Probiotic Substance in Combination with Zeolite Changes the Digestibility and Metabolism of Bulls

B S Nurzhanov¹, G K Duskaev¹
¹Federal Research Centre of Biological Systems and Agrotechnologies of the RAS, 29, 9 Yanvarya St., Orenburg 460000, Russia

E-mail: baer.nurzhanov@mail.ru

Abstract. In the course of laboratory studies, we found that the porosity (in volume) of zeolite acetone is 34.4%. The sorption capacity of the studied zeolite to the Bifidobacterium longum strain together with the MRS nutrient medium was determined. As a result of the experiment, it was found that 100 g of zeolite absorbs 34 ml of nutrient medium with bifidobacteria. The optimal dose of feeding a complex probiotic preparation (CPP) – 30.5 g/day included 82% zeolite and 18% probiotic strain of Bifidobacterium longum. The coefficients of digestibility of nutrients were slightly higher in bulls from the experimental groups compared to their peers from the control group. So for dry matter, respectively, by 1.87%, 4.68 and 3.58%; crude protein by 2.22%, 5.00 and 3.48%; BEV by 1.24%, 3.19 and 2.20%. The control animals absorbed less nitrogen compared to the bulls of the I, II and III experimental groups by 14.52%, 30.14 and 21.78%. According to the use of the nitrogenous part of the rations, the animals of the experimental groups outperformed their counterparts from the control group by 2.13%, 5.00%, and 3.38%, respectively. On average, during the experiment period, the bulls of the I - III experimental groups had an advantage over the animals from the control group in absolute live weight gain by 1.4%, 14.9 and 6.7%, and in average daily weight gain by 1.4%, 14.8 and 6.7%.

1. Introduction
It is known that zeolites — hydrated aluminosilicates of alkaline elements, are the most valuable minerals in the industry, having an open frame-cavity structure of the type [(Si, Al) O₄], which has a negative charge. The ion exchange capacity of zeolites is one of the main parameters that characterize their sorption and technological properties. The maximum ion exchange capacity corresponds to the complete substitution of one ion by another in all crystal positions, which corresponds to the maximum sorption capacity of the zeolite. According to Russian R&D Center GELNERUD and East Siberian Research Institute VostSibNIIGTtMNA [1], zeolites belong to the first group of natural materials in terms of thermal and acid resistance (i.e., they are highly resistant). Zeolites are recognized as non-toxic, mutagenic effects are not detected, and can be used without restrictions in all areas of the national economy.

Representatives of the microflora of the gastrointestinal tract of ruminants include bifidobacteria, lactobacilli, bacteroids, enterococci, Escherichia, yeast-like fungi. At the same time, the main part of it in healthy animals is made up of bifidobacteria. Normally, 10⁵-10¹² bifidobacteria are found in 1 g of the contents of the large intestines of animals; a decrease in their number less than 10⁷/g indicates violations in the intestinal microbiocenosis [2].

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Optical spectrometry and multiphoton microscopy (MPM) are effective approaches that can reveal the potential of natural zeolites in the controlled delivery of drugs and biomedical strains. This approach makes it possible to compare different mathematical models and uses more parameters to better characterize drug release profiles [3].

For the development of a highly selective and sensitive fluorescent biosensor based on poly (azomethine-urethane) and zeolite for detecting DNA molecules, zeolite was chosen to enhance the anionic or cationic functional groups in the polymer matrix and the interaction between the polymer and DNA [4].

The results of the study showed that cows treated with feed clinoptilolite (CPL) had an improved metabolic status due to a tendency to increase glucose levels and decrease beta-hydroxybutyrate values during early lactation. In addition, the acute phase response was lower (P <0.05) in cows receiving CPL. This result can be explained by the effect of CPL in the diet on the intensity and severity of the negative energy balance and inflammatory response in dairy cows [5].

The addition of zeolite during the prenatal period leads to significantly improved serum concentrations of CA after delivery and similar postpartum indicators compared to the control group, and it is effective in reducing hypocalcemia in Holstein multi-bred cows. In general, zeolite feeding had little effect on neutrophil gene expression and function; however, lower expression of neutrophil inflammatory mediator genes may be due to altered availability of dietary minerals before delivery and indicates that zeolite may control inflammation during the transition period. [6,7].

When 0.5% zeolite was introduced into the colostrum of newborn calves 30 hours after birth, the concentrations of γ-globulins, β-globulins and total protein in the calves group were higher than in the control group by 42.11% (p <0.05), 28.48% (p> 0.05) and 18.52% (p> 0.05) [8].

In studies [9, 10], the composition of biologically active substances in diets also had a positive effect on the productivity of animals.

The aim of the research was to study the technological properties of a complex probiotic preparation on a carrier-zeolite and its effect on the digestibility and metabolic processes in ruminants.

2. Materials and methods

The object of the study was: Nezhinsky Deposit zeolite, developed complex probiotic preparation, Kazakh white-headed bulls at the age of 12 months.

Laboratory studies were performed in the integrated analytical laboratory of the Federal State Budgetary Research Center Federal Research Center for Biological Systems and Agrotechnology under the Russian Academy of Sciences (accreditation of Gosstandart of Russia – Pocc. RU no. 0001/21 ПФ 59 of 29.08.2008) using a gas chromatograph "Crystal 4000", the method of differentiated centrophugation, the Kjeldahl method, the method for determining porosity by acetone, the macrodiffusion method and the standard method of serial dilutions on the MRS nutrient medium [17].

Animal care and experimental studies were performed in accordance with the instructions and recommendations of Russian Regulations, 1987 (Order No. 755 on 12.08.1977 the USSR Ministry of Health) and "The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)". When performing research, efforts were made to minimize animal suffering and reduce the number of samples used.

The physiological experiment was carried out on the farm of the Orenburg agricultural College of the Orenburg region on 12 Kazakh white-headed bulls divided by the method of analog pairs into 4 groups (n=3) at the age of 12 months. Scheme of studies involved a control group fed basic diet (BD), Experimental Group I – BD + CPP at a dose of 27.5 g/goal II – BD + CPP at a dose of 30.5 g/bird, III – BD + CPP at a dose of 33.5 g/head. On average, during the physiological experiment, the daily diet of calves consisted of 3 kg of Sudan grass hay, 15 kg of corn silage, 2.5 kg of concentrated feed, 0.038 kg of salt and 0.025 kg of premix.

Mathematical processing of the obtained data was performed by the program "SPSS Statistics Version 20".
3. Research results and discussion

In the course of laboratory studies, we determined the porosity of zeolite by acetone and its sorbing ability to create a new feed agent. It was found that the porosity (in volume) of zeolite acetone is 34.4%. The sorption capacity of the studied zeolite to the Bifidobacterium longum strain together with the MRS nutrient medium was determined. As a result of the experiment, it was found that 100 g of zeolite absorbs 34 ml of the nutrient medium with bifidobacteria.

Zeolites are natural frame aluminosilicates of a crystalline structure containing channels and voids. The pore size of the studied Nezhinsky zeolite was 4-25 Å. Chemical composition of zeolite (%): calcium – 1.9; potassium – 2.4; sodium – 1.23; phosphorus – 0.16; iron – 1.35; manganese – 0.04; aluminum – 13.45; silicon – 63.70; moisture – 15.77.

The essence of the development of a new drug is to immobilize a live culture of Bifidobacterium longum (0.5-1.3 microns in size) with a titer of 5*10^8 CFU/ml (CFU – colony-forming units) with zeolite. To do this, 100 g of the sorbent is added to a liquid nutrient medium with a 34 ml Bifidobacterium longum strain and thoroughly mixed. After 1 hour, the resulting paste is dried in a drying cabinet to a constant humidity of 15% at a temperature of 25-30 °C. Eubiotic cells are used in conjunction with components of the nutrient medium with 10^8-10^10 CFU/ml.

The optimal dose of feeding a complex probiotic preparation (CPP) – 30.5 g/day included 82% zeolite and 18% probiotic strain of Bifidobacterium longum.

In the course of physiological studies, it was found that feeding the studied drug improved the feed intake. The highest feed consumption was observed in experimental groups I and II and III.

With equal consumption of concentrated feed, the bulls of the experimental groups, compared with their counterparts from the control group, consumed more Sudan grass hay and corn silage, respectively, by 2.8%, 7.1 and 5.5%; 5.9%, 16.2 and 9.4%.

Experimental bulls that received the studied complex probiotic preparation used the nutrients of the diets better than their counterparts from the control group (table 1).

| Indicator                      | Group         | I Experimental | II Experimental | III Experimental |
|--------------------------------|---------------|----------------|-----------------|-----------------|
| Dry matter                     | control       | 62.92±0.57     | 64.79±0.34      | 67.60±0.48*     | 66.50±0.66*     |
| Organic matter                 |               |                |                 |                 |                 |
| Crude protein                  |               |                |                 |                 |                 |
| Crude fat                      |               |                |                 |                 |                 |
| Crude fiber                    |               |                |                 |                 |                 |
| Nitrogen-free extractive       |               |                |                 |                 |                 |
| Substances                     |               |                |                 |                 |                 |
| Note: *P<0.05; **P<0.01         |               |                |                 |                 |                 |

The coefficients of digestibility of nutrients were slightly higher in bulls from the experimental groups compared to their peers from the control group. For example, for dry matter it was, respectively, by 1.87%, 4.68 and 3.58%; for crude protein by 2.22%, 5.00 and 3.48%; for nitrogen-free extractive substances by 1.24%, 3.19 and 2.20%.

Between the groups, animals from experimental group II had higher indicators of nutrient digestibility. So, the difference between the bulls from the experimental groups was 2.81 and 1.10% dry matter, 2.78 and 1.52% crude protein, 0.54 and 0.84% crude fat, 2.56 and 0.45% crude fiber, 1.95 and 0.99% nitrogen-free extractive substances.
Bulls that received the probiotic drug under test made better use of the accepted nitrogen and deposited more of it in the body (Fig.1).

![Figure 1. Average daily nitrogen balance in experimental calves, g/head.](image)

(a – taken with food; b – excreted in the feces; C – digested; d – excreted in the urine; e – deposited in the body).

The nitrogen balance in the animals of all groups was positive. At the same time, nitrogen consumption with feed increased in the experimental groups, due to their higher feed consumption.

The control animals absorbed less nitrogen compared to the bulls of experimental groups I, II and III by 14.52%, 30.14 and 21.78%. According to the use of the nitrogenous part of the rations, the animals of the experimental groups outperformed their counterparts from the control group by 2.13%, 5.00%, and 3.38%, respectively.

The marked increases in the digestibility and use of nutrients in the feed rations had a positive effect on the growth rate of the experimental groups of bulls. At the beginning of the experiment, the live weight of all groups of animals was almost the same. In the future, the control animals that received the basic diet were inferior in live weight to their peers I, II, and III. The established trend continued until the end of the study, and bulls from the experimental groups outperformed their counterparts from the control group by 0.6%, 5.7%, and 2.4%, respectively.

On average, during the experiment, the bulls of experimental groups I, II and III had an advantage over the animals from the control group in absolute live weight gain by 1.4%, 14.9 and 6.7%, and in average daily weight gain by 1.4%, 14.8 and 6.7%. The highest gross growth was found in group II, and compared to the control group, I and III groups it differed by 12.9%, 11.7 and 7.1% respectively. In the available studies, there are data on the use of zeolite-containing substances similar to the studies conducted. In earlier research, the authors proposed artificial variants of carriers of biologically active substances, for example, zeolite imidazolate framework-8, which shows good thermal stability and regeneration ability in experiments on adsorption recirculation [11-13]. These carriers [14] are effective for extracting amino acids - 95.1% (tryptophan), 91.1% (tyrosine) and 90.1% (phenylalanine), respectively, and improving the enzymatic catalytic characteristics. Zeolite gives the enzyme improved resistance to harsh conditions (for example, high temperatures and organic solvents) [15]. Similar studies [16, 17] also showed that the activity of extracted α-chymotrypsin in the zeolite framework was well preserved at 93% of the initial activity. Dietary administration of clinoptilolite, especially with the smallest particle size (less than 0.15 mm), at the rate of 200 g per cow per day can effectively reduce the concentration of aflatoxin M1 (AFM1) in the milk of cattle and can be used as a
preventive measure to reduce the risks associated with the presence of aflatoxins in the milk of dairy cows [18].

4. Insights
During the research, a complex probiotic preparation for beef cattle was developed and tested. The research included the immobilization of a probiotic culture of Bifidobacterium longum bacteria on zeolite, characterized in that the preparation contains 82 wt.% zeolite and 18 wt.% of probiotic bacteria Bifidobacterium longum with a titer of 5*10^8 CFU/ml, and zeolite of Nezhinsky origin is used as a sorbent, the drug is fed as an additive to feed at a dose of 30.5 g per day, which contributes to the effective use of nutrients in the diet and increases the productivity of animals.

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