nitrogen metabolism in the body of bulls were significantly influenced by the use of protein and mineral supplements in their diets.

In the feeding diets of the 3-5 period that consumed zeolite, the concentration of calcium in the scar content increased by 1.2-1.48%, phosphorus by 1.21-1.60%, and magnesium by 8.2-23.4%. However, in period 1 and 2, the concentration of copper and zinc in animals was at the same level. And in the other periods, there was a significant increase in the concentration of copper, zinc, cobalt and manganese. So in animals that received only zeolite, the concentration of copper increased by 15.94%, zinc by 16; cobalt by 44.4 and manganese by 16%; and in period 4, consuming biotrine and zeolite in the ratio (1:1), respectively, copper was more by 21.74%; zinc – 17.80; cobalt by 1.67 times and manganese 27.0%, and in period 5, where the ratio of biotrine + zeolite was (0.3:0.7) increased by 26%; 21; 1.78 times and 48.3%. It follows that during fermentation, under the influence of zeolite ions, complex organic compounds of the feed mass are converted into the most accessible forms and are further absorbed in the underlying parts of the intestinal tract, which is confirmed by data on the balance and amount of retained elements in the body.
Table 3. Exchange and use of nitrogen in bulls, g

| Indicators                  | 1                | 2                | 3                | 4                | 5                |
|-----------------------------|------------------|------------------|------------------|------------------|------------------|
| Taken with food             | 176.56 ± 2.10    | 177.95 ± 2.18    | 180.23 ± 1.99    | 178.56 ± 2.05    | 177.64 ± 2.10    |
| Highlighted with feces      | 66.92 ± 1.91     | 61.57 ± 2.05     | 60.56 ± 2.13     | 58.03 ± 2.15     | 55.60 ± 0.22     |
| Digested                    | 109.64 ± 2.51    | 116.38 ±        | 119.67 ± 1.64**  | 120.53 ± 1.88**  | 120.4 ±         |
| Excreted in the urine       | 34.46 ± 0.75     | 77.15 ± 1.93     | 43.67 ± 0.52**   | 45.21 ± 0.23**   | 74.24 ± 0.21     |
| Held in the body            | 39.23 ± 0.67*    | 24.23 ± 0.05     | 25.32 ± 0.09     | 26.91 ± 0.10     | 39.20 ± 0.10*    |
| Used                        | 19.52 ± 0.32     | 22.00 ± 0.16     | 36.50 ± 0.13     | 37.51 ± 0.21     | 26.91 ± 0.05*    |
| % of accepted               | 31.43 ± 0.68     | 33.75 ± 0.10     |                  |                  |                  |

Note: * P < 0.05, ** P < 0.01.

The balance of calcium, phosphorus and trace elements – copper, cobalt, zinc and manganese in all periods of cultivation was positive. The largest amount of calcium was deposited in animals in 2-5 periods compared to the first, it was more in 2 – by 1.51 g, in 3 – by 2.16, in 4 – by 2.08 and in 5 – by 2.85 g or 2.98; 4.65; 4.00 and 5.75%, respectively. Animals in the 3,4,5 period better used and assimilated phosphorus by 6,85; 11,05; 15,02% of the accepted amount and with high confidence.

The use of biotrine in the diets of bull calves of the 2nd period, which contains not only trace elements, but also vitamins A, D, E and amino acids, had a positive effect on the exchange of cobalt, copper, zinc and manganese and their better assimilation. Animals in period 3 that received only zeolite had higher assimilation compared to period 1 and 2: cobalt – by 8.2-10.0%; copper – 10.7; zinc – 18.3-12.5 and manganese – 7.6-1.7%. The use of zeolite improved the absorption of trace elements.

Animals in the 4th and 5th periods that consumed biotrine and zeolite in different ratios, significantly better used cobalt, copper, zinc, and manganese than from the first and exceeded it by 1,6-11,1; 9,7-14,0; 18,8-19,8; 7,1-9,1%. Thus, the use of biotrine and zeolite in a combination of 1:1 and 2:1 provided a positive balance of macro-and microelements, which contributed to their better use in the body's metabolic processes.

**Morphological and biochemical parameters of blood.** As a result of interaction of zeolite ions and vitamin complexes A, D, E and B contained in biotrin, positively influenced the morphological composition, since iron, copper and cobalt ions are involved in the processes of hematopoiesis and there is not only quantitative, but also qualitative transformation of hemoglobin and shaped elements. In General, the concentration of hemoglobin in period 2-5 increased: in 2-by 6.34%; in 3-by 11.40; in 4-by 11.53 and in 5-by 14.85%, and red blood cells – by 11.00; 13.81; 15.60; and 19.00%, respectively.

The main indicator of natural resistance of the animal body is phagocytic activity, which to some extent characterizes the content of white blood cells in the blood. They exceeded 2-5 periods in animals – by 7.9; 6.6; 8.0 and 5.2% and were in the upper limit of physiological norms, which is explained by the influence of biotrine and zeolite on their phagocytic activity. It should be noted a significant improvement in the blood's biochemical parameters in 2-5 periods, in particular, reserve alkalinity, total protein and their fractions, the concentration of glucose, lipids, carotene and minerals, which characterize the fullness and balance of feeding diets.

Describing the dynamics of the concentration of total protein and its fractions, it can be noted that animals of the 2-5 period at the age of 9 months, who received biotrine and zeolite separately and in different combinations, showed an increase in total protein and its fractions and significantly differed from the first period, however, the content of total protein and its fractions did not go beyond the limits of physiological norms, and protein metabolism indicators corresponded to average daily increases in live weight. Thus, the intensity of metabolic processes in the animal body and the associated growth energy are directly dependent on the level of protein fractions.

In all periods, the concentration of trace elements-cobalt, copper, manganese, zinc and iron in the blood at the beginning of the experiment did not have significant differences between peers and were in...
the lower limit of physiological norms, but in bulls of 2-5 periods, receiving zeolite and biotrine both separately and in combination, there was an increase in the concentration of the studied trace elements.

4. Conclusion
Application biotrin and zeolite in feed rations optimized balance of protein and bioactive substances, which contributed to the improvement in microbial protein synthesis in ruminants precluded, normalized concentration of macro-and micronutrients, increased the nutrient digestibility of feed by stimulating hematopoiesis.

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