Technological aspects of getting essential elements using probiotic preparations based on bacteria of the genus *Bacillus*

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Abstract. The paper presents data on getting bioavailable forms of essential elements on the example of zinc using probiotic strains. Probiotic strains form the basis of probiotic preparations: "Sporobacterin" (*B. subtilis* 534) and "Bactisubtil" (*B. cereus* 5832). The experiment was carried out on laboratory animals (rats). To confirm the technology of obtaining bioavailable forms of zinc, studies of blood biochemical parameters (alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase) were carried out, and also the data on the delivery of zinc in the tissue of laboratory animals were reflected. The data of the conducted study indicate small changes in the biochemical parameters of blood against the background of a mineral deficient state. The concentration of the studied biochemical parameters was within the reference values. Probiotic strains did not affect blood biochemical parameters adversely. Evaluation of the accumulation of bioavailable forms of zinc showed that inactivated Bacillus strains were capable of converting into a bioavailable form and delivering zinc to the tissues of laboratory animals. It can be concluded that one of the possible options for regulating the elemental status of the organism of animals and humans is the technology of using biotic preparations based on the metabolic by-product of representatives of transient microflora with high sorption characteristics.

1. Introduction

Minerals that enter the body of animals with food are involved in digestion, metabolism, hematopoiesis, and also affect the body's defenses. Zinc is one of the most important trace elements in the body, which is responsible for numerous structural, catalytic and biochemical functions [1].

In real conditions, the absolute deficit of any macroelement or microelement (at zero level) does not exist. In this regard, all manifestations of mineral deficiency can be represented in the form of simple deficiency with characteristic clinical and morphological signs [2]. Latent (partial) insufficiency is a kind of "threshold state" of the body. A small deficit of an element does not cause typical symptoms in this case, but it does affect the totality of signs that determine productivity [6]. Conditional (secondary insufficiency) is a fairly common and difficult to diagnose condition, since its clinical manifestations can significantly deviate from the classic symptoms of simple insufficiency [3].

Zinc deficiency can be caused by low intake of zinc from food, insufficient absorption of zinc, increased excretion of zinc or increased demand for zinc (for example, in children and pregnant women). Zinc deficiency is associated with stunted growth, poor appetite, dermatitis, alopecia,
hypogonadism and impaired immune function, which can lead to frequent diarrhea and/or upper respiratory tract infection [4, 5].

One of the possible ways to regulate the elemental status of animals and humans is to use biotic preparations based on the vital products of probiotic microorganism strains with high sorption characteristics [6–8].

2. Materials and Methods

2.1. Object of study

The following probiotic strains were selected for the experimental part: B. subtilis 534 (Sporobacterin, LLC Bakoren, Orenburg (Russia), B. careus 5832 (Baktisubtil, Marion Merrel, France. Zinc sulfate (ZnSO₄) salt acted as a regulatory factor.

The studies were performed on the model of analog groups of laboratory animals (rats) of the Wister breed. The experimental part was performed on the basis of the experimental biological clinic (vivarium) of Orenburg State University.

Animal maintenance and experimental studies were performed in accordance with the instructions and recommendations of Russian Regulations, 1987 (Order No. 755 on 08/12/1977 the USSR Ministry of Health) and "The Guide for Care and Use of Laboratory Animals (National Academy Press Washington, DC 1996). In carrying out the research, efforts were made to minimize animal suffering and reduce the number of samples.

2.2. Experimental scheme

To perform experimental studies, it was necessary to cultivate isolated microorganisms from probiotic preparations with a metal salt in a liquid nutrient medium (meat and peptone bouillon). In order to prepare a salt solution of the metal under study, a sample was weighed (based on calculations) and dissolved in 100 ml of distilled water at a concentration of 0.05 M/ml. The previously obtained metal salt solution and probiotic strains were added to the liquid nutrient medium, followed by cultivation for 24 hours in an incubator at a temperature of 37 °C. After that, centrifugation was performed at 3000 rpm for 10 minutes (with the aim of sedimentation of biomass), followed by removal of the supernatant. The resulting biomass was placed in an autoclave (VK-30-01) (Tyumen Plant of Medical Equipment and Instruments, Russia) and sterilized for 40 minutes. This procedure is necessary for the inactivation of bacteria of the genus Bacillus. The biomass obtained after sterilization was introduced into animals per os.

To accomplish the task of assessing the prospective use of probiotic strains of microorganisms as a regulatory factor of elemental status in the development of mineral deficient states, we formed 4 groups: 2 control and 2 experimental groups of 24 animals each. The first control group of intact animals was on the diet in accordance with the requirements of the instructions and recommendations of the Russian regulations (Order of the Ministry of Health of the USSR No. 163 of 03/10/1966) and acted as a criterial assessment of the physiological norm (K₁). The next control group was on a mineral-deficient diet (K₂). The experimental groups, like the K₂ group, received a mineral diet for 20 days leading to the development of metal deficient states. In the first experimental group (O₁), biomass of inactivated B. subtilis 534 (Sporobacterin) was introduced into the diet within 10 days from the beginning of the experiment after cultivation in a liquid nutrient medium in the presence of an excess amount of metal salt in dosages of 1 ml per animal for group O₁. In the second experimental group (O₂), B. cereus 5832 (Bactisubtil) was used as a source of metal ions with a similar administration schedule and dosage.

The study was carried out using a comparative method of biological research, i.e. animals were in identical maintenance conditions and one time period.

Biochemical analysis of blood was carried out using an automatic biochemical analyzer CS-T240 (Dirui Industrial Co., Ltd., China). Biochemical analysis was performed using commercial biochemical kits for veterinary medicine DiaVetTest (Russia) and Randox (USA). The list of blood
biochemical parameters included alanine aminotransferase (AlAt), aspartate aminotransferase (AsAt) and alkaline phosphatase.

Quantitative analysis of the metal was carried out using atomic absorption spectrometry. The atomic absorption spectrophotometer was an AAS-1 (GDR) instrument with a set of spectral lamps (LUMEX, Russia).

2.3. Statistical processing
The samples were taken every five days (background, fifth day, tenth day and fifteenth day). Statistical analysis was performed using the Microsoft Office Excel (Microsoft, USA) with data processing Statistica 10.0 (Stat Soft Inc., USA). The data obtained were subjected to statistical processing using Student’s t-test.

3. Results
Antemortem study of blood biochemical parameters allows fully assessing the degree of influence of the studied strains (drugs) on individual organs and organ systems, as well as on the body as a whole. One of the main criteria for the study of probiotic drugs is the absence of a toxic effect on the body. For this purpose, we throughout the experiment conducted a study of GPT, GOT, alkaline phosphatase.

When decoding the biochemical blood parameters of GPT and GOT in laboratory animals, it was found that the studied strains did not significantly affect the studied parameters throughout the experiment. Analyzing the data obtained in the study of GPT in the deficit control group compared with the control on the 5th, 10th and 15th days of the experiment, an increase of 10.5, 13.2 and 12.6 % was observed (p < 0.05). In the experimental group O₂, on the 5th day of the experiment there was an increase of 1.2 %; on 10 and 15 days of the experiment in this experimental group, a decrease was observed compared to previous values.

Changes in GOT in the deficit control group relative to background values at days 5, 10, and 15 of the experiment were expressed in an increase of 3.36, 1.3 and 1.5 %. A slight increase was recorded in the O₂ experimental group on the 10th day of the study by 2.3 %; on the 15th day of the experiment, a decrease in the O₂ group was observed.

In the study of alkaline phosphatase, interest is a significant increase in the indicator in the deficit control group compared to the background value on the 10th day of the experiment by 7.11 % (p<0.001) and by 0.76 % on the 15th day of the experiment (p<0.001). It was recorded that on day 5 of the study, a slight increase was observed in all experimental groups. This picture was present at all periods of the study. The changes were unreliable.

Evaluation of the effectiveness of the use of probiotic strains of the genus Bacillus was carried out by determining the content of zinc ions in the tissues of laboratory animals. An analysis of the obtained data indicates that in groups using inactivated microorganisms on the 15th day of the experiment, the amount of zinc in the tissues exceeds the amount of zinc in the deficiency group (K₂) (Table 1).

The results of our studies showed that on the 5th day of the experiment in the skin in all experimental groups there was an increase in the amount of zinc in O₁ (21.0 %) (p < 0.001) and O₂ (25.5 %) (p < 0.001); on the 10th day of the study, an increase in the amount of zinc in O₁ (15.3 %), O₂ (16.6 %); on the 15th day of the study, an increase in the amount of zinc by 10.0 and 9.5 %.

A similar situation in the analysis of muscle tissue on the 5th day of the study in relation to the control group in all experimental groups was an increase in the amount of zinc by 18.6 % (p < 0.001) and 15.7 %; on the 10th day of the study, 1.1 and 8.0 %; on the 15th day of the study, 7.7 and 2.1 %.

The same can be said when examining bone tissue. On the 5th day of the study, in relation to the background group, in all experimental groups there was an increase in the amount of zinc by 30.0 % (p < 0.001) and 35.8 %; on the 10th day of the study, an increase of 5.7 and 5.6 %, as well as on the 15th day of the study, an increase in the amount of zinc by 5.4 and 8.9 %.
Table 1. Zinc ion concentration in tissues of laboratory animal at different periods of study

| Groups | Background results | After 5 days | After 10 days | After 15 days |
|--------|--------------------|--------------|---------------|---------------|
|        | Concentration of zinc ions in skin |              |               |               |
| K₁     | 0.44±0.003         | 0.42±0.005   | 0.43±0.006    | 0.42±0.003    |
| K₂     | 0.21±0.003         | 0.22±0.003   | 0.22±0.003    | 0.23±0.003    |
| O₁     | 0.21±0.005         | 0.31±0.005*** | 0.40±0.006*(1) | 0.37±0.008***(1) |
| O₂     | 0.22±0.008         | 0.35±0.008***(1)***(2) | 0.44±0.008     | 0.38±0.003****(1) |
|        | Concentration of zinc ions in muscle tissue |              |               |               |
| K₁     | 0.44±0.008         | 0.44±0.005   | 0.43±0.005    | 0.44±0.008    |
| K₂     | 0.25±0.003         | 0.26±0.005   | 0.25±0.003    | 0.24±0.005    |
| O₁     | 0.22±0.003****(2)  | 0.39±0.008***(1)***(2) | 0.40±0.008     | 0.37±0.005***(1) |
| O₂     | 0.26±0.005         | 0.38±0.005***(1) | 0.45±0.006*(1) | 0.43±0.003    |
|        | Concentration of zinc ions in bone tissue |              |               |               |
| K₁     | 0.35±0.008         | 0.33±0.006   | 0.31±0.003    | 0.31±0.005    |
| K₂     | 0.19±0.005         | 0.18±0.008***(1) | 0.19±0.003     | 0.19±0.003    |
| O₁     | 0.20±0.005         | 0.32±0.005***(2) | 0.35±0.008*(1) | 0.32±0.003    |
| O₂     | 0.19±0.003         | 0.33±0.003   | 0.36±0.003****(1) | 0.31±0.005 |

*p < 0.05; **p < 0.01; ***p < 0.001
(1) Comparing group K₁ and experimental groups
(2) Comparing group K₂ and experimental groups

4. Discussion
A biochemical blood test is a recognized informative test that reflects the general condition of animals [9]. The data obtained during the biochemical analysis of blood revealed small changes in the studied blood parameters against the background of a mineral deficient state of the body. Most likely, this may be due to the adaptation mechanisms of the organism of laboratory animals to the introduction of large concentrations of bioavailable zinc, which considers high concentrations of the element used as a xenobiotic factor. The organism adaptation hypothesis can be confirmed by a decrease in all the studied parameters on the 10th day of the experiment. This allows concluding that there are prospects in the development of the use of biologically active micronutrient preparations based on probiotic strains in the system for correcting the elemental status of a macroorganism in geochemical provinces that determine the development of mineral deficient states [9].

It has been established that micronutrient deficiency is a fairly common phenomenon in Russia. Therefore, at present, more and more attention is paid to the use of complex preparations, which contain not only the necessary element, but also other mineral elements [10]. Our data on the delivery of zinc to the tissues of laboratory animals allowed increasing the concentration of the test compound by the 10th day of the experiment. This is due to the fact that probiotics promote the absorption of trace elements, including zinc [11]. Previously, we have already obtained data on increasing the iron content in animals using inactivated probiotic strains [12].

5. Conclusion
Summarizing the above, it can be noted that one of the possible options for regulating the elemental status of the animal organism is the use of biotic drugs based on the vital products of representatives of transient microflora with high sorption characteristics.

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