Yogurt enriched to correct intestinal microflora in dysbiosis

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Abstract. The high incidence of dysbiotic disorders, growing threat of new intestinal bacterial pathogens with multidrug resistance and high virulence, and high level of xenobiotic contamination of food products and the environment require the search for alternative non-medicinal products aimed at improving and maintaining health of the intestinal tract. The authors developed a recipe and technology of two-layer yogurt enriched with a prebiotic complex, containing dietary fiber of wheat bran, indigestible food components that promote the growth of normal intestinal microflora (bifidobacteria and lactobacilli), an inactivated yeast culture of Saccharomycescerevisiae (vini), and blueberry and cornelian cherry puree—a source of polyphenolic compounds. The yoghurt culture used in the technology contains freeze-dehydrated strains of probiotic cultures, i.e., Streptococcus thermophilus, Lactobacillus delbrueckiisobulgarianus, Lactobacillus acidophilus, Bifidobacterium lactis, and lactose. The authors studied the effect of the developed yogurt on the intestinal microflora and their ability to prevent and correct dysbiosis. To this end, a model of experimental antibiotic-associated dysbiosis was reproduced using a broad-spectrum antibiotic Gentamicin in white mice. The antibiotic was being injected intraperitoneally for 5 days. After withdrawal of gentamicin, a considerable increase in opportunistic enterobacteria (Enterobacter aerogenes, Enterobactercloacae, and Citrobacterfreundii), Proteussp, and Candidasp. was observed. There was also a decrease in the quantity and quality of Lactobacillus sp. E.coli and a decrease in the content of Bifidobacteriumsp. Enriched yogurt in the diet of mice allowed the microbiological evidences of dysbiosis to be eliminated in a short time, prevented clinical symptoms of dysbiosis, i.e., decreased physical activity and poor appetite, stool softening, constipation, flatulence, and worsening the appearance. Thus, the developed enriched yogurt can be used to prevent and correct dysbiotic intestinal disorders.

1. Introduction
The global deterioration of the environment and human health is a key component that adversely affects global health trends. Noncommunicable diseases such as diabetes, cancer, and heart diseases increase worldwide and account for more than 70% of deaths, i.e., about 41 million people each year, including 15 million premature deaths at the age of 30-69 years [1]. All of the above diseases can be considered nutritional-related, that is, associated with food contamination with residual amounts of pesticides, agrochemicals, food additives, and hormonal and antibacterial drugs used in crop production and animal
husbandry. These substances not only cause endogenous intoxication, but also integrate into the metabolic reactions of the body, and disrupt the functions of organs and systems, worsening pathological conditions [2]. So, the main task of the food industry is the development of food products that have not only high nutritional value, but also protective properties that prevent the adverse impact of harmful factors on human health [3].

The intestinal tract is the main barrier to pathogens and xenobiotics. The main components of the intestinal barrier are immunocytes produced by lymphoid tissues and their products – immunoglobulins and antibodies (including secretory ones), antimicrobial peptides, cytokines, etc. About 80% of all immune cells are associated with the intestinal mucosa [4]. Even a slight decrease in the concentration of lymphocytes can lead to a considerable decrease in the speed of the immune response [5].

The human gastrointestinal tract is known to contain trillions of microbes represented by 4000 strains of microorganisms that make up the intestinal microbiota. Commensal bacteria (CB) of the intestine induce the maturation of dendritic cells and release of cytokines that provide a mature T-cell immune response and are responsible for the maturation of B-lymphocytes, producing secretory IgA that controls microbial and antigenic penetration. Due to these mechanisms, CB prevent various chronic inflammatory diseases of the gastrointestinal tract [6, 7].

The microbiome is involved in vital physiological and immunological processes, including energy homeostasis, metabolism, synthesis of vitamins and other nutrients, regulation of endocrine and immune functions, protection against colonization by enteropathogens, and metabolism of xenobiotic compounds. Qualitative and quantitative changes in the intestinal microflora lead to dysbiosis, disorder of microbiome functions, and some pathological conditions in the body [8]. Thus, the high incidence of dysbiotic disorders, growing threat of new intestinal bacterial pathogens with multidrug resistance and high virulence, and high xenobiotic contamination of food products and the environment require search for alternative non-medicinal products aimed at improving and maintaining health of the intestinal tract. In this regard, an urgent and promising trend is the development of new fermented dairy food – pre- and probiotic functional products designed to prevent dysbiotic conditions.

The authors developed a recipe and technology of two-layer yogurt enriched with a prebiotic complex, containing dietary fiber of wheat bran, indigestible food components that promote the growth of normal intestinal microflora (bifidobacteria and lactobacilli), an inactivated yeast culture of Saccharomycescerevisiae (vini), and blueberry and cornelian cherry puree – a source of polyphenolic compounds. The yoghurt culture used in the technology contains freeze-dehydrated strains of probiotic cultures, i.e., Streptococcus thermophilus, Lactobacillus delbrueckiissp.bulgariicus, Lactobacillus acidophilus, Bifidobacterium lactis, and lactose.

2. Purpose and objectives
The purpose of the study was to experimentally study the effect of the yoghurt developed on the intestinal microflora. For this, there were accomplished the objectives, i.e., to reproduce a model of experimental antibiotic-associated dysbiosis, using a broad-spectrum antibiotic Gentamicin on white mice; correct dysbiotic disorders, using the yogurt developed; compare and assess the dynamics of clinical and microbiological parameters; and evaluate the effectiveness of the developed product as a protector of dysbiotic disorders.

3. Materials and methods
To accomplish the objectives of the experimental study, we performed the following steps:

a) research objects selected
   The object of the study was white outbred mice, weighing 18-20 g. The animals were randomized into 2 groups, namely, Group I (n=10) received gentamicin; Group II (n=10) received gentamicin and the product developed.

b) antibiotic-associated dysbiosis modeled
   Antibiotic-associated dysbiosis was reproduced simultaneously in both groups by oral supplementation of 0.1 ml of a solution of 2.9 mg of gentamicin for 5 days once every 24 hours.
c) dysbiotic disorders corrected with yogurt

On day 3 after starting the gentamicin course, Group II mice received the developed product (the puree layers and yogurt were homogenized) orally for 21 days, 0.2 ml per animal. Group I mice were not given the product.

d) the dynamics of microbiological and clinical indicators compared and assessed

For a quantitative and qualitative assessment of microorganisms, suspensions of white mice’s feces were seeded on differential diagnostic media produced by the nutrient media produced by the Federal State Institution of NIIMP Rospotrebnadzor (Rostov) and RA Nutrient Media (Makhachkala). The number of isolated microorganisms was expressed in lg CFU/g of biological material weight. Feces were studied simultaneously in both mice groups before the gentamicin course (baseline data), then in Group I on days 3, 10, 17, and 24 after the gentamicin course, and in Group II on days 1, 8, 15, and 22 after the start of the developed product, i.e., on days 4, 11, 18, and 25 after antibiotic withdrawal. The experiments were repeated three times. Clinical evidences were evaluated visually.

d) statistical analysis

Experimental data were analyzed and processed, using the analytical package of Excel 2010. The statistical significance in average values was calculated by the t-test after checking the normality of the values distribution, using parametric methods of data calculation. When assessing the significances of the compared data, the significance was considered p<0.05.

4. Results and discussion

4.1. Microflora composition changed under intestinal dysbiosis due to gentamicin

Under experimental dysbiosis, changes in the qualitative and quantitative composition of the microbiocenosis of the large intestine of experimental animals were recorded. The results are presented in Table 1.

| Microorganisms                  | Initial background | Day after withdrawal of gentamicin (lg/CFU, g) |
|--------------------------------|--------------------|-----------------------------------------------|
|                                | M±m                | 3        | 10     | 17     | 24     |
| Lactobacillus sp.              | 7.19±0.2           | 50% RAFA* | 5.12±0.2*** | 5.35±0.3*** | 5.91±0.2*** |
|                                | 6.23±0.1***        |          |        |        |        |
| Bifidobacterium m sp.          | 9.63±0.4           | 7.78±0.1*** | 5.44±0.3*** | 6.11±0.3*** | 7.56±0.8*** |
| E.coli                         | 7.83±0.4           | 50% REA* | 100% СФА* | 50% СФА* | 6.48±0.3 |
|                                | 6.25±0.3***        |          |        |        |        |
| Staphylococcus sp.             | 3.23±0.1           | 2.12±0.1*** | 3.14±0.2 | 3.26±0.4 | 3.15±0.4 |
| Candida sp.                    | 0                  | 4.22±0.3*** | 2.15±0.2*** | 1.02±0.1*** | 0       |
| Opportunistic enterobacteria   | 1.51±0.1           | 4.25±0.3*** | 8.41±0.6*** | 8.05±0.3*** | 7.17±0.4*** |

* REA is E.coli with reduced enzymatic (lactase) activity
** RAFA is Lactobacillus sp. with reduced acid-forming ability
*** p <0.05 compared to the initial background

The animals induced with dysbiosis had a statistically significant decrease in Lactobacillus sp. lg CFU/g, being registered throughout the experiment. On day10, there was recorded the maximum low value (5.12±0.2) combined with a decreased acid-forming ability; the difference with the base value was 28.8%. Lactobacilli should be noted not to recover to the background level (5.91±0.2 versus 7.19±0.2), the difference was 17.8% (p<0.05) at the end of the experiment.
Dysbiotic disorders due to gentamicin were accompanied with a decrease in *Bifidobacterium* sp., with the maximum value being 5.44±0.3 on day 10. In relation to the background, the difference was 43.51%. By the time the experiment was completed, the bifidobacteria content had slightly increased, but remained reduced by 21.5% (p<0.05) in comparison with the initial indices.

Severe dysbiotic disorders in Group I animals were confirmed by a decrease in *E. coli* lg CFU/g. There was observed not only a quantitative decrease in these bacteria with a maximum value of 34.1% compared with the background on day 10 of the experiment, but also qualitative indicators of microorganisms. In particular, a decrease in enzymatic (lactase) activity was found to be up to 50% on day 3 after the withdrawal of gentamicin, up to 100% on day 10, and up to 50% on day 17.

Against the background of the protective components deficiency of the intestinal microbiocenosis, animals in Group I were observed to have a significant increase in opportunistic enterobacteria, i.e., *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Citrobacter freundii*. Their maximum increase was registered on day 10 after intake of gentamicin (lg 8.41±0.6 against the initial lg of 1.51±0.1). On day 24 of the experiment, lg CFU/g of opportunistic enterobacteria was 5 times higher than the background. This indicated significant and persistent biotope colonization and a pronounced dysbiotic disorder associated with a deficiency of bifidobacteria and lactobacilli, a sharp decrease in the *E. coli* lactase activity, and *Lactobacillus* sp. with reduced acid-forming ability to competitively inhibit the opportunistic enterobacteria growth.

*Candida* sp. showed a slightly different picture. It was completely absent in the initial state; by the third day of gentamicin intake, the lg CFU/g value was 4±0.3, which was typical for drug dysbiosis. Further observation revealed a decrease in lg CFU/g of *Candida* sp. two times by day 10, and four times by day 17; complete elimination of *Candida* was observed by the end of the experiment.

Changed microbiological points in Group I were accompanied by nonspecific and specific clinical evidences of dysbiosis. On days 10-17 after the gentamicin withdrawal, 60% of the animals changed their coat that became dull and “needle-like” and suffered from decreased physical activity and poor appetite. There were noted specific disorders—liquid stool in 80%, constipation in 20%, and bloating in 80% of individuals. Severe clinical evidences had decreased by day 24, and microbiological points of feces improved.

### 4.2. Dysbiotic disorders corrected with enriched yogurt

The developed yogurt enriched with a prebiotic complex, containing dietary fiber of wheat bran, an inactivated yeast culture of *Saccharomyces cerevisiae (vini)*, and blueberry and cornelian cherry puree, led to earlier restoration of normal microflora in mice. The results are presented in table 2.

**Table 2.** Microflora composition changed under intestinal dysbiosis due to gentamicin and corrected by the product developed (Group II).

| Microorganisms          | Initial background lg CFU g | Day after withdrawal of gentamicin (lg CFU, g) (day of enriched yogurt intake) |
|-------------------------|-----------------------------|---------------------------------------------------------------------------------|
|                         |                             | 4 (1)                              | 11 (8)                              | 18 (15)                             | 25 (22)                             |
| *Lactobacillus* sp.     | 7.21±0.1                    | 50% RAFA**                         | 7.15±0.2                            | 7.44±0.4                            | 8.85±0.4***                         |
| *Bifidobacterium* sp.   | 9.51±0.3                    | 7.88±0.3***                        | 9.46±0.5                            | 10.15±0.2                           | 11.71±0.5***                        |
| *E.coli*                | 7.91±0.6                    | 50% REA*                           | 30% CFDA*                           | 7.59±0.3                            | 7.81±0.4                            |
| *Staphylococcus* sp.    | 3±                          | 6.78±0.2***                        | 7.14±0.4***                         | 0                                    |
| *Candida* sp.           | 0                           | 3.78±0.1                           | 0                                   | 0                                    |
| *Opportunistic* enterobacteria | 1.47±0.1           | 4±0.2                              | 2.34±0.1                            | 1.25±0.1                            | 1.20±0.2                            |

*REA is *E.coli* with reduced enzymatic (lactase) activity
** RAFA is Lactobacillus spp. with reduced acid-forming ability
*** p <0.05 compared to the initial background

On the background of enriched yogurt taken, mice with dysbiosis had the maximum decrease in the lactobacilli log CFU/g and a decrease in the acid-forming ability by 50% on day 1 of the product intake (dysbiosis day 4) (6.56±0.5, p<0.05). After a week, the number of lactobacilli and their functional activity were restored to the background values, and the log CFU/g exceeded the baseline by 23.0% at the end of the experiment (three-week intake of the product). A statistically significant decrease in log CFU/g for Bifidobacterium spp was noted on day 4 of dysbiotic disorders; the difference with baseline was 21% and was considered statistically significant. The bacteria restored their initial value after yogurt intake for a week and exceeded the background by 18% (p<0.05) by the end of the experiment.

Lactobacillus spp and Bifidobacterium spp’s significant excess over background was due to the regular intake of small doses of these microorganisms as a part of yogurt, and the prebiotic component in the form of wheat bran fibers considerably stimulated growth of these probiotic cultures, comparable in efficiency to the classic probiotic prebiotc lactulose.

Moreover, bifidobacteria and lactobacilli exhibited pronounced antagonistic activity against opportunistic and pathogenic microorganisms due to specific enzymes, various bactericidal and bacteriostatic substances and antibiotic-like components, as well as a faster reproduction rate, and, consequently, more active competition for nutritional substrates in their cells [9]. Antagonistic effect of Lactobacillus spp was due to their ability to produce lactic acid that had a bactericidal effect due to a significantly decreased pH of the medium. In addition, peroxidase helped lactobacilli to activate the peroxide compounds, such as thiocyanate and hypothiocyanate that had a strong bactericidal effect, especially against Staphylococcus spp [10]. This was confirmed by Staphylococcus spp and Candida spp microorganisms, having rapidly eliminated from the intestines, and severe inhibition of the opportunistic enterobacteria growth.

The active growth of probiotic bacteria in the intestines of animals contributes to the stabilization of the intestinal cell wall and prevents endogenous intoxication [11]. In our experiment, this was confirmed by the clinical symptoms of dysbiosis, absent in 100% of Group II animals, namely, poor appetite, decreased physical activity, liquid stool, constipation, flatulence, and worsening of the animals’ appearance.

5. Conclusions

Based on the study, we can conclude that the developed yogurt enriched with a prebiotic complex, containing dietary fiber of wheat bran, an inactivated yeast culture of Saccharomyces cerevisiae (vini), and blueberry and cornelian cherry puree, to correct microflora in experimental antibiotic-associated dysbiosis of mice allowed complete normalizing the qualitatively-quantitative changes in the composition of the intestinal microflora due to oral supplementation for 21 days. Thus, the developed enriched yogurt can be used as a health-promoting product to prevent and correct dysbiotic intestinal disorders. The treatment dysbiosis with enriched yogurt should take into account the antagonistic relationship between exogenous probiotic strains and indigenous ones that can displace food probiotics from the human intestine immediately after withdrawal of the product enriched [10]. To preserve a high population of probiotic strains in the gastrointestinal tract, we recommend a constant consumption of the probiotic-enriched product. The wheat bran fibers in its composition normalize gut microbiome not only due to stimulating the growth of fermented milk yogurt flora, but also due to the restoration of indigenous lactobacilli.

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