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BOGDAN VICTORIA

THE PHYSIOLOGICAL ROLE OF INTESTINAL ENTEROCOCCI IN MAINTAINING THE HEALTH OF THE DIGESTIVE TRACT

165.01. Human and animal physiology

Abstract of doctoral thesis in biological sciences

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The doctoral thesis and abstract can be consulted at the National Library of the Republic of Moldova, the „Andrei Lupan" Central Scientific Library (Institute), the Central Library of Moldova State University (MD 2009, Chișinău, 60 Alexei Mateevici Street), on the ANACEC website (http://www.anacec.md), and on the MSU website (http://www.usm.md).

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CONCEPTUAL RESEARCH FRAMEWORK

The actuality of the investigated topic. The health of the intestinal tract and its efficient functionality are important factors determining overall organism health. Research over recent decades has reaffirmed the significance of the gastrointestinal microbiota (GI) in both physiology and overall health. At the beginning of the 21st century, the journal „Science” predicted that „research on the human microbiome would become a new hot topic globally” (Lederberg J., 2000). This prediction led to major research projects such as the US Human Microbiome Project (HMP) (HMPC, 2012) and the European Metagenomics of the Human Intestinal Tract (MetaHIT) (Qin J. et al., 2010), among others, which have demonstrated the beneficial functions of normal intestinal flora on physiological processes and health, down to the genetic level (Jandhyala S.M. et al., 2015).

The intestinal microbiota itself represents a consortium of bacteria that establishes individually for each organism, remains relatively stable over time, and has coevolved with its host species (human or animal), developing mechanisms of beneficial coexistence (commensalism) throughout this coevolutionary history. The benefits provided by intestinal bacteria depend on their adaptation to specific conditions within the digestive tract, bacterial interspecies communication, and the mechanisms dictating their interactions with the host organism. Each bacterial species plays a role in maintaining the functionality and health of the gastrointestinal tract (Albenberg L.G., Wu G.D., 2014; Kim C.S., Claud E.C., 2019; Khaledi M. et al., 2024).

Enterococci, despite being a rather controversial group of bacteria not only in terms of their pathogenicity but also, until recently, from a systematic point of view, are indispensable bacteria of the indigenous intestinal microbiota. They are among the first colonizers of the digestive tract immediately after birth, participate in the modulation of the immune system in humans and animals, and have the property of creating symbiotic relationships with other bacteria and with the host organism (Schleifer K.H., Kilpper-Bälz R., 1984; Sanders M.E. et al., 2010). Due to properties such as bacteriocin production (enterocins), enhanced adhesive capacity, and high antagonistic activity against pathogens, some species and strains of enterococci have demonstrated probiotic properties beneficial for health (Hanchi H. et al., 2018; Krawczyk B. et al., 2021; Wu Y. et al., 2022; Im E.J. et al., 2023). This has also been established in research conducted at the Institute of Physiology and Sanocreatology, regarding the physiological role of the digestive tract and the intestinal microflora, and specifically the significance of commensal bacteria (Timoșco M. et al., 2010; 2011). These studies are also in line with the research direction of the Institute – sanocreatology, which aims to develop the theory and practice of forming and maintaining health in accordance with lifestyle and ecological and living factors (Фурдуй Ф.И. et al., 2010; 2011 a, b; 2016; Timoșco M. et al., 2010; 2011).

In this context, focusing on the „non-infectious” behavior of enterococci could elucidate their significance for host organism health, particularly in preventing and treating intestinal infections. Such research aligns with global and European scientific studies regarding antibiotic resistance and healthcare-associated infections.

The aim of the research was to elucidate physiological aspects regarding the significance of enterococci for digestive tract health and to highlight the probiotic potential of certain enterococcal strains.

Objectives of the study:

- Investigating the incidence and quantitative levels of intestinal enterococci in the human and animal digestive tract according to the physiological status of the organism.
- Studying the impact of dietary factors on the content of intestinal enterococci compared to other bacterial groups and highlighting their role in maintaining beneficial intestinal microbial balance.
- Isolating, identifying, and selecting the most advantageous strains of intestinal enterococci with health-promoting potential and elucidating their probiotic properties.
- Prospecting and testing new enterococcal microbial compositions designed to maintain digestive tract health and overall organism health.

Research hypothesis: Enterococci, as part of the intestinal microbiota established during digestive tract colonization, contribute as commensals to maintaining intestinal microbial balance, and pure and safe enterococcal strains can be used as probiotics to restore intestinal flora, thereby impacting organism health.

The scientific novelty and originality consist of revealing of the enterococci quantitative level correlation with that of the beneficial bacteria in conditions where the development of intestinal dysfunctions is not attested, which denotes about of their role in maintaining the sanogenic intestinal microbial balance, demonstrated by the elucidation of the quantitative changes of enterococci in the intestinal contents depending on the physiological status of the body and the action of the food factor. Under conditions of severe pathological processes, enterococci may lose their health-promoting action and potentially become pathogens in association with other pathobionts. Based on the correlation of enterococci with the beneficial bacteria of intestinal microflora, it is possible to deduce about the intestinal microbial balance and the health state of digestive tract. The probiotic potential of certain strains of enterococci has been proven, which is identical or more advantageous compared to the recognized probiotic potential of some obligate bacteria, and the effectiveness of new enterococcal microbial compositions in maintaining the health of the digestive tract and the whole organism has been tested.

The results obtained by the author contribute to solving an important scientific problem. Elucidating the role of intestinal enterococci at the action of factors determining the physiological status of the digestive tract and, respectively, of the organism, especially the dietary factor expressed through a different percentage structure of basic macronutrients – proteins, lipids, and carbohydrates, on the one hand, revealed the beneficial correlation of enterococci with obligate intestinal bacteria, contributing to maintaining a healthy intestinal environment, on the other hand, it highlighted the possibility of modulating the composition of the intestinal microbiota in a health-promoting aspect through the administration of diets with different nutritional value and caloric structure, as prebiotics. The possibility of modifying and fortifying the intestinal microflora through diet could enhance its capabilities to inhibit the development of intestinal pathogens, without the use of antibiotics or other medical preparations. Determining the relevant probiotic properties of certain strains of enterococci, their compatibility with obligate intestinal bacteria, and their action on the intestinal microflora and natural resistance indicators of the organism, supports the inclusion of tested enterococcal strains in probiotic microbial compositions, confirming the problem of ensuring intestinal health when administering probiotics.

The theoretical significance of the study consists in expanding of knowledge regarding the role of intestinal enterococci in gastrointestinal health depending on environmental conditions, the physiological status of the digestive tract and of the organism, dietary factors, and their compatibility with other commensal bacteria. It has been established that the health-promoting effect of enterococci is maintained under conditions where their numerical effectiveness does not exceed that of beneficial commensal bacteria, and its correlation controls the growth of pathogenic bacteria.

The practical value of the study. The probiotic potential of isolated enterococci strains (pure strains) that express more beneficially in association with bifidobacteria and lactobacteria has been demonstrated, their use as probiotics has been recommended, and microbial compositions containing tested strains of enterococci with sanogenic action have been developed.

Scientific research results have been implemented in the research process at the Institute of Physiology and Sanocreatology, Moldova State University. The use of novel microbial compositions developed on the basis of the tested enterococci strains was stopped at the experimental sample and patent stage due to considerations of further verification of the safety aspects of the bacterial strains.

Synthesis of methodology and justification of chosen research methods: In order to achieve the aim, the objectives and to prove the research hypothesis, the following methods were used: classical microbiological methods for culturing strains of enterococci and other bacterial groups; methods for assessing the viability and quantification of the cultured strains (species) of bacteria and methods for statistical processing of the results.

THESIS CONTENT

The thesis comprises an introduction, 4 chapters, general conclusions and recommendations, bibliography with 360 titles, totaling 250 pages, including 172 pages of main text, 4 annexes, 19 figures, and 36 tables. The obtained scientific results have been disseminated through 22 scientific papers.

In Chapter 1, **„The significance of intestinal microbiota in organism functionality and health. The physiological role and probiotic potential of enterococci”**, the literature analysis emphasizes the importance of intestinal microbiota, including enterococci, in the normal functioning and health of the host organism. It highlights the formation of bacterio-ecological niches during the colonization of different segments of the digestive tract, based on bacterial compatibility and sophisticated communication mechanisms between the flora and the host organism, which subsequently determine the organism's healthy physiological status. The physiological role of enterococci is underscored, and arguments are presented regarding the distinction between commensal and nosocomial strains. As integral components of indigenous microbiota in humans and animals, enterococci participate in modulating the immune system and controlling the population of pathogenic bacteria, particularly through the production of enterocins. The text includes discussions on the use of enterococcal strains as probiotics and addresses safety considerations, including regulatory provisions governing the widespread application of enterococcus-based probiotics.

Chapter 2, **„Materials and research methods”,** presents the methods and research protocols for investigating the incidence of enterococci bacteria in various biological samples with different physiological statuses, adhering to microbiological examination rules according to SM SR ISO 7218 and the principle of using genetically unmodified microorganisms with natural biological potential (non-pathogenic). The investigation of enterococci was conducted in comparison with other groups of intestinal bacteria – bifidobacteria, lactobacilli, and *E. coli* – which are significant in maintaining digestive tract health.

Classic microbiological methods and tests were employed for pure culture isolation, identification, and selection of microorganisms, following modifications by Bogdanov V. (1959), Bannikova L. (1975), and identification methods as per Bergey (Bergey D.H., Holt J.G., 1994). Bacterial growth activity was assessed by counting colony-forming units (CFU) on Petri dishes following successive dilutions. CFU counts are expressed in decimal logarithms (lg 10) per 1 g of intestinal content (Гармашева И.Л., 2011).

The chapter outlines experimental designs involving various animals (laboratory and agricultural), which elucidated changes in intestinal bacteriocenosis due to dietary factors and highlighted the benefits of isolated enterococcal strains as probiotics. The efficacy of researched enterococcal strains and their probiotic potential were tested based on the action of microbial compositions (including enterococci) on physiological indices in laboratory and agricultural animals. In the experimental process, the requirements and principles of setting up scientific experiments were respected. Animal experiments were conducted in accordance with Directive 86/609/EEC of November 24, 1986, concerning the protection of animals used for scientific purposes and were approved by the Methodological Committee of the Institute of Physiology and Sanocreatology, Moldova State University.

Chapter 3, **„Incidence, quantitative level, and physiological role of enterococci in the human and animal digestive tract”,** includes research results regarding changes in the numerical value of enterococci in various animal species and in humans, depending on the host's physiological status: age, living environment, digestive tract health status, and dietary factors.

The proportion of enterococci among lactic acid bacteria (lactobacilli) – *Enterococcus*, *Lactococcus*, and *Streptococcus* – was determined in human subjects of different ages, establishing lower incidence compared to streptococci and higher compared to lactococci in early life, and higher levels compared to both groups of bacteria during adulthood, indicating widespread distribution of these bacteria.

Quantitative levels of enterococci also vary among different animal groups – pets, laboratory animals, farm animals, and captive wild animals. Higher values of these bacteria were detected in pets – dogs and cats – exceeding their levels in farm animals by 2.7 units, in laboratory animals by 1.6 units, in wild animals by 2 units, and in wild birds by 1.2 units. The low correlation of enterococci indices with beneficial bacteria (bifidobacteria and lactobacilli) and high correlation with pathogenic bacteria (*E. coli*) in pets confirms literature data on the widespread presence of enterococci in this group of animals due to close contact with humans and shared living environments, potentially leading to cross-transmission of these bacteria, including pathogenic forms (Nilsson O., 2012; Wu S., 2022). Based on the living environment, a higher level of enterococci was found in rural birds compared to urban birds. Urban birds showed a more positive correlation between enterococci, bifidobacteria, and lactobacilli in maintaining a low level of *Escherichia coli*, compared to rural birds. This suggests that the intestinal bacteriocenosis state in rural birds is less favorable compared to urban birds and zoo animals, indicating that in rural environments, contact with other domestic animals and humans leads to modifications in intestinal microflora with a dominance of pathogenic bacteria.

Another research objective aimed to highlight the **quantitative level of enterococci depending on age and digestive tract health status** in human subjects during the first year of life and aged 1 to 80 years, and in juvenile farm animals.

Investigating the level of enterococci in healthy infants and those with intestinal dysfunctions during the first year of life will elucidate aspects related to establishing the „healthy" level of enterococci and the „critical" periods for these bacteria in cases of dysbiosis. For this purpose, the incidence (numerical indices) of enterococci in healthy children (Group I) and those with intestinal dysfunctions (Group II) was studied across age groups up to one year (Table 1).

Table 1. Quantitative indices of intestinal enterococci in young children (up to 360 days) according to the health status of the digestive tract

Age, days	The number of microbial cells per 1 gram of intestinal content, decimal logarithms (lg)		The difference between group I and II, %
	I (healthy subjects)	II (subjects with intestinal disorders)	
0-6	5.93±0.15	6.63±0.12**	+11.80
7-30	5.76±0.10	6,84±0.12***	+18.75
31-60	5.23±0.14	7.17±0.16***	+37.09
61-90	6.19±0.13	6.57±0.14	+6.13
91-120	6.00±0.11	6.04±0.13	+0.66
121-180	5.92±0.10	5.65±0.11	-4.56
181-240	6.53±0.09	6.49±0.12	-0.61
241-300	5.63±0.12	6.38±0.17**	+13.32
301-360	5.53±0.07	6.63±0.11***	+19.89

Note: ** - p≤0.01 (statistically significant); *** - p≤0.001 (statistically highly significant).

In the first year of life, in healthy human subjects (children), the content of enterococci is quite high even in the first days after birth, but the highest values are observed in the periods of 61-90 days and 181-240 days.

In subjects with intestinal dysfunction (Group II), there is an increase of 6%-37% in the quantitative level of enterococci. The greatest difference between healthy subjects and those with intestinal dysfunction was found in the period of 31-60 days after birth. Thus, we can deduce that this period is more „critical" in terms of disrupting intestinal bacterial homeostasis. At other periods, even against the background of intestinal dysfunction, the quantitative level of enterococci remains uniform.

To elucidate the „behavior" of enterococci in subsequent periods of life in cases of intestinal dysfunction, it was proposed to analyze the content of these bacteria in the intestinal contents of healthy human subjects (Group I), subjects with intestinal dysbiosis (Group II), and subjects with diarrheal intestinal dysfunction (Group III) (Table 2).

The analysis of the variation in enterococci content in healthy human subjects of different ages reveals: between the ages of 1-16 years, the level of enterococci is not stable and varies between 3 to 4 years and from 10 to 14 years; at the age of 20-50 years, the level of microbial cells is relatively stable, and in the period of 60-80 years, the level of enterococci is in a constant increase (Table 2).

Table 2. Quantitative indices of enterococci in human subjects (children and adults) depending on the health status of the digestive tract (Timoșco et al., 2014)

Source	Age, days	The number of microbial cells per 1 gram of intestinal content, decimal logarithms (lg)		
		Group I	**Group II**	**Group III**
Children	1	5.53±0.12	7.49±0.11***	8.45±0.18***
	2	5.60±0.07	7.90±0.13***	8.30±0.19***
	3	5.92±0.11	7.88±0.17***	8.46±0.20***
	4	5.20±0.25	6.30±0.46	8.65±0.46***
	6	5.38±0.15	7.20±0.40**	8.77±0.48***
	8	5.46±0.22	7.53±0.32***	8.17±0.50***
	10	5.88±0.22	7.86±0.32***	8.43±0.51***
	12	5.90±0.15	7.92±0.17***	8.04±0.22***
	14	6.13±0.13	7.76±0.18***	7.88±0.21***
	16	6.00±0.16	7.54±0.14***	7.64±0.25***
Adults up to 50 years old	20	7.54±0.14	8.43±0.18**	9.38±0.22***
	30	7.62±0.10	8.54±0.20**	9.49±0.23***
	40	7.74±0.12	8.64±0.19**	9.54±0.21***
	50	7.90±0.11	8.49±0.17*	9.60±0.22***
Adults over 50 years	60	8.20±0.13	8.92±0.18*	9.77±0.20***
	70	8.64±0.15	8.96±0.21	9.80±0.23**
	80	8.77±0.17	9.20±0.20	9.88±0.19**

Note: * - p≤0.05 (statistically significant); ** - p≤0.01 (statistically highly significant); *** - p≤0.001 (statistically very highly significant).

In the case of intestinal dysmicrobism (Group II), insignificant changes in the quantitative indices of intestinal enterococci were recorded, with an increase of approximately 1.2-1.4 times compared to healthy subjects. The most drastic changes were recorded in subjects with diarrheal disorders (Group III), where a 1.3-1.7 times increase in the numerical value of these microorganisms was established. The greatest difference is observed in children with diarrheal intestinal dysfunctions (Group III), especially at the age of 4 years, with an increase of 66.3% compared to healthy subjects (Table 2). In adults over 50 years old, the numerical value of enterococci increased by 1,0 time in subjects from Group II and by 1.1-1.2 times in subjects from Group III. The smallest difference in quantitative indices between groups was found in subjects aged 80 years (Table 2). Thus, the obtained results indicate a high sensitivity of intestinal enterococci to changes in the intestinal environment in children, especially in the first 4 years of life, while in elderly human subjects, changes in enterococci content are largely due to lifestyle and physiological changes that occur with aging, which directly affect the intestinal microbiota.

The analysis of the results obtained in farm animals: chicks, piglets, and calves showed that with growth, the numerical population of enterococci increases in healthy animals. In the case of digestive health disorders, the level of these bacteria, compared to healthy animals, decreases in chicks and piglets and increases in calves. These changes may again be caused by the physiological specificity of the digestive tract (phylogenetically established), as well as by the nutrition mode and controlled maintenance conditions (Table 3).

Table 3. Quantitative indices of intestinal enterococci in different groups of economically important animals depending on the health status of the digestive tract (Timosco et al., 2014)

Source	Age, days	The number of microbial cells per 1 gram of intestinal content, decimal logarithms (lg)		
		Group I	Group II	Group III
Chicks	10	5.43±0.12	8.54±0.18***	9.38±0.22***
	20	5.88±0.10	8.79±0.17***	8.92±0.19***
Piglets	10	5.25±0.13	7.88±0.19***	8.72±0.21***
	20	5.64±0.12	8.73±0.16***	9.41±0.20***
	30	6.34±0.11	8.92±0.21***	9.86±0.23***
	45	6.80±0.11	8.14±0.16***	8.53±0.23***
	75	6.66±0.11	8.04±0.21***	8.30±0.19***
	105	6.53±0.10	7.08±0.17*	8.07±0.21***
	135	6.49±0.13	7.46±0.20**	7.82±0.20***
Calves	10	6.53±0.14	8.49±0.14***	9.23±0.19***
	20	6.43±0.12	8.30±0.12***	8.94±0.17***
	30	6.20±0.13	8.43±0.11***	8.79±0.15***

Note: * - $p \leq 0.05$ (statistically significant); ** - $p \leq 0.01$ (statistically highly significant); *** - $p \leq 0.001$ (statistically very highly significant).

The obtained data allows us to assume that in the first days, months, and years of life, the commensal forms or strains of enterococci can acquire pathogenic/virulent characteristics when the respective conditions of infection (intestinal dysmicrobism and diarrheal intestinal dysfunctions) are created.

According to the objective „**The influence of dietary intake on the process of multiplication and development of intestinal enterococci and the role of these bacteria in maintaining beneficial intestinal microbial balance**" two series of experiments were conducted.

In the first series of experiments, the influence of diets with different nutritional values on the incidence of intestinal enterococci was tested. The nutritional value of the diets was determined based on the proportion of the main nutrients in the diet, with three diets administered: one with an excess of proteins, one with an excess of lipids, and one with an excess of carbohydrates. The protein sources were chicken meat and egg whites; the lipid source was lard; and the carbohydrate source was bread. The effect of these diets was monitored over time: after 5, 10, and 15 days of administration, and after 7 days of returning to a standard diet. The analysis of enterococci under the influence of these diets was compared with the content of bifidobacteria, lactobacteria, and *E. coli* to provide a broader view of how dietary factors influence the intestinal microbiota. The recording was done for each animal individually, which was kept separately in cages, according to established norms.

It was found that among all the investigated groups of bacteria, the numerical value of enterococci increased significantly with the administration of a diet with excess proteins, both compared to the initial data and to the control (Figure 1 A.).

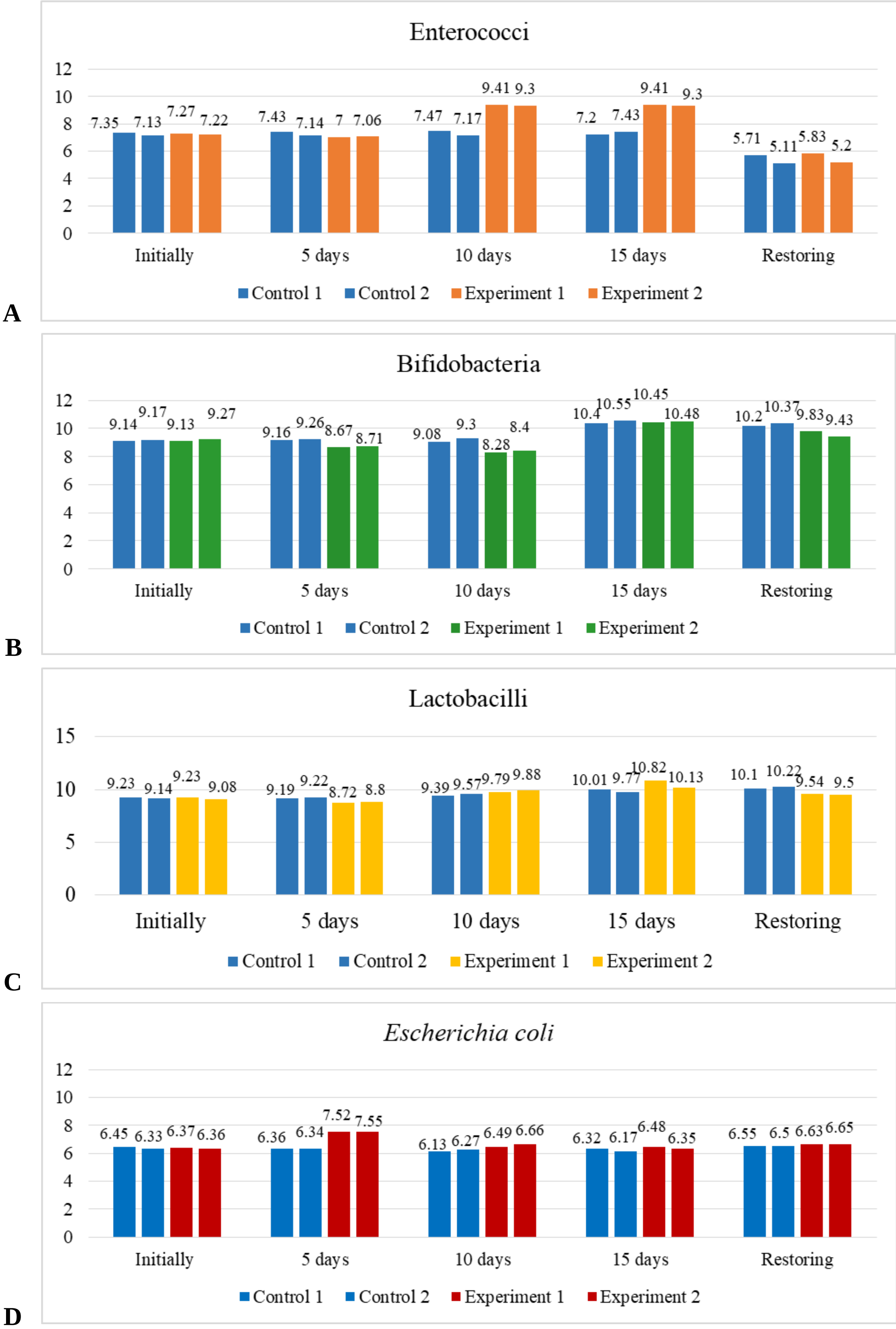


Figure 1. **The content of bacterial of enterococci (A), bifidobacteria (B), lactobacteria (C), and *E. coli* (D) cells (lg per 1 g of intestinal content) under the dynamic action of a protein-excess diet**

Legend: Control (1, 2) – laboratory white rats fed with the standard diet; Experiment (1, 2) – laboratory white rats fed with a protein-excess diet.

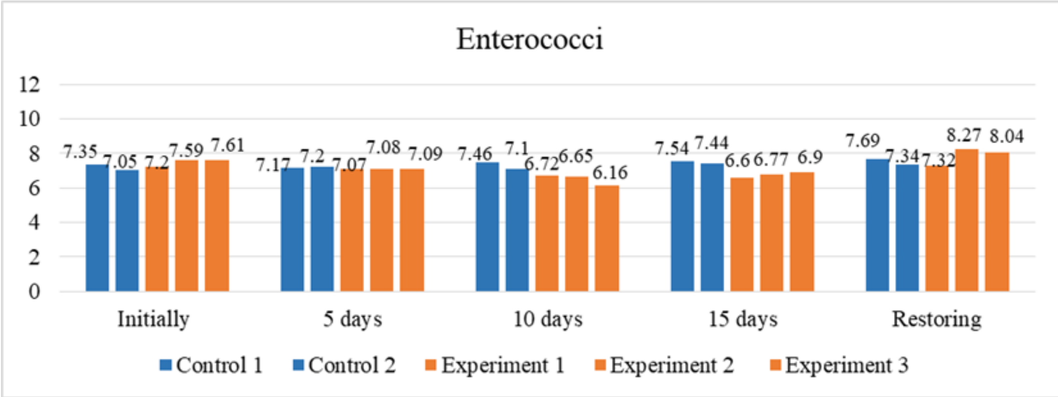
Thus, a protein-rich diet during the first 5 days of administration induces changes in the numerical values of the investigated bacteria by reducing the content of enterococci by 3.7% and 2.2%, respectively. This correlates with changes in bifidobacteria and lactobacteria and an increase in the level of *E. coli* (by approximately 1.2 times). With the longer administration of a protein-excess diet (10 and 15 days), a significant increase in the population of enterococci (by approximately 29.4% and 28.8% compared to initial data) is observed, along with that of both beneficial and pathogenic bacteria. After 7 days of re-establishment of normal diet, the numerical values of the investigated bacteria return to the initial data levels or to values identical to the control group (Figure 1).

An excess of fats in the diet (lard) induces a reduction in the content of enterococci in the intestinal content by approximately 1.8%-19.1% along with bifidobacteria (up to 11%) and lactobacilli (up to 20.6%) and an increase in the content of *E. coli* (up to 22-23%), indicating an imbalance in the intestinal microbiocenosis. It is important to note that the „negative effect” of a fat-rich diet persists even after stopping the diet administration, as evidenced by the reduced level of beneficial bacteria – bifidobacteria and lactobacilli – after 7 days of normal diet installed after 15 days of a lipid-excess diet (Figure 2).

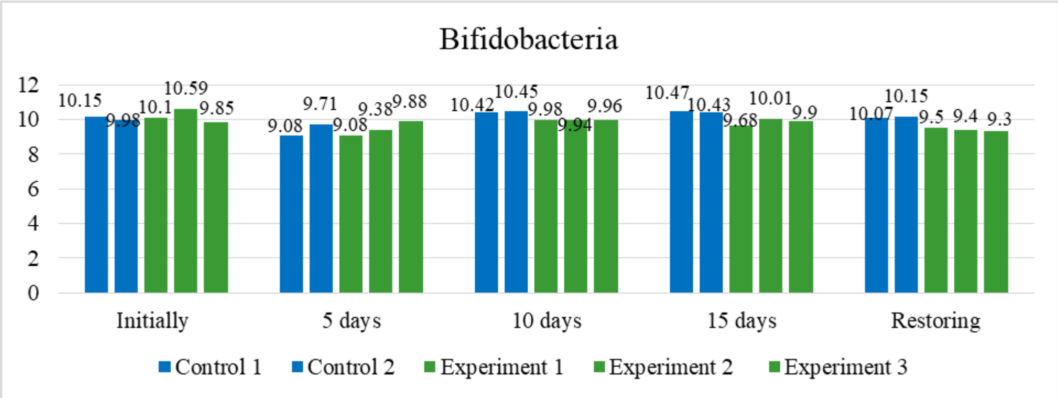
The action of a carbohydrate-rich diet had the greatest effect (compared to other diets) in inhibiting the enterococci population. After 5 days of administering the carbohydrate-excess diet, a reduction in the enterococci level by 14.6%-16.2% was observed. Maintaining the carbohydrate-rich diet (after 10 days) resulted in the most significant decrease in the numerical value of enterococci by 19.7%-21.0% compared to the initial data recorded before the experimental diet was administered. After 15 days of feeding laboratory animals with excessive carbohydrates, a smaller decrease in the enterococci level was observed, by 11.1%-14.9% compared to the earlier monitoring stages (Figure 3).

The bifidobacteria and lactobacilli were also sensitive to the introduction of excess carbohydrates in the animal feed: the content of these bacteria decreased by 2.6%-22.2% and 7.9%-18.0%, respectively. *E. coli* responded with a more pronounced increase in population in the early stages (days) of carbohydrate-rich diet administration (but not to the extent that the beneficial bacteria reacted), showing an increase of 10.6%-10.9% after 5 days, 1.9%-3.9% after 10 days, and 5.5-6.7% after 15 days of carbohydrate diet.

Returning to the normal diet (after 7 days) determined the restoration of the enterococci, bifidobacteria, and lactobacilli levels, and the reducing of *E. coli* one, in the intestinal content of experimental animals to the levels before the tested diet administration. Thus, the excess carbohydrates in the diet, specifically processed carbohydrates (bread), led to a decrease in enterococci, bifidobacteria, and lactobacilli, and an increase in *E. coli* counts, indicating disruption of the beneficial balance of intestinal microflora.



A



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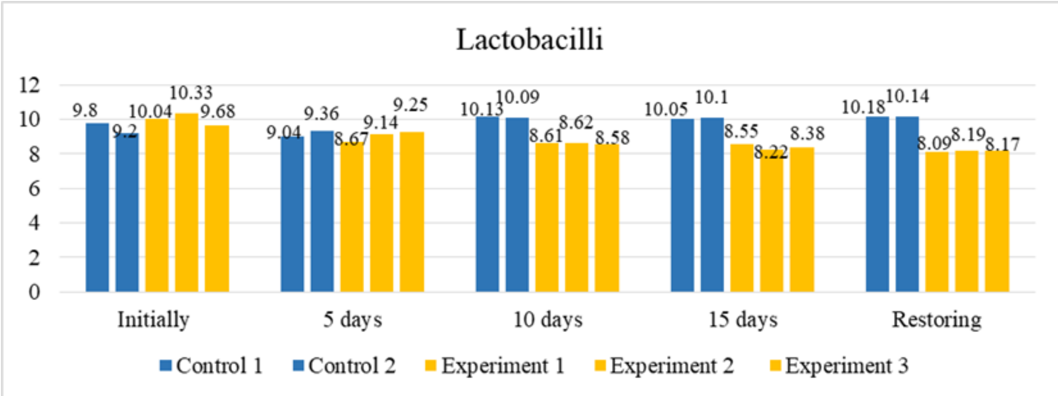

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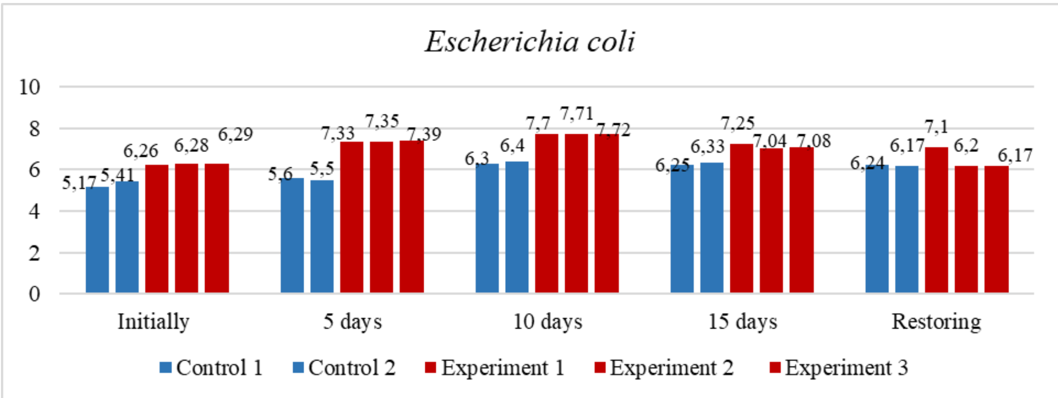
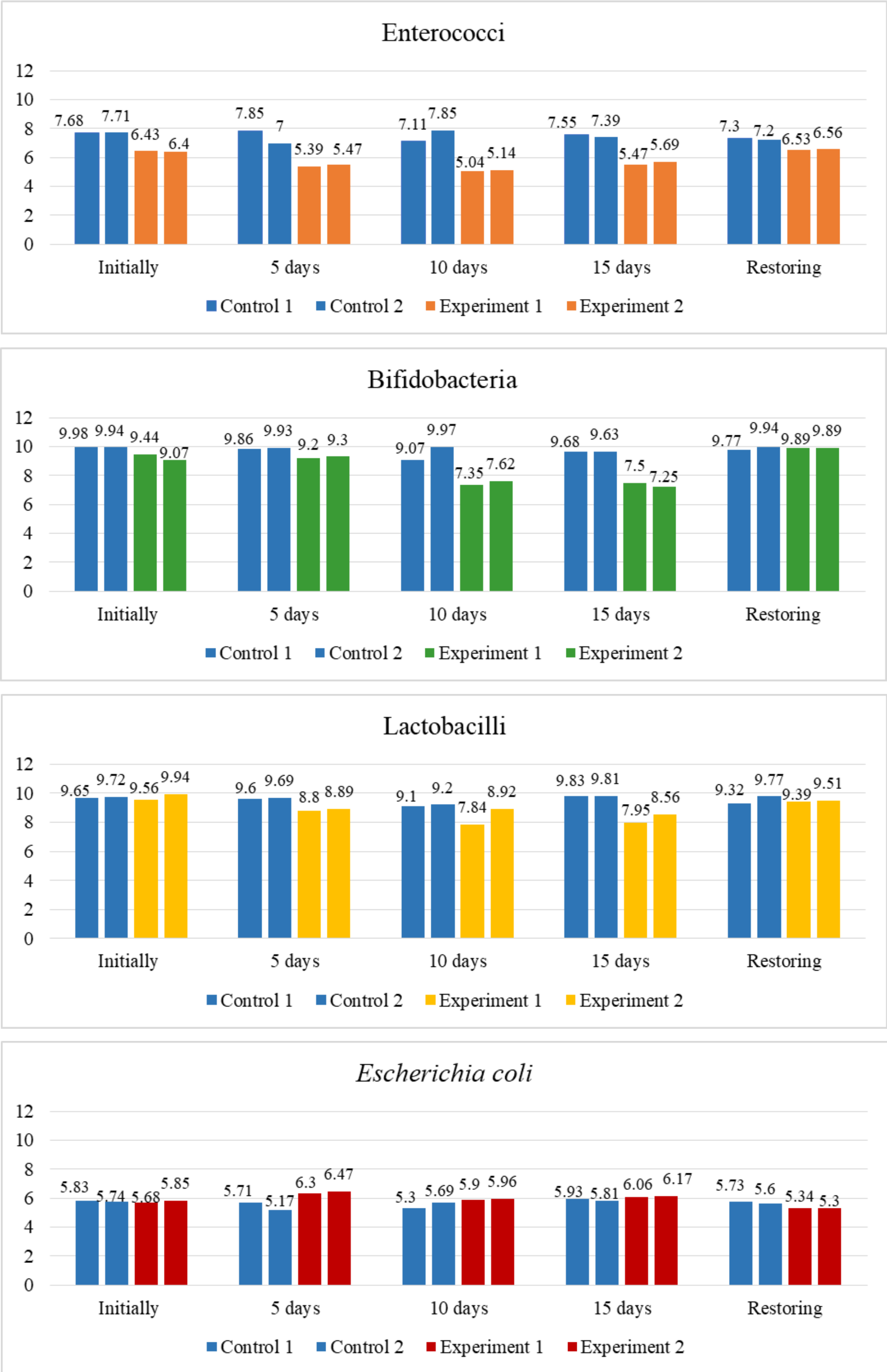


D

Figure 2. Content of bacterial of enterococci (A), bifidobacteria (B), lactobacteria (C), and *E. coli* (D) cells (lg per 1 g of intestinal content) under the dynamic action of a diet with excess of lipids

Legend: Control (1, 2) – laboratory white rats fed with a standard diet; Experiment (1, 2, 3) – laboratory white rats fed with a high-fat diet.



A

B

C

D

Figure 3. **The content of enterococci (A), bifidobacteria (B), *lactobacilli* (C), and *E. coli* (D) cells (lg per 1 g of intestinal content) under the dynamic action of a diet with excess carbohydrate**

Legend: Control (1, 2) - white laboratory rats fed a standard diet; Experiment (1, 2) - white laboratory rats fed a carbohydrate-rich diet.

The obtained and analyzed results definitely indicate that the consumption of rations in which a certain macronutrient predominates differentially influences the number of enterococci and, respectively, the intestinal microbiocenosis, and the excess of some of them has negative effects on the intestinal microbial representatives that can also affect the health of the host's body. Quantitative changes of enterococci in the intestinal content against the background of the administration of different nutritional value rations reveal their commensal character, as their numerical values correlate positively with those of the beneficial intestinal bacteria and negatively with pathogenic microbes.

In **two series of experiments**, the action of diets with different caloric structures was investigated. Initially, six diets (developed at the Institute of Physiology and Sanocreatology) with different caloric structures were tested *in vitro*, and as a result, four diets were selected that had varying effects on the numerical indices of enterococci: diet 1 (containing 8% protein, 35% fat, and 57% carbohydrates), which had the highest numerical inhibitory effect on the bacteria; diets 4 (containing 11% protein, 29% fat, and 60% carbohydrates) and 6 (containing 14% protein, 25% fat, and 61% carbohydrates), which contributed to a lesser extent to the numerical decrease of these microorganisms. Diet 5 (containing 12% protein, 27% fat, and 61% carbohydrates) acted as a stimulant, contributing to the increase in the number of these bacteria.

To confirm the results obtained *in vitro*, *in vivo* experiments were conducted on Wistar white laboratory rats, grouped into four experimental Groups: the first Group received diet 1; Group II – diet 4; Group III – diet 5; and Group IV – diet 6. Based on the data obtained, it is observed that under *in vivo* conditions, the nature of the changes in the studied bacteria differs from that established *in vitro* (Figure 4).

Thus, the final numerical indices of microorganisms from the *Enterococcus* genus increased by 69.27% in Group I when ration no. 1 was administered. In animals from experimental Groups II, III, and IV during the administration of the tested rations, a minor increase in the quantity of microbial cells was observed, specifically by 19.36%, 16.39%, and 17.97% respectively. The differences in data obtained under *in vivo* and *in vitro* conditions indicate that in the intestines of animals, bacteria of various obligatory or facultative microorganism genera do not act separately but in association. This is evident when comparing the numerical indices of enterococci with those of bacteria from the genera *Bifidobacterium*, *Lactobacillus*, and *E. coli* (Figure 4). Prior to feeding with rations (initially), a certain ratio is observed between the numerical indices of enterococci and those of other bacterial groups, which is nearly identical in all experimental Groups. After feeding with rations (final), correlations between the investigated bacterial groups change, namely: a substantial increase (by 51.7% and 69.3%) of enterococci and *E. coli* compared to bifidobacteria and lactobacilli in Group I; a greater increase (on average by 13-14%) in the numerical indices of bifidobacteria and lactobacilli and approximately 17% increase of enterococci levels and a decrease of *E. coli* content in Groups II, III, and IV. It was observed that the feed rations with a higher percentage of protein (rations nos. 4, 5 and 6) had the most beneficial influence on the numerical indices of enterococci and bacteria studied in comparison (this was also established when testing rations of different nutritive value).

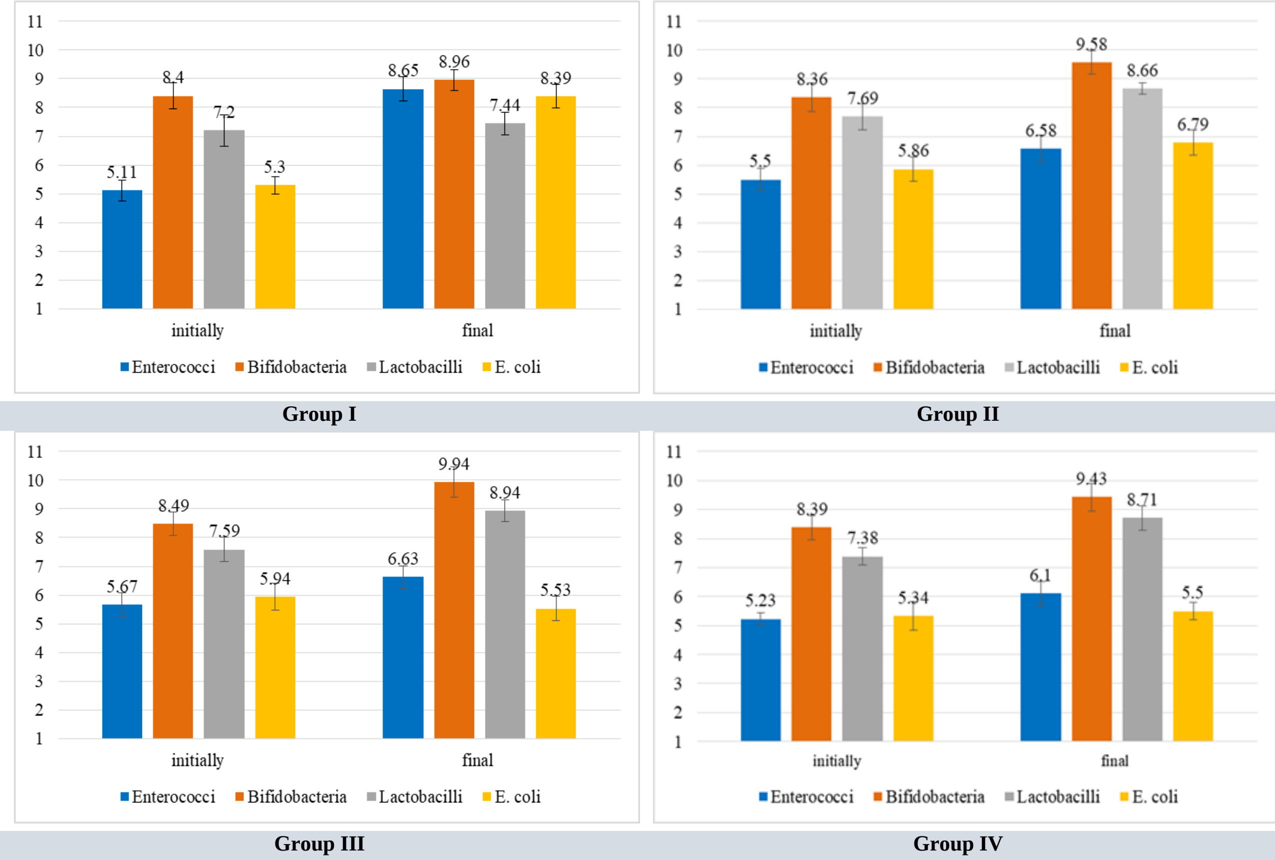


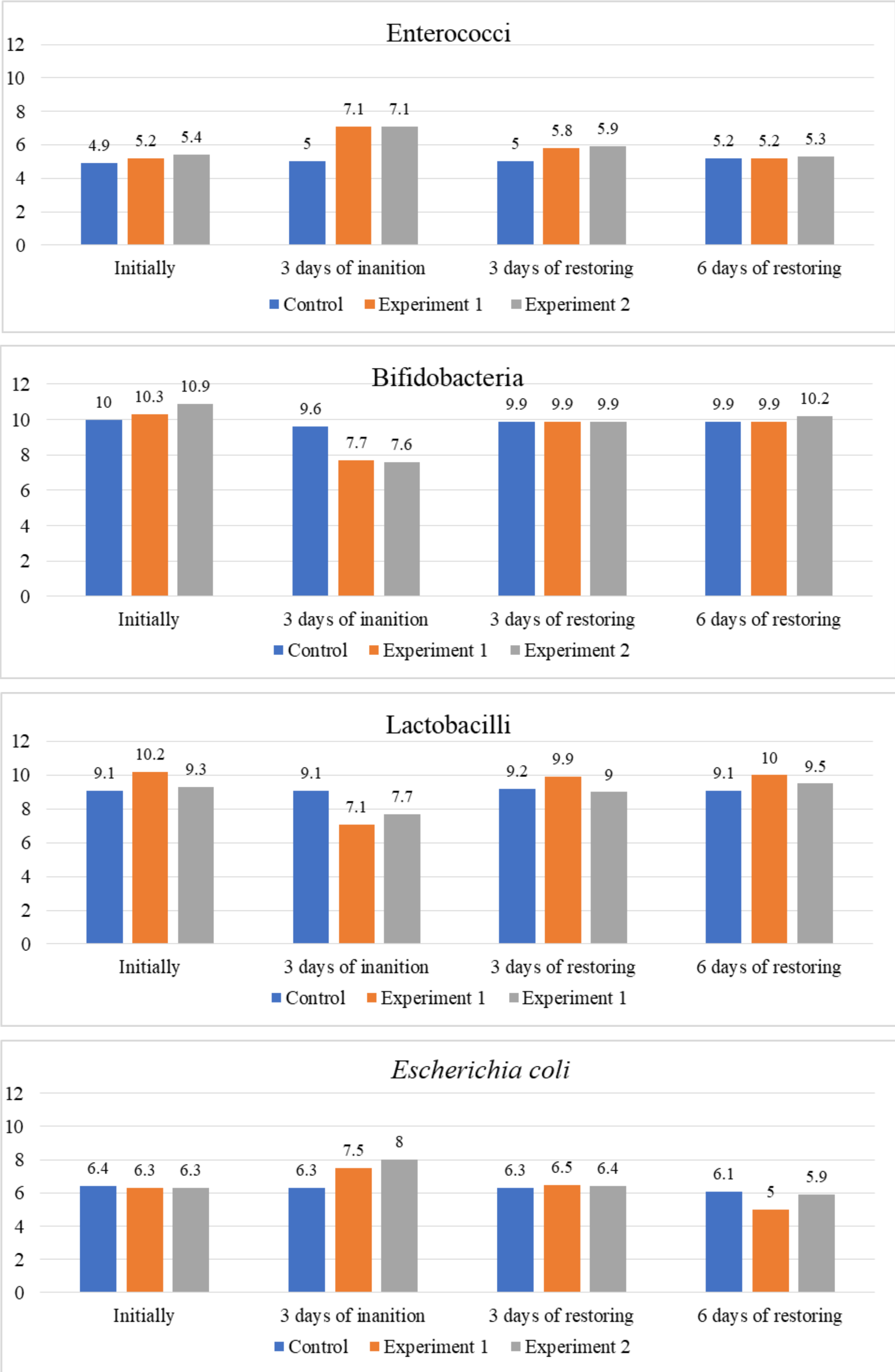
Figure 4. Changes in numerical indices of enterococci compared to other intestinal bacteria in rats depending on the administration of diets with varying caloric structure (lg per 1 g of intestinal content)

Identical results were obtained in experiments on guinea pigs, which reported the same correlation between the numerical value of enterococci and that of bifidobacteria, lactobacilli, and *E. coli* when administered rations with different caloric structures, indicating a tendency for cooperation between enterococci and bifidobacteria/lactobacilli in inhibiting the multiplication of *E. coli*, especially in animals fed a ration with moderate levels of protein, lipids, and carbohydrates.

Therefore, it was observed that rations with different caloric structures exhibit different effects on the multiplication and development processes of intestinal enterococci, suggesting that the numerical level of enterococci and other intestinal bacteria can be regulated and maintained not only through microbial preparations with probiotic action, but also through the use of dietary rations that exhibit prebiotic action.

The numerical value of enterococci in correlation with the physiological status of the host organism was studied under starvation conditions, correlating with the physical status (body weight) of the model animals.

The change in intestinal enterococci count under starvation conditions was tested on laboratory animals – Wistar white rats, each housed individually in separate cages and deprived of food for 72 hours. Data monitoring was conducted individually for each experimental animal. Animals fed with the approved standard diet served as controls (Figure 5).



A

B

C

D

Figure 5. The change in the quantity of bacterial cells of enterococci (A), bifidobacteria (B), lactobacilli (C), and *E. coli* (D) (log per 1 gram of intestinal content) in rats subjected to starvation (inanition)

Legend: Control (- white laboratory rats fed a standard diet; Experiment (1, 2) - white laboratory rats subjected to starvation during 72 h.

It was established that total food deprivation for 72 hours led, on one hand, to an increase of 1.3-1.4 times in enterococci content and 1.2-1.3 times in *E. coli* levels, and on the other hand, to a reduction of 1.34-1.44 times in bifidobacteria count and 1.2-1.5 times in lactobacilli count. The described results, namely the increase in enterococci and *E. coli* and the reduction in bifidobacteria and lactobacilli (lactobacteria) levels, indicate that starvation creates, at the intestinal microbiota level, conditions that promote dysbiosis development in a relatively short time. Return to a normal diet induces changes again in the intestinal microbiota, suggesting restoration of facultative and obligate bacterial counts to a level that does not pose a health risk to the digestive tract (Figure 5).

The elucidation of the correlation between the numeric value of enterococci, body mass, and diet presented interest. Animals with lower body weight (average of 193.8 g) showed a higher value of enterococci (8.45 microbial cells (lg) per 1g of intestinal content) compared to animals with higher body mass (average of 297.2 g), where the enterococci value was lower (5.75 microbial cells (lg) per 1g of intestinal content). Regarding the significance of enterococci values in animals with lower and higher body mass, it can be inferred by comparing the numeric indices of enterococci with those of other groups of intestinal bacteria - bifidobacteria, lactobacilli, and *E. coli*. The data indicate a correlation between enterococci with bifidobacteria and lactobacilli in the group of animals with lower body weight. In these animals, the numeric value of enterococci is approximately at the same level as bifidobacteria (8.45 vs 8.3) and higher compared to lactobacilli (8,45 *vs* 8,3) and *E. coli* (8,45 *vs* 7,3). Although the level of enterococci is higher, this does not necessarily indicate a pathogenic state, as the content of *E. coli* is lower compared to that of beneficial bacteria. In the case of animals with higher body mass, no correlation was observed between enterococci with bifidobacteria and lactobacilli, especially in maintaining a lower level of *E. coli*, with the numeric indices of enterococci being approximately 1.6 times lower compared to bifidobacteria (5,75 *vs* 9,45), 1.4 times lower compared to lactobacilli (5,75 *vs* 8,3), and 1.2 times lower compared to *E. coli* (5,75 *vs* 6,68).

To highlight the correlation between the number of enterococcus cells with body mass and diet, white Wistar rats were fed specially formulated diets designed to correct body weight. For animals with lower body weight (group I), diets were developed with the protein increased content from 14% to 20%, and lipid decreased content from 28% to 23%. For animals with higher body weight (group II), the diets similarly increased the percentage ratio of proteins from 20% to 30% and decreased the percentage ratio of lipids from 25% to 21%. The carbohydrate content did not change significantly. The control group animals received standard diets designed for laboratory animal (Table 4).

Table 4. Quantitative characteristics of the basic components of food rations fed to rats of different body mass

Nutrient components in food rations, %	Experimental groups/subgroups			
	Group I (low body mass)			
	Subgroup 1	**Subgroup 2**	**Subgroup 3**	**Subgroup 4**
Proteins	14	16	18	20
Lipids	28	26	25	23
Carbohydrates	58	58	57	57
	Group II (high body mass)			
Proteins	20	22	25	30
Lipids	25	23	22	21
Carbohydrates	55	55	53	49

It has been found that laboratory animals with different body weights at the intestinal microflora level have different sensitivities to the caloric structure of nutrients in their diets. Specially designed diets for correcting body weight in animals with low weight caused fluctuations of the enterococci content, indicating the influence of other factors on the intestinal microbiocenosis state. In animals with higher weight, manipulating the nutrient structure of the diet – specifically, increasing the percentage of proteins and decreasing that of lipids and carbohydrates (subgroups 3 and 4 from Group II) – contributed to the increase of the number enterococci cells in their intestinal content (Figure 6).

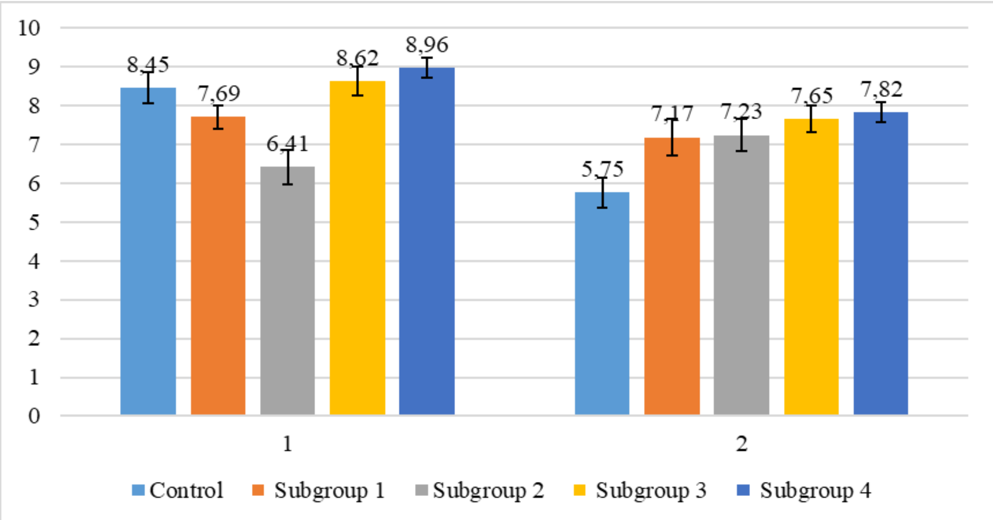


Figure 6. The amount of microbial cells of enterococci (lg per 1 g of intestinal content) in rats with lower (1) and higher (2) body mass when receiving rations with different ratio of nutritional components (subgroups 1-4)

The obtained data attest that nutrition, through various diets, food groups, and macronutrients is the main factor in modifying and remodeling the intestinal microbiota, both in terms of health and disease. The composition of the microbiota, including the level of intestinal enterococci, undergoes changes depending on the physiological status of the host organism, determined either by malnutrition or body weight.

In Chapter 4, „**Prospecting New Strains of Enterococci as Probiotics**" the data regarding the isolation of enterococci strains and the study of their adhesion capacity and antagonistic activity against pathogens for their use as probiotics are described. This includes the compatibility of enterococci with other bacteria specific to the digestive tract microbiocenosis and the testing of the efficacy of new microbial compositions based on enterococci on the state of intestinal microbiota.

To identify beneficial strains of enterococci and characterize the properties underlying their use as probiotics, strains of enterococci and other coliform bacteria (streptococci and lactococci) were isolated from healthy animals and human subjects (children). From healthy human subjects – children aged 1-16 years – 834 strains of coliform bacteria were isolated, of which 301 were enterococci. From healthy animals (piglets and calves), 407 and 120 strains of coliform bacteria were isolated, respectively, of which 209 and 54 strains were enterococci. The isolated strains of *Enterococcus*, *Lactococcus*, and *Streptococcus* were tested for their adhesion properties and antagonistic activity against pathogenic bacteria *in vitro*, which are crucial properties for colonizing the digestive tract, interacting with the host organism's intestinal epithelium, and influencing its immune system, and which are important in defining probiotic preparations.

It was noted that the adhesive properties and the antagonistic activity against pathogens of enterococci strains were more evident compared to the indices obtained for streptococci and lactococci. These properties are more evident in enterococci strains specific to the human digestive tract compared to those specific to the animal digestive tract (Table 5).

Table 5. The adhesive capacity and antagonistic activity against pathogenic bacteria of some strains of enterococci, streptococci, and lactococci, isolated from the gastrointestinal tract of human subjects (children), calves, and piglets with a healthy digestive tract status

The number of tested monocultures		**The genus of bacteria**	**Adhesion index**	**Antagonistic activity (%) against pathogens:**		
				E. coli	***Proteus vulgaris***	***Staphylococcus aureus***
Human subjects (children)						
834	301	*Enterococcus*	4.58-4.77	85.45-89.35	71.25-77.57	78.40-85.70
	297	*Streptococcus*	3.66-4.37	72.35-79.69	69.64-75.40	74.55-82.60
	236	*Lactococcus*	3.16-3.70	68.75-70.37	59.45-65.60	71.25-76.75
Calves						
120	54	*Enterococcus*	4.28-4.66	81.39-85.62	64.40-68.59	69.27-75.37
	37	*Streptococcus*	3.13-3.46	65.71-68.44	57.32-61.18	64.65-70.20
	29	*Lactococcus*	2.62-3.04	58.39-63.25	63.46-66.31	56.78-61.58
Piglets						
407	209	*Enterococcus*	4.38-4.50	82.35-86.48	66.75-70.20	70.60-77.45
	127	*Streptococcus*	3.25-3.62	67.46-69.37	62.57-68.32	66.51-71.25
	71	*Lactococcus*	2.90-3.30	62.50-66.35	58.64-63.47	60.72-65.54

Each strain of microorganisms can have distinct properties, and bacterial-origin remedies, including probiotics, contain certain strains of microorganisms that meet advanced criteria regarding these preparations/remedies, and, as well as safety requirements (being non-pathogenic). From the lactic acid bacteria isolated strains, certain ones have been selected and highlighted, which were subsequently re-tested for their adhesive capacity and antagonistic activity. The principle of strain selection was the certainty that the strain belonged to a particular group of acid-lactic bacteria. It should be noted that among the total selected strains, single strains of enterococci and lactococci were included, for the purpose of comparative testing of these two groups of bacteria (as lactococci have proven health-promoting potential and are successfully included in probiotic preparations), regarding their adhesive capacity and antagonistic/antibacterial activity against individual pathogens (Table 6).

All isolated strains of enterococci showed a certain characteristics that defined probiotics, expressed by increased adhesion capacity and high antimicrobial property (antagonistic activity). Based on the analysis of data regarding adhesion capacity and antagonistic (antibacterial) activity, it was found that the highest indices were shown by the enterococcus strain no. 116 from human intestinal content and the enterococcus strain no. 77 from animal intestinal content, which can be recommended for further application in the composition of probiotic effect formulations.

Table 6. The adhesion capacity of lactic acid bacteria strains (enterococci and lactococci) specific to the human and animal digestive tract

Isolate strian number	Adhesion index	Antagonistic activity (%)against pathogens:	
		Escherichia	*Salmonella*
From the intestinal content of human subjects			
Monocultures of enterococci			
18	3,18	29,74	19,94
49	4,14	23,24	19,14
58	4,12	22,27	19,45
70	4,16	26,28	25,42
89	3,98	20,95	25,85
108	4,10	22,85	19,78
112	4,16	24,34	23,07
116	**4,67**	**32,16**	**25,53**
Monocultures of lactococci			
25	**4,27**	**24,60**	**23,09**
32	4,15	24,47	19,36
46	4,10	21,96	21,45
67	4,08	21,41	20,44
82	4,11	23,87	19,76
85	4,14	25,30	23,43
93	4,12	24,01	22,37
From the intestinal contents of animals			
Monocultures of enterococci			
13	3,18	24,60	23,09
21	3,59	24,47	19,36
43	3,24	21,96	21,45
55	2,81	21,41	20,44
74	3,72	23,87	19,76
77	**3,85**	**25,30**	**23,43**
97	3,57	24,01	22,37
129	3,65	24,60	23,09
Monocultures of lactococci			
37	**3,45**	**18,13**	**17,02**
64	3,44	17,72	15,91
101	3,12	14,35	13,56

On the basis of the described properties, bacterial compositions containing strains of enterococci and bacteria of the genera Bifidobacterium and Lactobacillus were developed and tested in comparison with other commercially available microbial preparations. The testing was conducted based on the modification of quantitative indices of some intestinal microbial representatives with sanogenic and pathogenic action (*Bifidobacterium*, *Lactobacillus*, *Enterococcus*, *Escherichia*, *Proteus*) in the intestinal content of laboratory white mice (Table 7).

The association developed for the first time based on strains of microorganisms from the *Enterococcus* genus (which exhibited the best adhesion capacity and antagonistic activity) and those from the *Bifidobacterium* and *Lactobacillus* genera had the most beneficial effect on the intestinal microbiota, proven by increasing the numerical indices of obligate bacteria. The new microbial composition proved to have a greater inhibitory action against pathogenic bacteria (*Escherichia* and *Proteus*). The increase in enterococci effectiveness in this case cannot be associated with certain intestinal dysfunctions, as it correlates with high values of bifidobacteria and lactobacilli and decreased levels of *Escherichia* and *Proteus* (Table 7).

Table 7. Quantitative indices of certain intestinal bacteria (in white mice) upon administration of various microbial preparations

Experimental groups, administered preparation	The evaluated groups of microorganisms	Number of microbial cells per 1 g of intestinal content, decimal logarithm (lg)		Difference (%) compared to:	
		initially	*at the end*	initial data	control
I Control	*Bifidobacterium*	7.20±0.12	7.82±0.10*	+8.61	
	Lactobacillus	6.11±0.11	6.77±0.11**	+10.80	
	Escherichia	8.53±0.11	9.49±0.12***	+11.25	
	Proteus	3.04±0.10	4.14±0.08***	+26.18	
	Enterococcus	**6.49±0.12**	**7.23±0.13****	**+11.40**	
II Bifidobacterin	*Bifidobacterium*	7.17±0.08	9.41±0.07***	+31.24	+20.64
	Lactobacillus	6.88±0.12	7.72+0.09***	+12.20	+14.30
	Escherichia	8.84±0.13	7.46±0.12***	-15.61	-21.39
	Proteus	3.11±0.09	2.07±0.10***	-33.44	-50.00
	Enterococcus	**6.77±0.11**	**7.90±0.14*****	**+19.99**	**+9.26**
III Lacidofil	*Bifidobacterium*	7.53±0.11	8.04±0.13*	+8.05	+2.81
	Lactobacillus	6.32±0.12	8.32±0.10***	+31.64	+22.89
	Escherichia	8.96±0.10	7.54±0.14***	-15.84	-20.54
	Proteus	3.00±0.13	1.23±0.12***	-59.00	-70.28
	Enterococcus	**6.84±0.15**	**7.04±0.13**	**+2.92**	**+2.62**
IV Bifiform	*Bifidobacterium*	7.23±0.13	9.20±0.12***	+18.03	+17.64
	Lactobacillus	6.63±0.11	7.70±0.13***	+16.13	+13.73
	Escherichia	8.68±0.12	6.82±0.10***	-21.42	-28.13
	Proteus	3.07±0.11	1.17±0.10***	-61.88	-71.73
	Enterococcus	**6.54±0.14**	**8.62±0.10*****	**+31.80**	**+19.22**
V New experimental composition containing E. faecium	*Bifidobacterium*	7.60±0.10	9.47±0.13***	+24.60	+21.09
	Lactobacillus	6.50±0.11	8.65±0.09***	+33.07	+27.76
	Escherichia	8,46±0,12	5.79±0.11***	-28.25	-38.98
	Proteus	3.17±0.08	0***	-100.0	-100.0
	Enterococcus	**6.32±0.13**	**8.88±0.12*****	**+40.50**	**+22.82**

Note: * - p≤ 0.05 (statistically significant); ** - p≤ 0.01 (statistically highly significant); *** - p≤ 0.001 (statistically very highly significant).

Another important aspect reflecting the properties of bacterial species and strains that can be used as probiotics is the degree of compatibility between intestinal bacteria. Thus, an objective of research aimed at determining and identifying strains of enterococci with probiotic properties focused on studying the compatibility of intestinal enterococci with other representatives of the digestive tract microbiota, especially those from genera considered as primary and obligatory for this cavity of the macroorganism (*Bifidobacterium* and *Lactobacillus*), compared to coliform bacteria belonging to the *Lactococcus* and *Streptococcus* genera as discussed earlier. It was established that enterococci (*E. faecium*) have a high level of compatibility with representatives of obligatory genera (*Bifidobacterium* and *Lactobacillus*), comparable to lactococci (*L. lactis*) and greater than of streptococci (*S. thermophilus*), and in some cases, even a stimulatory action on this type of bacteria was observed. The compatibility or interaction of enterococci with other representatives of intestinal microflora is of interest due to the dual significance of these bacteria – as commensals and nosocomial pathogenic agents.

In experiments on guinea pigs, newly developed microbial associations with probiotic action were tested. These associations contained strains of enterococci and lactococci, as these groups of bacteria demonstrated increased compatibility with bifidobacteria and lactobacilli. For this purpose, the animals were divided as follows: in group I (control), the animals did not receive any bacterial remedy; in group II, the animals received a microbial composition consisting of one strain of lactococci, one of lactobacilli, and two of bifidobacteria, while those in group III received a microbial composition consisting of one strain of enterococci, one of lactobacilli, and one of bifidobacteria. All preparations were administered orally in the context of intestinal dysbiosis (characterized by lower levels of bifidobacteria and lactobacilli and higher levels of *Escherichia* and *Proteus*) (Table 8).

Table 8. Quantitative indices of intestinal microorganisms in guinea pigs upon administration of newly developed microbial associations

Animal groups	The genera of bacteria	Number of microbial cells per 1g of intestinal content, decimal logarithms (lg)		Difference, compared to: %	
		initially	***at the end***	**initial data**	**control**
I	*Bifidobacterium*	4.34±0.14	4.50±0.13	+3.68	
	Lactobacillus	4.17±0.15	4.59±0.14	+10.07	
	Escherichia	8.83±0.16	8.88±0.15	+0.56	
	Proteus	5.11±0.15	5.23±0.14	+2.34	
	Enterococcus	8.74±0.14	8.92±0.13	+2.05	
II	*Bifidobacterium*	4.47±0.12	8.36±0.11***	+87.02	+85.77
	Lactobacillus	4.32±0.15	8.64±0.08***	+100.00	+88.23
	Escherichia	8.81±0.13	5.73±0.15***	34.96	35.47
	Proteus	5.04±0.11	1.17±0.07***	76.78	77.62
	Enterococcus	8.80±0.16	6.49±0.13***	26.25	27.24
III	*Bifidobacterium*	4.53±0.15	9.04±0.09***	+99.55	+100.88
	Lactobacillus	4.43±0.13	9.17±0.07***	+100.00	+99.78
	Escherichia	8.90±0.16	5.32±0.14***	40.22	40.09
	Proteus	5.20±0.12	1.07±0.08***	79.42	98.08
	Enterococcus	8.77±0.14	6.41±0.11***	26.90	28.13

Note: * - p≤0.05 (statistically significant); ** - p≤0.01 (statistically highly significant); *** - p≤0.001 (statistically very highly significant).

It was established that the tested microbial association containing enterococci (lot III) increased the quantitative levels of bifidobacteria and lactobacilli by approximately 100%, both compared to the initial values (at the beginning of the experiment) and compared to the values recorded in the control group of animals. It also exhibited the highest inhibition action against *Escherichia* (by about 40%) and *Proteus* (by about 79% compared to initial data and 98% compared to the control). The level of enterococci decreased in animals from lot III, showing a similar inhibition rate as in lot II (approximately 26-28%) (Table 8).

These changes in the count of obligate and facultative bacteria denote the beneficial action of the tested microbial compositions and demonstrate the opportunity to include not only obligate bacteria (*Bifidobacterium* and *Lactobacillus*) but also of commensals such as enterococci and lactococci in probiotic (sanogenic) remedies. These commensals, being part of the tested microbial associations, have contributed to the elimination of dysmicrobism and the establishment of intestinal eubiosis.

The effectiveness of microbial compositions based on monocultures and microorganism associations containing strains of enterococci has been demonstrated in young farm animals (calves and piglets) under industrial farm conditions. Their beneficial effect has been shown in preventing bacterial gastroenteritis, thereby enhancing the natural resistance and productivity of the animals.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

The scientific results obtained within the doctoral thesis **„The physiological role of intestinal enterococci in maintaining the health of the digestive tract",** in accordance with the proposed research aim and objectives, allowed for the formulation of the following general conclusions:

1.Quantitative changes in enterococci in the intestinal content of human and animal subjects, under different conditions and depending on the physiological status of the digestive tract and of the body, show a correlation with the values of bifidobacteria and lactobacilli, indicating a possible cooperation of these bacteria in maintaining *E. coli* bacteria count at a level that does not allow the development of intestinal pathogens [chap. 3].

2.The incidence of enterococci in different animal species depends on their living environment, being higher in pets and rural birds compared to urban ones. The low correlation between enterococci levels and beneficial bacteria (bifidobacteria and lactobacilli) and high correlation with pathogens (*E. coli*) in pets, some farm animals, and rural birds reveals that close contact with humans and shared environments modify the intestinal microflora with a predominant dominance of pathogenic bacteria [chap. 3, sub. 3.2.1.].

3.The content of enterococci is significantly influenced by the health status of the digestive tract, being documented the increase of amounts of these bacteria in human subjects and agricultural animals with intestinal dysmicrobism and diarrheic bowel dysfunction. The greatest differences between healthy human subjects and those with intestinal homeostasis disorders were observed in the age group of 31-60 days and 1-4 years, and in farm animals during the first 30 days of life, which allows to conclude that in the early days, months, and years of life, commensal forms or strains of enterococci can acquire pathogenic/virulent characteristics if appropriate infection conditions (intestinal dysbiosis and diarrheal dysfunctions) are created [chap. 3, sub. 3.2.2.].

4.Quantitative changes in enterococci in the intestinal content due to the administration of diets with varying nutritional values (excess of proteins, lipids, or carbohydrates) reveal their commensal nature, as the numerical values of them correlate positively with those of beneficial intestinal bacteria and negatively with pathogenic microbes. The differentiated effect of tested diets with varying caloric structures on the multiplication and development of intestinal enterococci indicates that their content can be regulated and maintained not only through microbial preparations with probiotic action but also through food rations that exhibit prebiotic action [chap. 3, sub. 3.3.].

5.The different susceptibility of enterococci to changes in the physiological status of the organism has been highlighted and the possibility of influencing their effectiveness by varying the caloric value of nutrients with repercussions on the health of the digestive tract and the organism has been established [chap. 3, sub. 3.4.2.].

6.The increase in enterococci population under starvation conditions along with *E. coli* bacteria and the reduction in bifidobacteria and lactobacilli levels demonstrate that the lack of food quickly creates conditions for the development of dysbiosis, suggesting that pathogenic bacteria can form "coalitions" with enterococci, which under normal conditions do not exhibit pathogenicity [chap. 3, sub. 3.4.1.].

7.Enterococci strains isolated for the first time from the intestinal content of human subjects (children) and animals demonstrated probiotic properties, confirmed by high values of antagonistic activity and adhesive capacity, being more pronounced compared to lactococci strains and more marked in human-origin strains than animal-origin ones [chap. 4, sub. 4.1.].

8.It was established that enterococci (*E. faecium*) have a high level of compatibility with obligatory microbial representatives (*Bifidobacterium* and *Lactobacillus*) comparable to lactococci (*L. lactis*) and higher than streptococci (*S. thermophilus*). The compatibility or interaction of enterococci with other representatives of the intestinal microflora is significant in manifesting their commensal or nosocomial pathogen aspect and in justifying the association of enterococci with other bacteria in microbial compositions with probiotic action [chap. 4, sub. 4.2.].

9.Experimentally, the probiotic (health-promoting) action of microbial compositions, developed based on associations of obligatory bacteria (Bifidobacterium and Lactobacillus) and tested enterococci strains, was established, which have led to the maintenance of a healthy intestinal balance, conditioned by a certain correlation of enterococci with representatives of both obligate beneficial and pathogenic bacteria, thus contributing to the elimination of dysmicrobism and the establishment of intestinal eubiosis [chap. 4, sub. 4.1. and 4.2.].

10. Compositions based on monocultures and associations of microorganisms containing tested enterococci strains were developed and tested on young farm animals (calves and piglets), which have shown a beneficial effect on animal growth and development under industrial farm conditions, a direct relationship being established between the content of enterococci in the digestive tract of calves and piglets, natural resistance indicators of farm animals in experimental groups, and their productivity, pointing the opportunity to use enterococci, bifidobacteria, and other lactic acid bacteria in the growth and nutrition process of young piglets and calves in industrial farms to increase their natural resistance and productivity [chap. 4, sub. 4.3.].

Practical Recommendations

The applicative aspect of the research and the obtained results target the testing of safe enterococci strains with demonstrated antibacterial properties and high adhesive indices and their potential use in developing mixed microbial compositions with probiotic action, leading to the following recommendations:

- Inclusion in probiotic microbial compositions of enterococci strains with enhanced antagonistic activity against pathogenic bacteria and high adhesive capacity, ensuring safety for digestive tract health;
- New microbial compositions based on enterococci strains can be used, after testing their beneficial effects on the intestinal microflora and physiological and physical parameters of the organism and demonstrating safety for health;
- The application of new microbial compositions must comply with normative requirements for enterococci strains recommended as probiotics.

Personal Contribution

The author conducted the experiments during the doctoral program, performed microbiological investigations, quantified, systematized, and analyzed collected data using appropriate research methods, and interpreted and generalized the data and conclusions according to the addressed topic and established goal.

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ADNOTARE

BOGDAN Victoria „Rolul fiziologic al enterococilor intestinal în menținerea sănătății tubului digestiv", teză de doctor în științe biologie, Chişinău, 2024.

Structura tezei: introducere, patru capitole, concluzii generale şi recomandări, bibliografie cu 360 de titluri, volumul tezei este de 250 de pagini, 172 de pagini de text de bază, 4 anexe, 19 de figuri și 36 de tabele. Rezultatele obţinute sunt publicate în 22 de lucrări științifice.

Cuvintele-cheie: enterococi, rol fiziologic, microbiotă intestinală, sănătate, factor alimentar, probiotic.

Scopul lucrării a constat în elucidarea unor aspecte fiziologice privind semnificația enterococilor pentru sănătatea tubului digestiv și evidențierea potențialului probiotic al unor tulpini de enterococi.

Obiectivele cercetării: investigarea incidenței și nivelului cantitativ al enterococilor intestinali în tubul digestiv uman și animal în funcție de statutul fiziologic al organismului; studierea acțiunii factorului alimentar asupra conținutului enterococilor intestinali și evidențierea rolului acestora în menținerea echilibrului microbian intestinal benefic; izolarea, identificarea și selectarea celor mai avantajoase tulpini de enterococi intestinali cu potențial sanogen și elucidarea proprietăților probiotice ale acestora; prospecțiunea și testarea noilor compoziții microbiene elaborate pe bază de enterococi cu semnificație în menținerea dirijată a sănătății tubului digestiv și a organismului.

Noutatea şi originalitatea ştiinţifică constă în evidențierea corelării nivelului cantitativ al enterococilor cu cel al bacteriilor benefice în condiții în care nu se atestă dezvoltarea disfuncțiilor intestinale, ceea ce denotă despre rolul enterococilor în menținerea echilibrului microbian intestinal sanogen, fapt demonstrat prin elucidarea modificărilor efectivului enterococilor din conținutul intestinal în funcție de statutul fiziologic al organismului și la acțiunea factorului alimentar. La inducerea drastică a proceselor patologice, enterococii își pierd acțiunea sanogenă și pot deveni agenți patogeni în coaliție cu alți patobionți. În baza corelării enterococilor cu bacteriile benefice microflorei intestinale se poate de dedus despre echilibrul microbian intestinal și starea sănătății tubului digestiv. A fost dovedit potențialul probiotic al unor tulpini de enterococi, care este identic sau mai avantajos în comparație cu potențialul probiotic recunoscut al unor bacterii obligative și testată eficacitatea compozițiilor noi microbiene enterococice în menținerea sănătății tubului digestiv și a organismului.

Rezultatele obținute care contribuie la soluționarea problemei științifice importante: A fost stabilită corelația benefică a enterococilor cu bacteriile obligative intestinale în menținerea unui mediu intestinal sanogen și reliefată posibilitatea modulării componenței microbiotei intestinale în aspect sanogen la administrarea rațiilor alimentare de diferită valoare nutritivă și structură calorică, ceea ce ar permite prin acțiune prebiotică a macronutrienților din rații de a fortifica capacitățile microflorei de a stopa dezvoltarea patogeniilor intestinale. A fost argumentată experimental posibilitatea includerii unor tulpini de enterococi în compoziții microbiene cu acțiune probiotică.

Semnificaţia teoretică constă în dezvoltarea cunoştinţelor referitor la rolul enterococilor intestinali în sănătatea tractului gastrointestinal în funcție de mediul de trai, statutul fiziologic al tubului digestiv și al organismului, de acțiunea factorului alimentar și de compatibilitatea lor cu alte bacterii comensale. A fost stabilit efectul sanogen al enterococilor, care se menține în cazurile, când efectivul numeric al acestora nu depășește cel al bacteriilor benefice și se formează o corelație cu aceste bacterii, ce controlează dezvoltarea bacteriilor patogene.

Valoarea aplicativă a lucrării: A fost demonstrat potențialul probiotic al unor tulpini izolate de enterococi (tulpini pure) ce se exprimă mai benefic în asociație cu bifidobacteriile și lactobacteriile, recomandată utilizarea lor în calitate de probiotice și elaborate compoziții microbiene ce conțin tulpini testate de enterococi cu acțiune sanogenă.

Implementarea rezultatelor științifice: Rezultatele cercetării au fost aplicate în procesul de cercetare în cadrul Institutului de Fiziologie și Sanocreatologie, Universitatea de Stat din Moldova.

ANNOTATION

BOGDAN Victoria „ The physiological role of intestinal enterococci in maintaining the health of the digestive tract", doctoral thesis in biological sciences, Chişinău, 2024.

Thesis structure: The thesis consists of an introduction, 4 chapters, conclusions and recommendations, a bibliographic list of 360 titles, 4 annexes, 250 total and 172 pages of the main text, 19 figures and 36 tables. The results are presented in 22 published scientific papers.

Key words: enterococci, physiological role, intestinal microbiota, health, food factor, probiotics.

The aim of the study consists in elucidating some physiological aspects regarding the significance of enterococci for the health of the digestive tract and highlighting the probiotic potential of some strains of enterococci.

Research objectives: investigation of the incidence and quantitative level of intestinal enterococci in the human and animal digestive tract depending on the physiological status of the organism; studying the action of the food factor on the content of intestinal enterococci and highlighting their role in maintaining the beneficial intestinal microbial balance; the isolation, identification and selection of the most advantageous intestinal enterococci strains with sanogenic potential and the elucidation of their probiotic properties; prospecting and testing of new elaborated microbial compositions based on enterococci strains with significance in maintaining the digestive tract and the body health.

The scientific novelty and originality consist of revealing of the enterococci quantitative level correlation with that of the beneficial bacteria in conditions where the development of intestinal dysfunctions is not attested, which denotes about of their role in maintaining the sanogenic intestinal microbial balance, demonstrated by the elucidation of the quantitative changes of enterococci in the intestinal contents depending on the physiological status of the body and the action of the food factor. Under conditions of severe pathological processes, enterococci may lose their health-promoting action and potentially become pathogens in association with other pathobionts. Based on the correlation of enterococci with the beneficial bacteria of intestinal microflora, it is possible to deduce about the intestinal microbial balance and the health state of digestive tract. The probiotic potential of certain strains of enterococci has been proven, which is identical or more advantageous compared to the recognized probiotic potential of some obligate bacteria, and the effectiveness of new enterococcal microbial compositions in maintaining the health of the digestive tract and the whole organism has been tested.

The obtained results that contribute to the solution of the important scientific problem: The beneficial correlation of enterococci with obligate intestinal bacteria in maintaining of a sanogenic intestinal environment was established and the possibility of modulating the composition of the intestinal microbiota in a sanogenic aspect when administering food rations with a different nutritional value and caloric structure was highlighted, which would allow, through the prebiotic action of the macronutrients in the rations, to strengthen the capacities of the intestinal microflora to stop the development of intestinal pathogens. The possibility of including some strains of enterococci in the composition of microbial preparations with probiotic action was experimentally argued.

The theoretical significance of the study consists in expanding of knowledge regarding the role of intestinal enterococci in gastrointestinal health depending on environmental conditions, the physiological status of the digestive tract and of the organism, dietary factors, and their compatibility with other commensal bacteria. It has been established that the health-promoting effect of enterococci is maintained under conditions where their numerical effectiveness does not exceed that of beneficial commensal bacteria, and its correlation controls the growth of pathogenic bacteria.

The practical value of the study. The probiotic potential of isolated enterococci strains (pure strains) that express more beneficially in association with bifidobacteria and lactobacteria has been demonstrated, their use as probiotics has been recommended, and microbial compositions containing tested strains of enterococci with sanogenic action have been developed.

Implementation of scientific results: The research results were applied in the research process within the Institute of Physiology and Sanocratology, Moldova State University.

АННОТАЦИЯ

БОГДАН Виктория «Физиологическая роль кишечных энтерококков в поддержании здоровья пищеварительного тракта», диссертация на соискание ученой степени кандидата биологических наук, Кишинев, 2024.

Структура диссертации: введение, 4 главы, выводы и рекомендации, список литературы из 360 наименований, 4 приложения, 172 страниц основного текста, 19 рисунков и 36 таблиц. Результаты изложены в 22 научных работах.

Ключевые слова: энтерококки, физиологическая роль, микробиота кишечника, здоровье, пищевые факторы, пробиотики.

Цель работы: выявление некоторых физиологических аспектов значения энтерококков для здоровья пищеварительного тракта и пробиотического потенциала отдельных штаммов энтерококков.

Задачи исследования: изучение наличия и количественного уровня кишечных энтерококков в пищеварительном тракте человека и животных в зависимости от физиологического состояния организма; изучение действия пищевых факторов на содержание кишечных энтерококков и выявление их роли в поддержании полезного микробного баланса кишечника; выделение, идентификация и селекция наиболее перспективных штаммов кишечных энтерококков, обладающих саногенным потенциалом, и изучение их пробиотических свойств; выявление и тестирование новых микробных композиций разработанных на основе энтерококков, обладающих наибольшей эффективностью для поддержания здоровья организма и пищеварительного тракта.

Научная новизна и оригинальность заключается в выявление корреляции численности энтерококков с уровнем полезных бактерий в условиях эубиоза, что свидетельствует о роли энтерококков в поддержании саногенного микробного баланса кишечника, доказанной количественными изменениями в содержимом кишечнике энтерококков в зависимости от физиологического состояния организма и действия пищевых факторов. При резкой индукции патологических процессов энтерококки теряют свое саногенное действие и могут стать возбудителями кишечных заболеваний в коалиции с другими патобионтами. На основании корреляции количества энтерококков с уровнем полезных кишечных бактерий, можно судить о микробном балансе кишечника и состоянии здоровья пищеварительного тракта. Доказан пробиотический потенциал некоторых штаммов энтерококков, по сравнению с признанными пробиотическими свойствами облигатных бактерий, и тестирована эффективность новых микробных композиций на основе энтерококков для поддержания здоровья пищеварительного тракта и всего организма.

Полученные результаты, способствующие решению важной научной задачи. Установлена благоприятная взаимосвязь энтерококков с облигатными кишечными бактериями в поддержании саногенной среды кишечника и подчеркнута возможность модуляции состава кишечной микробиоты в саногенном аспекте в условиях рационов питания с различной пищевой ценностью и структурой калорийности, что позволит за счет пребиотического действия макронутриентов, усилить возможности кишечной микрофлоры противостоять действию возбудителей кишечных заболеваний. Экспериментально обоснована возможность включения некоторых штаммов энтерококков в состав микробных композиций пробиотического действия.

Теоретическая значимость заключается в развитии знаний о роли кишечных энтерококков в здоровье желудочно-кишечного тракта по отношению к среде обитания, физиологическому состоянию пищеварительного тракта и организма, действию пищевого фактора и их совместимости с другими комменсальными бактериями. Установлено саногенное действие энтерококков, которое сохраняется в тех случаях, когда их численность не превышает таковую полезных бактерий и в случае, когда их корреляция контролирует развитие болезнетворных бактерий.

Практическая ценность работы: продемонстрирован пробиотический потенциал некоторых штаммов энтерококков, которые более эффективно проявляют себя в ассоциации с бифидо- и лактобактериями и разработаны микробные композиции, на их основе, обладающие саногенным действием.

Внедрение научных результатов: Результаты исследования используются в исследовательском процессе Института физиологии и санократологии Государственного университета Молдовы.

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