

The influence of the culture *Bacillus subtilis* 534 and *Bifidobacterium longum* on the strength of laboratory animals' tubular bones

Kvan Olga¹, Fomina Marina¹, Lebedev Svyatoslav¹, Miroshnikov Sergey², Bykov Artem¹, Sizentsov Aleksei¹, Baranova Oksana¹, Davydova Natasha¹.

¹FSEE HPE «Orenburg state university», Contact: avenue Pobedy, 13, h. 16, r. 307, Orenburg, Russia, 460018, tel: 89225485657. E-mail: kwan111@yandex.ru.

²SSI All-Russian Scientific Institute beef cattle RAAS, 460018, Orenburg, January 9th Street, e-mail: vniims.or@mail.ru, phone: 8(3532) 774641

kwan111@yandex.ru

Abstract: Objective: With help of pairs-analogues method there were formed 3 groups (n=20) of two month old animals, which by the end of preparatory period were transferred to the regimen of the main accounting period (21 days) which supposed the keeping of the first group animals (control) on a diet balanced according to the recommendations of the Institute of Nutrition (RAMN) [Obolsky O.L., 2005], individuals of the second and third groups on a diet deficient in minerals [Miroshnikov S.A., 2006]. **Materials and methods:** The research on the study of the probiotic preparations took place in the experimental-biological clinic (vivarium), FSEE HPE "Orenburg State University" according to the recommendations [Yermolov A.S, Abakumov M. M., 2007]. The ration of the second and third group animals consisted in a boiled, polished rice (rice cooking in distilled water for 15 minutes followed by pouring of the rice water and washing it), with the supplement of vitamins A,D,C,K,E,B₁,B₂,B₃,B₄,B₅,B₆,Bc,B₁₂, which were approved by the recommendations of the Institute of Nutrition RAMN. Distilled water was used as a drink. After the preparatory period (25 weeks) and keeping on a diet balanced by the recommendations of the Institute of Nutrition RAMN, they were transferred to the main accounting period (4 weeks). The experimental technique supposed keeping animals on a diet deficient in minerals with the only difference that the animals of the second group in addition to per os were getting a liquid probiotic preparation which included the culture *Bifidobacterium longum* and the animals of the third group were getting per os a liquid probiotic preparation which included the culture *Bacillus subtilis* 534, containing 10 of microbial bodies in 1ml of preparation, the optimal dosage according to P.I. Zhdanov is 2.5 ml/kg of a forage. In the beginning and in the end of the experiment there was made the study of the elementary composition of bio substrata of the animals. The analysis of the bio substrata of rats and forage under study was made in a laboratory ANO "Center of Biotic Medicine", Moscow (certificate of accreditation ГЦЭН.РУ.ЦОА.311, registration number in a public register РОСС RU.0001.513118 from 29th of May 2003) with a use of the atomic emission and mass spectrometric analysis with a inductively-coupled argon plasma (apparatuses ICAP-9000 «Thermo Jarrell Ash, CHIA, Perkin Elmer Optima 2000DV, USA). Sample preparation was fulfilled according to the recommendation 4.1.1482-03 and 4.1.1483-03, by the method of microwave decomposition on the apparatus Multiwave 3000 (A.Paar). The statistical treatment of the derived material was realized using the programme "Statistica 6.0". The significance level was considered proved at p<0.05 [Rebrova O.Yu., 2002]. **Results and Discussion:** According to our research keeping the rats on a diet deficient in minerals led to the reducing the length and diameter of animals' bones. The length of a thigh-bone of the second and third experimental group exceeded 13% and 15% (p<0.05) the control group, the diameter differed 7.8% only in a group which received in addition *Bifidobacterium longum* [Miroshnikov S.A., 2007]. Calcium and phosphorus are located in a bone tissue as phosphorus-calcium compounds, that's why the level drop of these elements will lead to the violation of the formation and the strength of the animals' bones. In our experiments the strength of thigh-bones was judged by the maximum compression force, during which the bone fracture occurred. The strength of thigh-bones of laboratory animals kept on a diet deficient in minerals (2 and 3 experimental groups) was 32% and 28% higher (p<0.05) than the strength of the first group animals. In order to find out the possible influence of different factors on the strength of laboratory animals' bones a correlation analysis was carried out. The strength of the bones depended on the bone length (r=0.58) and critical power which the bone could maintain to fracture r=0.63. A small negative correlation dependence was noticed between the diameter of the bone and its strength r=-0.09. **Annotation** The results of the experimental research regarding the influence of the probiotic cultures *Bacillus subtilis* 534 and *Bifidobacterium longum* on the strength of laboratory animals' tubular bones are performed in this work (the rats of Wistar line). During the analysis it was shown that probiotic preparation prevents from endogenous loss of Calcium and Phosphorus which increases the strength of laboratory animals' tubular bones.

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Key Words: endogenous loss, laboratory animals, probiotics.

1. Introduction

Based on the facts, the efforts of the organism during the process of digestion to bring the composition of the food to the desirable composition of the chymus duodenum-plazma realize through "the spill" of endogenous substances to the digestive tract. It's well known that the elemental component of the last ones often surpasses in a quantity of the substance of the exogenous ones [Borivant M., 2007].

Specifically, according to Ouwehend, A. C. et al., 2003, the quantity of the endogenous calcium, phosphorus etc, being released to the digestive tract often exceeds two times and more the intake of those elements with food. However, there is a little information about the influence of the probiotic preparations on the strength of the bones during the diet deficient in minerals.

The purpose of this research is the influence of the probiotic preparations on the strength of the laboratory animals' tubular bones.

2. Materials and methods

The research on the study of the probiotic preparations took place in the experimental-biological clinic (vivarium), FSEE HPE "Orenburg State University" according to the recommendations [Yermolov A.S, Abakumov M. M., 2007]. With help of pairs-analogues method there were formed 3 groups (n=20) of two month old animals, which by the end of preparatory period were transferred to the regimen of the main accounting period (21 days) which supposed the keeping of the first group animals (control) on a diet balanced according to the recommendations of the Institute of Nutrition (RAMN) [Obolsky O.L., 2005], individuals of the second and third groups on a diet deficient in minerals [Miroshnikov S.A. et al., 2006].

The ration of the second and third group animals consisted in a boiled, polished rice (rice cooking in distilled water for 15 minutes followed by pouring of the rice water and washing it), with the supplement of vitamins A,D,C,K,E,B₁,B₂,B₃,B₄,B₅,B₆,Bc,B₁₂, which were approved by the recommendations of the Institute of Nutrition RAMN. Distilled water was used as a drink.

After the preparatory period (25 weeks) and keeping on a diet balanced by the recommendations of the Institute of Nutrition RAMN, they were transferred to the main accounting period (4 weeks). The experimental technique supposed keeping animals on a diet deficient in minerals with the only difference

that the animals of the second group in addition to per os were getting a liquid probiotic preparation which included the culture *Bifidobacterium longum* (6.2 ml/kg of a live weight, 1ml of the preparation contains about 10 of microbial bodies), and the animals of the third group were getting per os a liquid probiotic preparation which included the culture *Bacillus subtilis* 534, containing 10 of microbial bodies in 1ml of preparation, the optimal dosage according to P.I. Zhdanov is 2.5 ml/kg of a forage [Zhdanov P.I., 1994].

The experimental animal study was made according to the Russian regulations, (1987) и «The Guide for the Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)».

In the beginning and in the end of the experiment there was made the study of the elementary composition of bio substrata of the animals. The analysis of the bio substrata of rats and forage under study was made in a laboratory ANO "Center of Biotic Medicine", Moscow (certificate of accreditation ГСЭН.РУ.ЦОА.311, registration number in a public register ПООС RU.0001.513118 from 29th of May 2003) with a use of the atomic emission and mass spectrometric analysis with an inductively-coupled argon plasma (apparatuses ICAP-9000 «Thermo Jarrell Ash, ЦИИА, Perkin Elmer Optima 2000DV, USA). Sample preparation was fulfilled according to the recommendation 4.1.1482-03 and 4.1.1483-03, by the method of microwave decomposition on the apparatus Multiwave 3000 (A.Paar).

The statistical treatment of the derived material was realized using the programme "Statistica 6.0". The significance level was considered proved at $p < 0.05$ [Rebrova O.Yu., 2002].

Experimental animal study was made according to Russian regulations, (1987) and «The Guide for the Care and Use of Laboratory Animals (National Academy Press Washington, D.C. 1996)».

3. Results and discussion

It's well known that the normal formation of bone tissue directly depends on the exchange of calcium and phosphorus in animals' bodies.

Two processes are always running: resorption of a bone substance followed by the output of the released calcium and phosphorus to the bloodstream, and the deposition of phosphorus-calcium salts to the bone tissue [Breitwieser G., 2008; Carafoli, E. 1991; Underwood E.J.6 1991].

It might be supposed that keeping animals on a diet deficient in minerals will affect the bone formation of the experimental animals.

According to our research keeping the rats on a diet deficient in minerals led to the reducing the length and diameter of animals' bones.

The length of a thigh-bone of the second and third experimental group exceeded 13% and 15% ($p < 0.05$) the control group, the diameter differed 7.8% only in a group which received in addition *Bifidobacterium longum* [Miroshnikov S.A., 2007].

Calcium and phosphorus are located in a bone tissue as phosphorus-calcium compounds, that's why the level drop of these elements will lead to the violation of the formation and the strength of the animals' bones.

In our experiments the strength of thigh-bones was judged by the maximum compression force, during which the bone fracture occurred.

The strength of thigh-bones of laboratory animals kept on a diet deficient in minerals (2 and 3 experimental groups) was 32% and 28% higher ($p < 0.05$) than the strength of the first group animals.

In order to find out the possible influence of different factors on the strength of laboratory animals' bones a correlation analysis was carried out.

The strength of the bones depended on the bone length ($r=0.58$) and critical power which the bone could maintain to fracture $r=0.63$.

A small negative correlation dependence was noticed between the diameter of the bone and its strength $r=-0.09$

4. Conclusion

1. Keeping animals on a mineral-deficient diet leads to decrease of the length and diameter of the bones and strength reduction. However, the probiotic supplement in the dietary intake of the animals prevents from the endogenous loss of calcium and phosphorus and helps the normal formation of the laboratory rats' bones [Zyla K., 2004; Driver J.P., 2005].

The similar effects of the probiotic preparations on the mineral metabolism of the animals were shown.

It was established by the authors that the additional insertion of the culture *Bifidobacterium longum* assists in the reduction of loss of mineral substances from the animals' bodies.

2. The positive effect of the probiotics on growth and development of the host can be explained with help of the following factors:

a) Stimulation of growth of representatives of the indigenous flora as a result of productions of vitamins and other growth-enhancing factors, Ph normalization, neutralization of toxins.

b) Change of microbial metabolism shown in increase and reduction of enzymes activity.

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Corresponding author:

Ph. D. Kvan Olga
FSEE HPE «Orenburg state university»
Contact: avenue Pobedy, 13, h. 16, r. 307,
Orenburg, Russia, 460018
tel: 89225485657.
E-mail: kwan111@yandex.ru

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