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# Gene-modified product (SOYA) consumption laboratory animals laboratory bowel microflora main representative level

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## Abstract

The aim of the study was a comparative study of the incidence of indigenous and facultative representatives of the normal microflora of the colon of experimental animals under the influence of GM-shade. To do this, we studied the encounter rate of 9 microorganisms from representatives of the colon microflora. The results showed that in both groups there were significant differences in the level of occurrence of representatives of this microflora. The main group of white rats with GM-soy added to the diet is indigenous microflora indigenous (*Bifidobacterium* spp., *Lactobacillus* spp., *Escherichia Coli*) and facultative (*Enterobacter* spp., *Proteus* spp., *Staphylococcus* spp., *Staphylococcus* spp., *Staphylococcus* spp.) the balance between the representatives was found to be disturbed. Decreased incidence of indigenous microorganisms has been shown to increase facultative microorganisms, increased lactose-negative *Escherichia Coli*, and increased incidence of *Candida* spp. It has been shown that the main factor that causes this is the GM-shadow.

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## RELEVANCE

The violation of the normal microflora of the colon under the influence of various external and internal factors is characterized by a qualitative and quantitative imbalance of the indigenous and facultative microflora in it and is called intestinal dysbiosis. Many physical, chemical, and biological factors can be examples of factors that lead to intestinal dysbiosis.

Today, a lot of scientific work has been done on the different effects of genetically modified (GM) products on the human body, and experts differ on this point, along with the opinion that these products have no adverse effects on the human body [2,11]. There are many proven works [3,8,10]. Subsequent scientific studies have shown that GM products have a negative effect on the immune system [1], liver and pan-

creas [9], thymus and spleen [12], as well as hematological, biochemical changes, mutagenic and reproductive activity [6,7], there are studies showing that it has a negative effect on bone marrow cells [13], the colon microbiocenosis of healthy white-bred rats not included in the GM- and Gm-free soy diet, in the standard vivarium diet, was studied and analyzed.

Given the above, **the aim of the study** was to compare the level of occurrence of indigenous and facultative representatives of the normal microflora of the colon of experimental animals under the influence of GM-shade.

## MATERIALS AND METHODS

For this purpose, a total of 90 male white rats were involved in the study, which were divided into 3 groups:

**Group 1** - intact white rats with a standard

vivarian diet, not fed with GM or without GM (n=30);

**Group 2** - white non-GM rats included in the standard vivariate diet without GM (n=30);

**Group 3** - white pedigree rats fed with GM-shade on a standard vivariate diet (n=30).

These groups were representative and differed from each other by only one character. Emphasis was placed on whether the studies were randomized and that principles of evidence-based medicine were followed. The study strictly adhered to the ethical principles of working with laboratory animals and the rules of biological safety [5].

Bergy's Manual Systematic Bacteriology (1997) using appropriate nutrient media (Blaurokk, SRM-4 (MRS-4), Endo, Saburo media, egg yolk agar, etc.).

Microorganisms were identified and differentiated:

- Bifidobacterium spp.,
- Lactobacillus spp.,
- Escherichia coli,
- Enterobacter spp.,
- Proteus spp.,
- Staphylococcus spp.,
- Streptococcus spp.,
- Candida spp.

Intergenerational and interspecific identification was performed using food media from HiMedia (India).

Statistical processing of the results was carried out using traditional variational statistical methods, and the principles of evidence-based medicine were followed in organizing and conducting the research.

## RESULTS AND DISCUSSION

The results obtained (Table 1) showed that the normal microflora indigenous microorganisms Bifidobacterium spp. and Lactobacillus spp. were detected in practically all laboratory animals - 93.3±4.6% (n=28) and 100.0% (n= 0, respectively).

It is noteworthy that Escherichia coli, which is important for the colon microflora and does not exhibit pathogenicity, was detected in 100.0 (n = 30) rats in all experimental rats. Pathogenic Streptococcus spp. also germinated in most laboratory animals - 90.0±5.5%, n=27. It should be noted that representatives of the facultative microflora are not found in all animals with a similar appearance in the normal state. Examples are Enterobacteria spp. and Proteus spp., members of the family Enterobacteriaceae. They found that the incidence rate was the lowest among all 9 microorganisms studied -

43.3±6.3% (n=13) and 33.3±8.6% (n=10), respectively.

Table 1  
Intact white pedigree rats colon microflora meeting rate

Microorganisms	In absolute numbers	In relative (%) numbers
Bifidobacterium spp.	28	93.3±4.6
Lactobacillus spp.	30	100.0
Escherichia Coli (lactose-positive)	30	100.0
Escherichia Coli (lactose-negative)	0	0
Enterobacter spp.	13	43.3±6.3
Proteus spp.	10	33.3±8.6
Staphylococcus spp.	26	86.7±6.2
Streptococcus spp.	27	90.0±5.5
Candida spp.	20	66.7±8.6

A similar result was observed for Candida spp. (66.7±8.6%, n=20). The detection rate of Staphylococcus spp. was similar to that of other gram-negative cocci (Streptococcus spp.) (86.7±6.2%, n=26).

Lactose-degrading (lactose-negative) intestinal bacilli did not germinate in white rats belonging to this group (0%). In our opinion, there were no factors that adversely affected the composition of the colon microbiocenosis, including Escherichia coli. As a result, the intestinal rod retains all its biological properties and does not become pathogenic.

Thus, the incidence rate of representatives of the colonic microflora of white non-GM rats with and without GM with soy diet remained unchanged and did not differ from the data presented in many publications [4].

Due to the need to study the effect of GM-shadow on the normal microflora of the colon, these parameters were compared with the indicators of intact laboratory animals.

It is clear from the results obtained (Table 2) that in both groups there were sharp differences in the level of occurrence of representatives of this microflora.

It was found that the incidence of indigenous microflora was low in the group consuming GM-soy - Bifidobacterium spp. 40.0±8.9% (n=12) and Lactobacillus spp. 46.7±9.1% (n=14), respectively (R<0,001). This is 2.33 and 2.07 times lower than in control group animals, respectively. A decrease in the rate of occurrence of these microorganisms has led to a violation of the balance of representatives of the normal

microflora of the colon, dysbiosis. It was proved that a convincing decrease in the encounter rate of these microorganisms ( $R < 0.001$ ) led to a decrease in their quantitative parameters.

Table 2

Comparative indicators of the rate of occurrence of representatives of the normal microflora of the colon of white rats consuming GM-soy

Microorganisms	Intact laboratory animals		Laboratory animals consuming GM-soy	
	Absolutely	(%)	Absolutely	(%)
Bifidobacterium spp.	28	93.3 ±4.6	12	40.0±8.9 * ↓
Lactobacillus spp.	30	100.0	14	46.7±9.1 * ↓
Escherichia Coli (lactose -positive)	30	100.0	4	13.3±6.2 * ↓
Escherichia Coli (lactose -negative)	0	0	26	86.7±6.2 * ↑
Enterobacter spp.	13	43.3 ±6.3	28	93.3±4.6 * ↑
Proteus spp.	10	33.3 ±8.6	22	73.3±8.1 * ↑
Staphylococcus spp.	26	86.7 ±6.2	29	96.7±3.3 * ↔
Streptococcus spp.	27	90.0 ±5.5	29	96.7±3.3 * ↔
Candida spp.	20	66.7 ±8.6	29	96.7±3.3 * ↑

Note: \* -convincing distinction relative to the control group; ↑, ↓ - directions of changes; ↔ - There are no differences.

Interestingly, this was observed in terms of the percentage of Escherichia coli germination. If in all cases (100.0%,  $n = 30$ ) lactose-negative intestinal rods with lactose-degrading properties were detected in the control group, in laboratory animals with GM-soy added to the diet, their germination rate decreased sharply (13.3±6.2%,  $n=4$ ), the percentage of lactose-negative Escherichia coli germination increased significantly - 86.7±6.2%,  $n=26$  ( $R < 0.001$ ). The difference between different strains of this microorganism was 6.52 times in favor of lactose-negative microorganisms.

Representatives of the family Enterobacteriaceae showed a sharp increase in the incidence of Enterobacter spp. and Proteus spp. relative to the control group (93.3±4.6%,  $n=28$  and 73.3±8.1%,  $n=22$ , respectively) is one of the signs of the formation of intestinal dysbiosis. It is recognized that these microorganisms increased significantly ( $R < 0.001$ ) in the main group (GM-soy consumed) by 2.15 and 2.20 times, respectively, compared to the control group.

Gram-negative cocci from indigenous microflora representatives - Staphylococcus spp. and Streptococcus spp. germination rates did not differ significantly from each other in the control and baseline groups - 96.7±3.3% against 86.7±6.2% ( $n=26$ ), respectively ( $n=29$ )  $R > 0.05$ . This indicates that the effect of GM-shade on the percentage of these microorganisms is small, and that these gram-positive microorganisms play a small role in the formation of colon dysbiosis.

The trend of changes in Candida spp. germination rates was similar to that of conditionally pathogenic enterobacteria and gram-negative cocci, with a significant increase in germination percentage in the main group compared to the control group by 1.45 times (66.7±8.6%,  $n=20$  versus 96.7±3.3%,  $n=29$ ,  $R > 0.05$ ).

Thus, GM-soy was added to the diet, the main group of white rodents rats indigenous microflora indigenous (Bifidobacterium spp., Lactobacillus spp., lactose positive Escherichia coli) and facultative (Enterobacter spp., Proteus spp., Staphylococcus spp., Staphylococcus spp.) was found to be disturbed. A decrease in the incidence of indigenous microorganisms and an increase in facultative microorganisms is the main sign of an increase in dysbiosis in the main group. The appearance of lactose-negative Escherichia coli in the main group is another key sign that dysbiosis has developed. Decreased indigenous microflora and increased facultative microflora have been shown to lead to large-scale growth of lactose-negative Escherichia coli, a convincing increase in the incidence of Candida spp. leading to colonic dysbiosis. It has been shown that the main factor that causes this is the GM-shadow.

It was taken into account that the above changes in the microbiocenosis of laboratory animals may be not only a GM-soy product, but also a product made from soybean plant. Therefore, soybeans grown in our country were added to the standard vivarium diet of another group of white rats (comparison group) ( $n = 30$ ). The results were compared with the results of the intact laboratory animals considered in the control group (Table 3).

Analysis of the above data showed that no changes in indigenous microflora germination rate were detected when GM-free soy was added to the standard vivarium diet. All 4 parameters studied did not differ significantly from the control group (intact) values.

In the next phase of our research, the results of all three studied groups were compared (Table 4).

**Table 3**

Comparative indicators of the rate of occurrence of representatives of the normal microflora of the colon of white rats consuming soy without GM

Microorganisms	Intact laboratory animals		Laboratory animals that consume soy without GM	
	Absolutely	(%)	Absolutely	(%)
Bifidobacterium spp.	28	93.3 ±4.6	28	93.3±4.6* ↔
Lactobacillus spp.	30	100.0	29	96.7±3.3* ↔
Escherichia coli (lactosa-positive)	30	100.0	29	96.7±3.3* ↔
Escherichia coli (lactose-negative)	0	0	0	0
Enterobacter spp.	13	43.3 ±6.3	25	83.3±6.8*↑
Proteus spp.	10	33.3 ±8.6	24	80.3±7.3*↑
Staphylococcus spp.	26	86.7 ±6.2	28	93.3±4.6* ↔
Streptococcus spp.	27	90.0 ±5.5	29	96.7±3.3* ↔
Candida spp.	20	66.7 ±8.6	27	90.0±5.5*↑

Note: \* -convincing distinction relative to the control group; ↑, ↓ - directions of changes; ↔ - There are no differences.

**Table 4**

Comparative indicators of the incidence rate of representatives of the normal microflora of the colon of white rats with and without GM soy diet

Microorganisms	Intact laboratory animals	Laboratory animals that consume soy without GM	Laboratory animals that consume soy with GM
Bifidobacterium spp.	28/93.3±4.6	28/93.3±4.6* ↔	12/40.0±8.9 *↓
Lactobacillus spp.	30/100	29/96.7±3.3* ↔	14/46.7±9.1 *↓
Escherichia coli (lactosa-positive)	30/100	29/96.7±3.3* ↔	4/13.3±6.2* ↓
Escherichia coli (lactose-negative)	0/0	0/0	26/86.7±6.2 *↑
Enterobacter spp.	13/43.3±6.3	25/83.3±6.8*↑	28/93.3±4.6 *↑
Proteus spp.	10/33.3±8.6	24/83.3±6.8*↑	22/73.3±8.1 *↑
Staphylococcus spp.	26/86.7±6.2	28/93.3±4.6* ↔	29/96.7±3.3 *↔
Streptococcus spp.	27/90.0±5.5	29/96.7±3.3* ↔	29/96.7±3.3 *↔
Candida spp.	20/66.7±8.6	27/90.0±5.5*↑	29/96.7±3.3 *↑

Note: quantitative, relative (%) indices in the figure; \* - convincing distinction sign for the control group; ↑, ↓ - directions of changes; ↔ - There are no differences.

The results of the study of the meeting rate of all 9 colon microflora representatives studied in this Table 4 are shown in detail. The directions of intergroup changes, differences in germination percentages, and intergenerational characteristics are detailed.

### CONCLUSIONS

First, it was acknowledged that the incidence of indigenous microorganisms in the colon of laboratory animals consuming GM soy decreased by 2.33–7.52 times compared to the control and comparison group, indicating that GM soy had a negative effect on their germination rates;

Second, in 86.7% of cases in the control and comparison groups, no gram-negative Escherichia Coli was detected in the biological material in the main group, which showed that the microorganism changed its properties under the influence of GM-soy and became pathogenic. The incidence of lactose-negative Escherichia Coli has been shown to decrease convincingly as a result of a sharp increase in the percentage of lactose-negative Escherichia Coli germination;

Third, the fact that all three groups of gram-positive cocci (Staphylococcus spp. and Streptococcus spp.) did not differ convincingly from each other in practice proved that their occurrence rate had no practical effect on the GM- and GM-free shade under study. This was explained by the specific biological properties of the strains of these microorganisms, with a high degree of resistance;

Fourth, representatives of the facultative microflora were significantly differentiated from the control group by the fact that the incidence of gram-negative enterobacteria (Enterobacter spp., Proteus spp.) did not differ from each other in the main and comparison groups. This was explained by the unfamiliarity of shade for the organism of non-white rats, the low resistance of both microorganism strains studied to environmental factors;

Fifth, the trend in the incidence of yeast fungi of the Candida genus was similar to that of facultative gram-negative enterobacteria. In it, too, the comparison and control groups differed convincingly from the control group, with no intergroup differences identified. However, since this difference was not significant, it was considered that GM-free and GM-free shade had practically no effect on the germination rate of Candida spp.

**Conflict of Interest** - The author declares no conflict of interest.

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**Compliance with the principles of bioethics** - all studies were carried out with the permission of the bioethical committee.

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