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Results of Determining the Level of Influence of Gene-Modified Soy on the Normal Microflora of the Colon of Laboratory Animals

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Abstract: Along with various microorganisms that enter the body from the external environment, there are also microorganisms that live in symbiosis with it in the human body, forming the normal human microflora. They are located in various biotopes and are important for the functioning of the body. One of such biotopes is the large intestine, the normal microflora of which, consisting of indigenous and facultative microorganisms, is of great importance for vital activity. As is known, the microbiocenosis of the large intestine consists of more than 450 microorganisms and participates in the metabolism of the "host" organism and the formation of colonization resistance in the intestine.

Keywords: Disturbance of the normal microflora of the large intestine under the influence of various external and internal factors is characterized by a qualitative and quantitative imbalance of indigenous and facultative microflora in it and is called intestinal dysbiosis. Factors leading to intestinal dysbiosis include many physical, chemical, and biological factors.

Introduction.

Today, many scientific studies have been conducted on the various effects of genetically modified (GM) products on the human body, and the opinions of experts on this matter differ. Along with the opinions that these products have no negative effects on the human body [2, 10], there are also many studies that have proven their negative effects on the body [3 7, 9]. Scientific studies that confirm the latter opinions include experimentally proven negative effects of GM products on the immune system [1], liver and pancreas [8], thymus and spleen [11], as well as studies that have shown hematological, biochemical changes, mutagenic and reproductive activity [5, 6], and negative effects on bone marrow cells [12].

The analysis of many scientific literature shows that there are few and scattered studies on the level of impact of GM products on the microbiocenosis of human biotopes, including colon microbiosis.

Taking into account the above, **the aim of the study** was to determine the level of influence of GM-soybeans on the colonic microbiocenosis of purebred rats.

Materials and methods. A total of 90 male white outbred rats were recruited for the study, which were divided into 3 groups: Group 1 - intact white outbred rats fed a standard vivarium diet, not fed GM or non-GM soy (n=30); Group 2 - white outbred rats fed a standard vivarium diet with non-GM soy (n=30); Group 3 - white outbred rats fed a standard vivarium diet with GM soy (n=30).

These groups were representative and differed from each other by only one characteristic. Attention was paid to the fact that the studies were randomized and followed the principles of

evidence-based medicine. The study strictly followed the ethical principles of working with laboratory animals and the rules of biosafety [4].

After the colon mass of the white-bred rats was delivered to the bacteriological laboratory, as a result of bacteriological examinations, the following microorganisms were identified and differentiated *according to Bergy's Manual Systematic Bacteriology (1997) using appropriate nutrient media (Blaurock, CPM-4 (MRS-4), Endo, Sabouraud media, egg yolk agar, etc.): Bifidobacterium spp, Lactobacillus spp, Escherichia coli, Enterobacter spp, Proteus spp, Staphylococcus spp, Streptococcus spp, Candida spp . Intergeneric and interspecific identification was performed using nutrient media from the HiMedia company (India).*

the results was carried out using traditional variational statistical methods, the principles of evidence-based medicine were followed in the organization and conduct of research.

Results and their discussion. The obtained results showed that there were convincing differences between the compared groups on the studied quantitative indicators (Table 1).

Table 1. Comparative analysis of quantitative indicators of the colonic microbiocenosis of intact and non-GM soy-fed laboratory animals, lg CBC/ml (M±m).

Microorganisms	Group 1, n=30	Group 2, n=30
Bifidobacterium spp.	5.10±0.2	4.00±0.1* ↓
Lactobacillus spp.	6.10±0.2	4.00±0.1* ↓
Escherichia coli (lactose positive)	5.15±0.2	5.00±0.2 ↔
Escherichia coli (lactose negative)	0	0 ↔
Enterobacter spp.	1.20±0.1	5.00±0.2* ↑
Proteus spp.	0.80±0.1	5.00±0.2* ↑
Staphylococcus spp.	4.10±0.1	5.00±0.2* ↑
Streptococcus spp.	6.30±0.3	4.00±0.2* ↓
Candida spp.	3.60±0.1	7.00±0.1* ↑

Note: * - a sign of a significant difference between groups; ↑, ↓ - directions of change; ↔ - no significant difference.

It can be seen that in 7 out of 9 studied representatives of the colonic microflora (77.78%), significant changes were detected, and it should be noted that the quantitative indicators of microorganisms changed in different directions compared to the norm (group 1). Only the indicators associated with *Escherichia coli* did not change significantly in both groups. The quantitative indicators of non-pathogenic *Escherichia coli (lactose-positive Escherichia coli), which forms metallic-shiny fuchsin-red colonies in the endo environment, breaks down lactose to acid and gas according to the Hiss series, and Escherichia coli (lactose-negative Escherichia* coli), which forms dark-colored, light-red (or colorless) colonies in the endo environment, does not have the property of breaking down lactose according to the Hiss series, and shows pathogenicity, were close to each other (P>0.05).

The analysis showed that in group 2 (consumed non-GM soy) compared to the control group (intact), a significant decrease in quantitative indicators was observed among representatives of the normal indigenous microflora of the colon. The quantitative decrease *in Bifidobacterium spp* was up to 1.28 times (P<0.05), while the quantitative decrease in *Lactobacillus spp* reached 1.53 times (P<0.05). This indicates that the processes leading to a dysbiotic state in the colon of laboratory animals in group 2 have begun, and this is the first sign of it.

A similar situation can be observed for another representative of the normal microflora, *Streptococcus spp.* , whose concentration in the large intestine decreased by 1.58 times (P<0.05). This situation was also interpreted as a prelude to dysbiotic processes. Considering that, unlike the control group, group 2 had only one external agent (soy), these changes were due to its influence, and since this was an unfamiliar product for the body of white-bred rats, the decrease

in the quantitative parameters of the indigenous intestinal microflora was interpreted as a temporary phenomenon.

When comparing the parameters of the control group with those of laboratory animals, it should be noted that the quantitative increase in the parameters of the group was mainly due to enterobacteria and coagulase-negative cocci, taking into account their inclusion in the facultative (transient) microflora of the colon and the fact that they manifest pathogenic properties under favorable conditions, an imbalance of indigenous and facultative microorganisms was observed in this biotope. For example, in group 2, a quantitative increase was observed in representatives of the Enterobacteriaceae family *Enterobacter spp* and *Proteus spp* - 4.17 times ($P < 0.001$) and 6.25 times ($P < 0.001$), respectively. The quantitative increase in these microorganisms was interpreted as the beginning of dysbiosis processes in the colon.

A similar result, but with a lower intensity, was observed for *Staphylococcus spp* . It should also be noted that the intensity of the tendency for this microorganism to decrease in quantity was lower than for Gram-negative bacteria. The difference between the control and comparison groups was 1.58 times, in favor of intact animals ($P<0.05$).

Thus, significant quantitative differences were detected in the normal microflora of the colon of white-bred rats fed a standard vivarium diet with non-GM soy compared to intact laboratory animals, with quantitative changes in different directions in 7 out of 9 representatives of the normal microflora (77.78%) ($P<0.05$). The main significant differences were observed in *Bifidobacterium spp* (1.28-fold decrease), *Lactobacillus spp* (1.53-fold decrease), *Enterobacter spp* and *Proteus spp* (4.16- and 6.25-fold increase). Such a violation of the balance between the habitual and facultative microflora of the colon is an initial sign of dysbiosis processes and does not indicate the development of full-fledged dysbiosis, since no intergroup differences were detected in pathogenic and non-pathogenic strains *of Escherichia coli, one of the main microorganisms of the intestinal microflora* , and lactose-negative strains did not grow. The cause of the quantitative changes was the consumption of soybeans, and the fact that it was unfamiliar to the animal organism was the main reason.

At the next stage of the scientific work, quantitative indicators of the colon microbiocenosis of purebred white rats with GM soy added to the standard vivarium ration were studied and the obtained numbers were analyzed. Intact (non-GM-soy-fed) laboratory animals were used for comparison. All results are presented in Table 2.

Table 2. Comparative analysis of quantitative indicators of the colonic microbiocenosis of GM-soybean-fed and intact laboratory animals, lg CBC/ml (M±m).

Microorganisms	Group 1, n=30	Group 3, n=30
Bifidobacterium spp.	5.10±0.2	2.10±0.1* ↓
Lactobacillus spp.	6.10±0.2	2.00±0.2* ↓
Escherichia coli (lactose positive)	5.15±0.2	0 ↓
Escherichia coli (lactose negative)	0	5.30±0.3* ↑
Enterobacter spp.	1.20±0.1	5.45±0.2* ↑
Proteus spp.	0.80±0.1	3.00±0.1* ↑
Staphylococcus spp.	4.10±0.1	6.15±0.2* ↑
Streptococcus spp.	6.30±0.3	4.30±0.2* ↓
Candida spp.	3.60±0.1	7.00±0.4* ↑

Note: * - a sign of a significant difference between groups; ↑, ↓ - directions of change; ↔ - no significant difference.

When analyzing the quantitative parameters of the microorganisms that make up the colon microflora in Table 2, it was found that all of them had intergroup differences. These differences were observed for all 9 microorganisms studied.

It is noteworthy that the most profound quantitative changes were observed in *Bifidobacterium spp,* a representative of the normal indigenous microflora of the colon , with a decrease of 2.43 times ($P < 0.001$). A similar result was obtained for *Lactobacillus spp* - the quantitative indicators showed the same decreasing trend and intensity as bifidobacteria, with an average quantitative decrease of 3.05 times ($P < 0.001$).

the number of indigenous microflora representatives in the main group (fed with GM soybeans) by 2.43-3.05 times compared to the control group (intact) was the beginning of dysbiotic processes in this biotope. These quantitative changes were confirmed as the effect of an external factor on indigenous microorganisms, and if we take into account that only GM soybeans acted on them as an external factor, then the results obtained are clearly its effect.

A different picture was observed when studying the quantitative indicator of *Escherichia coli,* another representative of the normal microflora of the colon . While these non-pathogenic gram-negative bacteria, capable of breaking down lactose, grew in the control group at a level of 5.15±0.2 lg CFU/ml, they did not grow in the biological material obtained from the colon of white outbred rats of group 3. However, it was recognized that pathogenic *Escherichia coli* strains grew in the level of 5.30±0.3 lg CFU/ml, while in the control group these strains were not detected at all. This is another main sign of the dysbiosis process developing in this biotope.

Other representatives of *the Enterobacteriaceae family, Enterobacter spp* and *Proteus spp,* also showed changes similar to lactose-negative *Escherichia coli* , i.e. their quantitative indicators exceeded the normal limits - 5.45±0.2 lg CFU/ml and 3.00±0.1 lg CFU/ml, respectively. These figures were characterized by a significant excess of the normal limits by 4.54 and 3.75 times ($P<0.001$). This situation was characterized by a quantitative decrease in indigenous microorganisms and an increase in opportunistic enterobacteria. This phenomenon is a sign of the formation of dysbiotic processes in the large intestine.

The above-mentioned sharp changes in Gram-negative bacteria were not observed in Gram-positive cocci, although the quantitative indicators differed between groups, but the intensity of the changes was low. If *Staphylococcus spp* in group 3 significantly increased by 1.50 times compared to group 1 (respectively 6.15±0.2 lg CFU/ml versus 4.10±0.1 lg CFU/ml, $P<0.05$), we witnessed the opposite picture for *Streptococcus spp* , i.e., the indicators in group 3 significantly decreased by 1.47 times compared to the control group (group 1) ($P<0.05$).

Candida spp ., a facultative microorganism, consisting of opportunistic pathogens. The number of microorganisms belonging to this genus of yeasts in the colon of white outbred rats fed GM soy was significantly higher (1.94 times, $P<0.001$) than in intact rats not fed GM soy.

The analysis of the obtained results showed that the symptoms of colon dysbiosis were observed at the end of the observation period in laboratory animals that consumed GM soy. This condition is manifested in:

Bifidobacterium spp and *Lactobacillus spp,* which are representatives of the normal microflora of the colon, were significantly reduced by 2.43 and 3.05 times in animals fed with GM soy compared to intact rats. This decrease in the number was interpreted as an external factor that negatively affected them, and in this experiment it was GM soy. This phenomenon was interpreted as the first element of dysbiosis formed in the colon biotope.

Secondly, in white outbred rats fed GM soy, unlike intact ones, lactose-negative *Escherichia coli* grew (they did not grow in intact animals), and accordingly, lactose-positive *Escherichia coli* did not grow, and the opposite was true in intact ones. The growth of lactose-negative strains and the absence of lactose-positive strains were proven to be a second element of colon dysbiosis.

Thirdly, in group 3 laboratory animals, it was found that the conditionally pathogenic representatives of *the Enterobacteriaceae family Enterobacter spp* and *Proteus spp* increased by 4.54 and 3.75 times, respectively, compared to the control group. Under experimental conditions, they were exposed only to GM soy as an external effect, which showed that this product was the

main cause of the imbalance of indigenous and facultative microorganisms, which proved to be the third element of colon dysbiosis.

Fourth, in elements 1-3 of colon dysbiosis, with the clear manifestation of these signs, no significant changes were detected in the indicators of gram-positive cocci in this biotope, while the representative of the indigenous microflora, non-pathogenic *Streptococcus spp,* significantly decreased by 1.47 times in the main group compared to intact laboratory animals, while the quantitative indicator of the representative of the facultative microflora, coagulase-positive *Staphylococcus spp,* significantly increased by 1.50 times. This intergroup discrepancy was interpreted as the fourth element of colon dysbiosis.

Candida spp, a representative of the facultative microflora of the colon, was shown to be significantly increased by 1.94 times in white crossbred rats fed GM soy (group 3) compared to those not fed this product (group 1), as the fifth element of colon dysbiosis.

After it was shown that dysbiotic processes develop in the colon of white outbred rats as a result of exposure to GM soybeans, it was necessary to assess the degree of changes in the quantitative indicators of indigenous and facultative microflora. For this purpose, the ratio of the quantitative indicators of the compared groups was studied. In group 3 (fed with GM soybeans), the quantitative indicators of indigenous microorganisms significantly decreased ($P<0.001$) compared to group 1 (not fed with GM soybeans), while the quantitative parameters of facultative microorganisms significantly increased ($P<0.05$-$P<0.001$). This was interpreted as another indicator proving that deep dysbiosis developed in the colon under the influence of GM soybeans.

was a comparative study of the quantitative indicators of representatives of indigenous and facultative microflora of the large intestine of laboratory animals fed soy without GM (group 2) and GM soy (group 3) to the standard vivarium diet . The obtained results are presented in Table 3.

Table 3. Comparative analysis of quantitative indicators of the colonic microbiocenosis of laboratory animals fed non-GM and GM soy, lg CBC/ml (M±m).

Microorganisms	Group 2, n=30	Group 3, n=30
Bifidobacterium spp.	4.00±0.1	2.10±0.1* ↓
Lactobacillus spp.	4.00±0.1	2.00±0.2* ↓
Escherichia coli (lactose positive)	5.00±0.2	0 ↓
Escherichia coli (lactose negative)	0	5.30±0.3* ↑
Enterobacter spp.	5.00±0.2	5.45±0.2* ↑
Proteus spp.	5.00±0.2	3.00±0.1* ↑
Staphylococcus spp.	5.00±0.2	6.15±0.2* ↑
Streptococcus spp.	4.00±0.2	4.30±0.2* ↓
Candida spp.	7.00±0.1	7.00±0.4* ↑

Note: * - a sign of a significant difference between groups; ↑, ↓ - directions of change; ↔ - no significant difference.

Although the results of both groups differed from the parameters of intact laboratory animals, the intensity and depth of changes were evident in group 3. However, it was important to determine the degree of variation between non-GM and GM soybeans.

Bifidobacterium spp and *Lactobacillus spp,* representatives of the indigenous microflora of the colon - a significant decrease of 1.90 and 2.0 times, respectively ($P<0.001$).

Of the 9 microorganisms studied, 2 (*Staphylococcus spp, Candida spp*) did not show any differences between the groups ($P>0.05$), and their quantitative values were close to each other. It is noteworthy that 1 of them belongs to the group of facultative microorganisms. No clear pattern was observed in this situation. However, it was found that indigenous microorganisms (

Bifidobacterium spp, Lactobacillus spp) decreased even more in group 3, while facultative microorganisms (*Enterobacter spp, Staphylococcus spp*) increased even more. The results obtained for lactose-negative and lactose-positive *Escherichia coli* showed a sharp difference between these groups. In other words, if laboratory animals fed with GM soy had all 5 listed elements of dysbiosis, they were not clearly manifested in rats fed non-GM soy.

In order to summarize all the obtained results, we found it necessary to compare the indicators of all three groups (Table 4).

This Table 4 clearly shows a significant difference between the groups, the analyzed figures showed that in laboratory animals not fed with non-GM soy (intact, control) there were practically no changes in the normal microflora of the colon, no signs of dysbiosis were detected; in laboratory animals that consumed non-GM soy (group 2), the balance between indigenous and facultative microorganisms was partially disturbed, there were signs of dysbiosis, but it was not formed and did not develop; in animals fed with GM soy (group 3), the balance between indigenous and facultative microorganisms was disturbed relative to each other, signs of dysbiosis were clearly observed, all 5 of its elements were detected, and total dysbiosis of the colon developed. This situation was interpreted as the effect of GM soy on the body of white crossbred rats. It has been proven that GM-soy has a negative effect on the normal microflora of the large intestine of laboratory animals, causing total dysbiosis.

Table 4. Quantitative state of the colonic microflora of white crossbred rats fed GM and non-GM diets, lg (M±m) (CFU/ml)

Microorganisms	Group 1, n=30	Group 2, n=30	Group 3, n=30
Bifidobacterium spp.	5.10±0.2	4.00±0.1	2.10±0.1*^↓
Lactobacillus spp.	6.10±0.2	4.00±0.1	2.00±0.2*^↓
Escherichia coli (lactose positive)	5.15±0.2	5.00±0.2	0 ↓
Escherichia coli (lactose negative)	0	0	5.30±0.3*↑
Enterobacter spp.	1.20±0.1	5.00±0.2	5.45±0.2*^↑
Proteus spp.	0.80±0.1	5.00±0.2	3.00±0.1*^↑
Staphylococcus spp.	4.10±0.1	5.00±0.2	6.15±0.2*^↑
Streptococcus spp.	6.30±0.3	4.00±0.2	4.30±0.2*↓
Candida spp.	3.60±0.1	7.00±0.1	7.00±0.4*↑

Note: * - significant difference between groups 1 and 3; ^ - significant difference between groups 2 and 3; ↑, ↓ - directions of change; ↔ - no significant difference.

To determine the state of the normal microflora of the colon, the degree of dysbiosis development, and its severity, many studies have created and presented criteria for assessing dysbiosis in clinical practice. Among these methods, we selected the one we considered the most appropriate and used it to assess dysbiosis.

This method was recommended by Uzbek researchers Garib F.Yu., Adilov Sh.K. and Narbayeva I.E. in 1995, and the changes in the colon microflora are assessed in 2 degrees:

In grade I dysbiosis, changes are observed only among representatives of the indigenous group, *Bifidobacterium spp* and *Lactobacillus spp are reduced relative to* lactose-positive *Escherichia coli* , and intestinal dysfunction is not manifested.

In grade II dysbiosis, the number of facultative opportunistic microorganisms increases along with the decrease in indigenous microorganisms, and the balance between them is disturbed, and signs of intestinal dysfunction become evident. These degrees are determined using the dysbacteriosis index (DI):

DI I = *E.coli* KXQB/g / Indigenous microorganisms, KXQB/g <0.1;

DI I I = Facultative microorganisms , KXQB/g / Indigenous microorganisms, KXQB/g ≤ 0.5.

If DI I > 0.1; DI If I I ≤ 0.5 , this is considered dysbiosis level I, and if DI II > 0.5, it is considered dysbiosis level II, regardless of the DI I value.

The results obtained during our research were as follows:

In group 1 - 0.31<0.1 (DI I); 0.37 < 0.5 (DI II);

In group 2 - 0.38<0.1 (DI I); 0.77 < 0.5 (DI II);

in group 3 - 1.29<0.1 (DI I); 3.56 < 0.5 (DI II).

The results fully confirmed the above-mentioned ideas, namely, intact laboratory animals (group 1) did not show signs of dysbiosis, those fed non-GM soy (group 2) showed weak signs of dysbiosis (grade I), and in white crossbred rats fed GM soy, signs of dysbiosis were clearly evident (grade II).

Conclusion.

1. In the normal colonic microflora of white non-GM soy-fed rats, significant quantitative differences were observed in *Bifidobacterium spp* (1.28-fold decrease), *Lactobacillus spp* (1.53-fold decrease), *Enterobacter spp* and *Proteus spp* (4.16- and 6.25-fold increase) compared to intact laboratory animals. These are initial signs of dysbiosis and do not indicate the development of full-fledged dysbiosis, since no intergroup differences were detected in lactose-negative and lactose-positive strains *of Escherichia coli* .

2. In laboratory animals fed GM soy, the quantitative indicators of *Bifidobacterium spp* and *Lactobacillus spp* significantly decreased by 2.43 and 3.05 times compared to intact rats. This decrease was interpreted as an external factor that negatively affected them, and in this experiment, it was GM soy. This phenomenon was interpreted as the first element of dysbiosis formed in the colonic biotope.

3. In white outbred rats fed GM soy, unlike intact rats, lactose-negative *Escherichia coli* grew, while lactose-positive *Escherichia coli did not grow, while in intact rats the opposite was true. The fact* that lactose-negative strains grew and lactose-positive strains were not detected was proven to be a second element of colon dysbiosis.

4. In laboratory animals fed GM soy *, Enterobacter spp* and *Proteus spp* were found to increase by 4.54 and 3.75 times, respectively, compared to the control group, proving that this is the third element of colon dysbiosis.

5. In elements 1-3 of colon dysbiosis, with pronounced signs of this condition, no significant changes were detected in the indicators of gram-positive cocci - *Streptococcus spp.* significantly decreased in the main group by 1.47 times compared to intact laboratory animals, while the quantitative indicator of coagulase-positive *Staphylococcus spp.* significantly increased by 1.50 times. This intergroup discrepancy was interpreted as the fourth element of colon dysbiosis.

6. The quantitative indicator of *Candida spp* was shown to be significantly increased by 1.94 times in white crossbred rats fed GM soy compared to those not fed this product, as the fifth element of colon dysbiosis.

7. While laboratory animals fed GM soy had all 5 of the above elements of dysbiosis, they were not as pronounced in rats fed non-GM soy.

8. Determination of the dysbacteriosis index, which indicates the I and II degrees of dysbacteriosis, gave the following results: in group 1 - 0.31<0.1 (DI I); 0.37 < 0.5 (DI II); in group 2 - 0.38<0.1 (DI I); 0.77 < 0.5 (DI II); in group 3 - 1.29<0.1 (DI I); 3.56 < 0.5 (DI II). In intact laboratory animals, there were no signs of dysbiosis, in those fed non-GM soy, dysbiosis signs were weakly developed (I degree), in those fed GM soy, dysbiosis signs were clearly manifested (II degree).

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