

Effects of Genetically Modified Food on Gut Microbiota in Animal Models: A Systematic Review

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Abstract

Background: There are some debates about the possible unintended effects of genetically modified (GM) crops on consumer health. The gut microbiota plays an important role in maintaining the health of the host, especially in gastrointestinal diseases. The current review focuses on the studies with the aim of evaluating whether their outcomes indicate any adverse effects of feeding genetically modified (GM) crops on alteration and the count of gut microbiota.

Methods: A structured literature search was performed independently by three authors on Scopus, Web of Science, PubMed, and Embase on the 1st of July 2019. In total, 333 publications were obtained by the search strategy, which decreased to 306 after excluding the duplicates. Furthermore, experimental studies that have designed a control group and were written in English were included in the review. After reviewing the full texts, 16 studies were included. To access the quality of articles, we used the Cochrane checklist.

Results: Ten publications (62.5%) used 50% or more genetically modified (GM) diet in the treatment group. In 11 studies (68.7%), the duration of animal feeding was 90 days or more. There were no significant differences in the experimental and control groups of both male and female rats on the 90th day; it can be concluded that non-genetically modified (GM) and genetically modified (GM) rice diets did not cause any changes in the gut bacteria. Data analysis of different animal models showed that the most changes in the microbial flora were observed in the chicken and the least in the rat. In the studies in this review, all microbial isolates were anaerobic, and the *Lactobacillus* and *Enterococcus* families were common organisms.

Conclusion: Based on our literature review, we claim that there is not any significant difference in gut microbiota between the control group and the group with a transgenic diet. The mechanisms of the effects of genetically modified (GM) foods on the gut microbiota in animals should be explicated in future studies. Nevertheless, this study provides valuable information for research on genetically modified (GM) foods and whether they are useful or detrimental to human health.

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Introduction

There has been an exponential growth in the subject of agricultural land cultivation with genetically modified (GM) crops since 1996. In 2016, 20 years later, 185.1 million hectares were cultivated with genetically modified (GM) crops throughout the world (The International Service for the Acquisition of Agri-biotech Applications (ISAAA), 2017).¹ As the cultivation area and marketing of genetically modified (GM) products extend, an increasing number of consumers are becoming familiar with them, which has caused a lot of concern about the possible inadvertent impact of these new products on the environment and public health.² Maize, cotton, soybean, and canola are the most common genetically modified (GM) crops.³ In these crops, a gene has been introduced to either cause insect resistance,⁴ such as in the case of *Bacillus thuringiensis* (Bt) maize, or induce herbicide tolerance, an example of which would be Roundup Ready (RR) soybean. Genetic modification has been done on *Bacillus thuringiensis* (Bt) crops to cause the expression of one or more Cry proteins from the bacterium *Bacillus thuringiensis* (Bt) to protect them against insects of the order Lepidoptera.^{1,5} Since 2013, one of the preconditions for authorizing GM crops in the European Union has been 90-day feeding studies on rodents.¹

Recently, the critical role of the gut microbiota in keeping the host healthy has gained worldwide recognition.⁶ There is a functioning and complex microbiota in the gastrointestinal tract of mammals that affects the health of their bodies by playing an important role in intestinal metabolism. For example, the gut microbiota is tasked with energy extraction from indigestible foods, the development of the gut immune system, and the synthesis of crucial vitamins.⁷ Numerous studies have shown that intestinal bacteria play an unparalleled role in physiology, intestinal morphology, development of the immune system, and digestion.^{5,8,9} As a complex and active ecosystem, the gut microbiota is responsible for many critical functions in the host, such as modulating metabolic processes, fermenting undigested energy, harvesting nutrients, and detoxifying toxic compounds.¹⁰

It is a widely recognized fact that many species of bacteria are beneficial, such as *Lactobacillus*, *Bifidobacterium* and some types of *Streptococcus*, while some other species such as *Bacteroides*, *Clostridium* and *Enterobacterium* can be dangerous due to their metabolic activities. There are even some species that can be potentially pathogens.² The composition of the eaten food can have long-term effects on the microorganisms living in the gastrointestinal tract, which can be characterized by the fecal microbial flora.⁷ Some research has shown that changes in the structure of the microbiota in the gastrointestinal tract play a role in several conditions, such as

inflammatory bowel disease, type 2 diabetes, colon cancer, brain abnormalities,⁶ and multisystem organ failure.¹¹⁻¹³ For instance, colonic cancer, constipation, and inflammatory bowel disease can be influenced by the composition and metabolic activities of the microbiota.² Hence, certain food safety evaluations have been carried out on genetically modified organisms (GMOs) to identify the intestinal microbiota as a genetically modified organism (GMO) food safety index.⁷ Therefore, it is crucial to carefully monitor the composition of gut bacteria in order to achieve a better safety evaluation of genetically modified organisms (GMOs) with regard to their impact on gastric health.² Previous studies have indicated some differences between animals fed with GM products and the ones on a non-genetically modified (GM) diet. It has been proposed that genetically modified organisms (GMOs) might influence the health of animals in unknown ways.⁷ Furthermore, it has also been suggested that randomly inserting a transgene into the genome of animals may lead to some unintended changes.² With the advent of transgenic technology, scientists have been able to cultivate several genetically modified (GM) crops using Cry proteins extracted from *Bacillus thuringiensis* (Bt)-targeting pests.¹⁰ In the past 20 years, there have been numerous trials on animals aiming to assess the safety of GM crops cultivated with Cry proteins.¹⁰

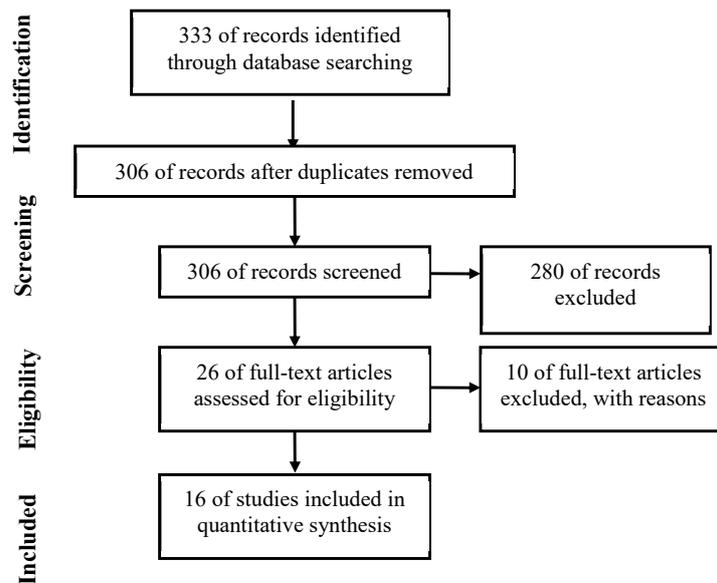
The present review focuses on previous studies in the field aiming to determine whether they reported any adverse effects on genetically modified (GM) crops about unintended changes and the gut microbiota count. To this end, we reviewed several experimental studies published in refereed scientific journals that involved feeding genetically modified (GM) crops to animals, rats and mice, pigs/sows, or poultry. Therefore, this study summarizes and discusses the observed effects of feeding genetically modified (GM) crops as reported in the literature.

Methods

Research strategy: The PICO elements for this study are P (problem): Genetically Modified Food; I (intervention): genetically modified (GM) feeding; C (comparison): Not genetically modified feeding; and O (outcomes of interest): Gut microbiota composition. A structured literature search was performed independently by three authors on Scopus, Web of Science, PubMed and Embase on 1 July 2019 (Table 1). In total, 333 publications were obtained by the search strategy, which decreased to 306 after omitting duplicates. Moreover, the review only included publications that were in English. The references included in the selected publications were used to find other peer-reviewed publications on the subject (“snowballing”). To access the quality of articles, we used the Cochrane checklist.

Table 1: Search strategy to study the impact of feeding GM crops on gut microbiota

Key words	
Gastrointestinal Microbiome” OR “Gut Microbiome” OR “Gut Microflora” OR “Gut Microbiota” OR “Gastrointestinal Flora” OR “Gut Flora” OR “Gastrointestinal Microbiota” OR “Gastrointestinal Microbial Community” OR “Gastrointestinal Microflora” OR “Gastric Microbiome” OR “Intestinal Microbiome” OR “Intestinal Microbiota” OR “Intestinal Microflora” OR “Intestinal Flora” OR “Enteric Bacteria	Genetically Modified Food” OR “GMO Food” OR “Genetically Modified Plant” OR “GMO Plant” OR “Genetically Engineered Plant” OR “Transgenic Plant” OR “Genetically Modified Organism” OR “GMO Organism” OR “Genetically Engineered Organism” OR “Transgenic Organism” OR “Genetically Modified crop” OR “Genetically engineered crop” OR “Genetic manipulated crop” OR “Transgenic crop” OR “transgenic food” OR “genetically altered food” OR “genetically altered crop” OR “genetically altered plant” OR “Bioengineered food” OR “Bioengineered crop” OR “Bioengineered plant

**Figure 1:** Flowchart of information through the different phases of a systematic review

The collected articles were considered and scored independently by three authors and each publication which had the eligibility criteria was included in our review.

Inclusion criteria: All experimental animal studies which assess the impact of genetically modified (GM) crops on the gut microbiota.

Exclusion criteria: Experiments without control group, the Cochran quality scale under 7 articles which were written in Persian or non-English language and articles the full text of which was not found.

In this regard, abstract screening was used to select 26 relevant publications. After the review of the full texts was done, 16 studies were included (Figure 1).

Results

We reviewed 16 scientific publications up to 1st of July 2019, which had our inclusion criteria. The characteristics of the articles are shown in Table 2. Ten publications (62.5%) used 50% or more genetically modified (GM) diet in the treatment group. The duration of treatment varied from 2 to 420 days. In 11 studies (68.7%), the duration of animal feeding was 90 days or more. Nine studies were conducted on rats, 4 on pigs and saw, 2 on broilers, and 1 on mice. In all, 8 studies fed their

animals with genetically modified rice, 3 maize, 2 corn, 1 apple, 1 soybean, and 1 canola. Most of the studies (75%) were performed in China, and a few of them were done in Ireland (3 studies) and New Zealand (1 study). All 3 studies in Ireland were done on pigs, all of which were fed with maize. One study in New Zealand was done on mice fed by apple. One experimental group included animals from multigenerational studies. Only 2 multigenerational studies were conducted on *Bacillus thuringiensis* (Bt) maize in saws¹⁴ and on rice in pigs.¹⁰ Results are shown briefly in Table 3 and reported separately based on bacteria family.

Enterobacteriaceae

Liu et al., Yuan et al. and Buzoianu et al. showed that there was a larger number of Enterobacteriaceae in the genetically modified (GM) group as compared with the non-genetically modified (GM) group.^{2, 10, 14} In Xu et al.'s study, the number of *Escherichia coli* (*E. coli*) was increased in non-genetically modified (GM) group, in contrast with the genetically modified (GM) one.¹⁵

In contrast to the mentioned studies, in the Zou et al.'s study, there was a significant reduction in all groups about the relative abundance of the *Escherichia coli* (*E. coli*) subgroup in males and

Table 2: Summary of the characteristics of studies reporting the effect of genetically modified (GM) crops on gut microbiota

Author	Year	Country	Sample size	Animal	Sex	Study duration	Genetically modified organisms (GMOs) Crop	Percent of genetically modified organisms (GMOs) in diet	
1	Wentao Xu	2011	China	36 male and 36 female. divided into 6 groups randomly (n=6 per group),	Sprague–Dawley rats	M/F	90 days	Rice	30%, 50%, 70%
2	Yanfang Yuan	2011	China	36 rats (18 male and 18 female) six groups (six rats per group)	Sprague–Dawley (SD) rats	M/F	90 days	T2A-1 rice	70%
3	Stefan G. Buzoianu	2012	Ireland,	40 (case=9 , control=8)	pigs	M	110 days	Maize	38.88%
4	Stefan G. Buzoianu	2012	Ireland,	18 (9 each treatment)	Crossbred pigs	M	31 days	Maize	38.88%
5	Stefan G. Buzoianu	2013	Ireland	9 non- genetically modified organism (GMO), 10 genetically modified organism (GMO)	The saw	M/F	36 weeks	Maize	38.88
6	Yanfang Yuan	2013	China	84 animals divided into 14 groups (six rats/group)	Rat	M/F	90 days	T2A-1 rice	70%
7	Richard V. Espley	2014	New Zealand	30 (each group n=10)	Swiss mouse	M	trial 1=7 days, trial 2=21 days	Apple	20%
8	Xiaozhe Qi	2014	China	Three groups (10 rats/sex/ group).	Sprague- Dawley rat	M/F	90 days	4-114-7 rice	50%
9	Lin Lu	2015	China	160, 20 cages (8 chicks per cage) with 10 cages (replicates) for each treatment.	Arbor Acres commercial male broilers	M	42 days	Corn (PTC)	54.0% during 1–21 days and 61.0% during 22–42 days
10	Kai Zhao	2016	China	Sixty (three groups, with 20 rats in each group (10 male and 10 female per treatment	SPF Sprague Dawley rats	M/F	90 days	Rice TIC-1	60%
11	Tianqi Lang	2017	China	140 (70 male and 70 female). 7 groups 20 rats/group (10 rats per sex).	Sprague- Dawley (SD) rats for	M/F	90 days	Canola RF3	2.5, 5 and 10% (w/w)
12	Penggao Li,	2017	China	Forty (20 female and 20 male)	Sprague- Dawley (SD) rats	M/F	10 weeks	Corn	75.0%, 76.5% and 76.2%,
13	Geng Lili	2018	China	140 (two groups)	Arbor Acres broilers	F	42 days	Rice TT51	100%
14	Qiang Liu,	2018	China	26 pigs (13 females and 13 males) (f0F control group, n=14) and a case group (f0Z group, n=12), 27 offspring of the first generation (f1). control group (f1F group, n=10) and a case group (f1Z group, n=17)	Inbred Wuzhishan pigs	M/F	Fed for f0=360 and f1=420 days	MH86 rice	56-66% by mass based on the composition of rice
15	Shiyong Zou	2018	China	140 Seven groups	Sprague- Dawley (SD) rat	M/F	90 days	DP-356043 soybeans	7.5%, 15% and 30% wt/wt
16	Xueqin Zhang	2019	China	140 (Seventy male and seventy female)- 7 groups (10 rats/sex/group)	Sprague- Dawley rats	M/F	90 days	Rice Lac-3	17.5, 35 and 70%

females by the end of the first month (by about 10%) as compared with the baseline, and the reduction continued up to the end of the study. However, these changes were not significant between the groups.⁷ Another study by Buzoianu et al.¹⁶ has shown that being fed with genetically modified (GM) maize has had no effect on any of the counts of the cultivable

bacteria from this family, whether enumerated in the feces, ileum, or caecum. There has also not been any significant impact on the composition of the caecal microbiota. According to the findings, it is safe to feed genetically modified (GM) maize to pigs in terms of the effects it has on their intestinal microbiota.

Table 3: The impact of feeding GM crops on animal gut microbiota

Author	Year	Method	Sample region	Bacteria family	Results
1 Wentao Xu	2011	Real-time quantitative PCR (RQ-PCR) based on genus-specific 16s rDNA primers	Cecal sample	Enterobacteriaacea Lactobacillaceae Clostridiaceae Prevotellaceae Rikenellaceae Bifidobacteriaceae Enterococcaceae total anaerobes	These results suggested that genetically modified (GM) had a complex effect on caecal microflora that may be related to the health of the host
2 Yanfang Yuan	2011	"1- real-time quantification method (RM)"	Fecal sample	Enterobacteriaacea Lactobacillaceae Clostridiaceae Bifidobacteriaceae Enterococcaceae total anaerobes	No adverse effects on the numbers of specific bacteria in rat faeces were observed as a result of GMR feeding
3 Stefan G. Buzoianu	2012	Culture-based methods	Fecal, cecal, ilial sample	Enterobacteriaacea Lactobacillaceae total anaerobes	Did not affect counts of any of the culturable bacteria enumerated in the feces, ileum or cecum
4 Stefan G. Buzoianu	2012	"Sequencing of 16S rRNA tags (V4 region; 239 bp long"	Fecal, cecal, ilial sample	Enterobacteriaacea Lactobacillaceae total anaerobes	Some of the differences observed within the cecal microbiotas
5 Stefan G. Buzoianu	2013	16S rRNA gene sequencing	Fecal, cecal, ilial sample	Enterobacteriaacea Lactobacillaceae total anaerobes	Confirms the lack of adverse effects of genetically modified (GM) maize on the intestinal microbiota of pigs
6 Yanfang Yuan	2013	"16S rRNA genes and denaturing gradient gel electrophoresis (DGGE)"	Fecal sample	Lactobacillus salivarius, Bifidobacterium longum, Enterococcus faecalis, Escherichia coli and Clostridium butyricum	Comparable differences were observed in the bacterial composition from GI tract content
7 Richard V. Espley	2014	"Quantified by real-time PCR and cultures"	Colonic content	Total bacteria, Bacteroides-Prevotella-Porphyrmonas group, Bifidobacterium spp., Lactobacillus spp.	High-flavonoid apple was associated with changes in gut microbiota
8 Xiaozhe Qi	2014	16S rRNA gene	Fecal sample		Have microbiota profile but not count
9 Lin Lu	2015	"16S rRNA PCR-denaturing gradient gel electrophoresis (DGGE) culture-based methods"	Cecal, ilial sample	Enterobacteriaacea Lactobacillaceae total anaerobes, total aerobes	No adverse effect on the quantity and diversity of gut microorganisms
10 Kai Zhao	2016	"16S rRNA genes and denaturing gradient gel electrophoresis (DGGE)"	Fecal sample	<i>Salmonella</i> , <i>Lactobacilli</i> , <i>Streptococcus</i> , <i>E. coli</i>	Cluster analysis of DGGE profiles revealed significant individual differences in the rats'
11 Tianqi Lang	2017	V4 region of the 16S rRNA was amplified by PCR and then sequenced with a MiSeq platform	Fecal sample	<i>Microbacterium</i> <i>Staphylococcus</i> <i>Clostridium</i> <i>Paracoccus</i> <i>Acinetobacter</i> <i>Psychrobacter</i> <i>Lactobacillus</i> or <i>Lactococcus</i> <i>Bifidobacterium</i> <i>Escherichia coli</i> (<i>E. coli</i>) or <i>Proteus</i>	Diets containing genetically modified (GM) canola did not disturb the balance of gut microbiota
12 Penggao Li,	2017	"V3-V4 regions of the bacterial 16S ribosomal RNA Illumina MiSeq Sequencing"	Fecal sample	<i>Lactobacillus</i> , <i>Barnesiella</i> , <i>Ruminococcus</i> , <i>Bacteroides</i> , <i>Clostridium XI</i> and <i>Clostridium XIVa</i> , <i>Ruminococcus 2</i> , <i>Enterococcus</i> , <i>Streptococcus</i> and <i>Oscillibacter Tannerella</i>	Similar effects on the fecal microbiota were observed after consuming a genetically modified (GM) GM- and non- genetically modified (GM) -corn-based diet for long periods
13 Geng Lili	2018	Sequencing the 16S rRNA	Cecal sample	Enterobacteriaacea Lactobacillaceae total anaerobes, total aerobes	No adverse effects on the broiler intestinal microbiota
14 Qiang Liu,	2018	16S rRNA gene sequencing	Fecal sample	Lachnospiraceae, Rumino-coccaceae, Prevotellaceae, Christensenellaceae, Streptococcaceae, Lactobacillaceae Enterobacteriaceae, Pseudomonadaceae and Pasteurellaceae	Bacillus thuringiensis (Bt) rice consumption has no adverse effects on the 13 gut microbiota

Furthermore, another study by Buzoianu et al., Lang et al., and Lili et al. reported that fecal, ilea, and ceca counts of Enterobacteriaceae were not affected by *Bacillus thuringiensis* (Bt) maize exposure. No significant differences were observed in *Escherichia coli* (*E. coli*) or *Proteus*.¹⁷⁻¹⁹ However, in Buzoianu et al.'s study, the pigs fed with *Bacillus thuringiensis* (Bt) treatment showed a lower cecal abundance of Succinivibrionaceae than those fed with isogenic treatment (0.17% vs. 1.80%; $P=0.08$).¹⁷

Lactobacillaceae

In some studies, the *Lactobacillus* group had a higher count in non-genetically modified (GM) males at the end of the third month compared with baseline.^{7, 15, 20} In Espley et al.'s²¹ study, relative to the total bacteria count in the colon, there was a significantly larger number of *Lactobacillus* spp. in the control group on the 2nd day as compared with any other dietary treatment ($P<0.01$, diet; $P<0.01$, day; $P<0.01$, diet 3 day). Also, in the Zou et al.'s⁷ study, there were several differences (in the form of decrease in the genetically modified (GM)-fed group in the relative abundance of the *Lactobacillus* group among the males between genetically modified (GM) and non-GM groups.

The number of *Lactobacillus* was decreased in the non-genetically modified (GM) group, but not in the genetically modified (GM) group, which suggests that GM might be putting the intestinal tract at a health risk.¹⁵ In the Li et al.'s⁶ research, there was a significant increase in *Lactobacillus* in the group of female rats following genetically modified (GM) corn-feeding as compared with the standard diet, while *Lactobacillus* was the largest genus in all fecal samples (36.13 vs. 12.02%, $P<0.05$).

In a study conducted by Yuan et al.,² compared with the standard group, both GM and non-genetically modified (GM) rice-fed groups showed a significantly higher count of *Lactobacillus* in feces on the 30th day although the count was decreased on the 60th and 90th days. Overall, several studies showed that *Lactobacillus* was not affected by maize, rice, corn in rats, pigs and birds in genetically modified (GM) and non-genetically modified (GM)-fed groups.^{2, 11, 17-19, 22}

Although some studies demonstrated this effect happened in the first week of the study, the difference at the end of the study weeks was non-significant.¹¹ Also, some studies showed that these differences depended on the gender and study area of the gastrointestinal tract, but, overall, it seems they could not find significant differences.

Enterococcus

The genetically modified (GM) and non-genetically modified (GM) groups had many differences in terms

of the relative abundance of *Enterococcus* in the males in the Zou et al.'s study,⁷ and in genetically modified (GM)-fed animals it was lower in both genders. Yuan et al., Buzoianu et al., and Yuan et al.^{2, 11, 17} showed significantly higher numbers of *Enterococcus* in genetically modified (GM)-fed groups compared with non-genetically modified (GM) groups. This difference was also seen in the groups fed with corn, maize and rice, and was also significant in rats and pigs. In other words, the difference observed was not dependent on the animal, gender, or the type of feeding differences.^{11, 15, 17, 19}

Clostridiaceae

The studies of the Clostridiaceae genus showed that gender was important in differences in the number of bacteria. In the Li et al.'s study,⁶ Clostridium IV was significantly increased in the genetically modified (GM) group of male rats compared with the control group (6.2 vs. 1.68%, $P<0.05$); however, said it was not the case with the female group.

In another study, *Clostridium sensu stricto* was increased in the corn-fed groups of female rats, while it reduced in the males ($P<0.05$). There was a significant decrease in the relative abundance of the *Clostridium perfringens* subgroup in both males and females at the end of the first month (by approximately 10%) as compared with baseline, and the decrease continued up to the end of the study.⁷

Peptostreptococcaceae

In the Lili et al.'s study,¹¹ there were larger numbers of Peptostreptococcaceae in isogenic rice-fed broilers ($P=0.02$).

Thermoanaerobacteraceae

In Lili et al.'s study,¹⁸ among the more scarce families, there were higher levels of Thermoanaerobacteraceae in genetically modified (GM) rice-fed broilers ($P=0.03$).

Prevotellaceae

In the Xu et al.'s study,¹⁵ there was not any clear regular pattern for the genome copies of the *Bacteroides-Prevotella* group among 3 subjects in the genetically modified (GM) and non-genetically modified (GM) groups. Espley et al.'s²¹ study concluded that regarding the *Bacteroides-Prevotella-Porphyrromonas* group, diet ($P=0.03$) and day ($P=0.05$) had significant impacts. In this respect, there was a higher bacteria count in the mice fed with genetically modified (GM) crops as compared with those on the non-genetically modified (GM) diet. In Zou et al.'s⁷ study, at the baseline, there was a minor increase in the relative abundance of the *Bacteroides-Prevotella* group in the female groups as time passed, while a

significant decrease was observed in the relative abundance of the same bacteria in the male groups down to about 10% by the end of the first month and 20% by the end of the study.

Bifidobacteriaceae

In Xu et al. and Lang et al.'s studies,¹⁹ there was not any clear regular pattern for the genome copies of the *Bifidobacterium* group among 3 subjects in the genetically modified (GM) and non-genetically modified (GM) groups.¹⁹ Buzoianu et al.¹⁷ reported a low prevalence of the Bifidobacteriaceae group, as they were only observed in the ceca of two pigs fed with the isogenic maize diet and five pigs fed with the genetically modified (GM) maize diet. Based on this finding, they concluded that the abundance of Bifidobacteriaceae tends to be higher in the ceca of pigs that received the genetically modified (GM) treatment than the pigs which received the isogenic treatment ($P=0.06$). Espley et al.'s²¹ study revealed a significantly higher level of *Bifidobacterium* spp. in the mice that received RG-F and Royal Gala apple flesh and peeled (RG-FP) diets as compared with any other diet in the experiment ($P<0.01$, diet; $P<0.01$, day; $P<0.01$, diet 3 day).²¹ In Li et al.'s study,⁶ there was also a significant increase in *Bifidobacterium* in the group of female rats fed with the GM diet (2.17% vs. 0.13%, $P<0.05$). In Zou, et al.'s⁷ study, a different trend of diversity was observed for the beneficial bacteria from the *Bifidobacterium* genus. In this regard, there was a significant increase of 1.2 fold in the relative abundance of bacteria from the *Bifidobacterium* genus in the genetically modified (GM) and non-genetically modified (GM) groups at the end of the study compared with baseline. Furthermore, there was a similar final abundance of bacteria from the *Bifidobacterium* genus between the males and females.

Barnesiellac (Porphyromonadaceae Family)

There was also an increase in the second largest genus in Li et al.'s study,⁶ *Barnesiellac*, following corn-feeding, and the increase was significant in the group of female rats on the genetically modified (GM) diet (23.42 vs. 9.31, $P<0.05$), while a significant decrease was observed in *Tannerella* in the same group (1.03 vs. 7.90%, $P<0.05$), but not in the males.

Akkermansiaceae (Verrucomicrobia)

Li et al.⁶ reported an increased level of the *Akkermansia* genus from the Verrucomicrobia phylum in corn-fed groups of male rats, which was significant in the non-genetically modified organism (GMO) group (5.74 vs. 0.32%, $P<0.05$). However, in male animals, the proportion of Firmicutes in the genetically modified organism (GMO) group decreased significantly compared with the group which received the standard diet (59.59 vs. 80.25%,

$P<0.05$). There was also a significant increase in the proportions of *Verrucomicrobia* and *Candidatus Saccharibacteria* in the non-genetically modified organisms (GMOs) group (5.74% and 1.85% vs. 0.32% and 0.19%, respectively, $P<0.05$).

Total Bacteria

In Lili et al.'s study,¹⁸ no differences were observed in the total number of aerobes or anaerobes in the ceca of broilers on isogenic rice or *Bacillus thuringiensis* (Bt) rice diets for 42 days ($P>0.05$). Moreover, there were no significant differences between broilers-fed *Bacillus thuringiensis* (Bt) -rice and isogenic-rice diets. There were also no significant differences in the relative abundance of bacterial phyla in the cecum of broilers on *Bacillus thuringiensis* (Bt)-rice and isogenic rice diets. Overall, 31 bacterial families were detected in the DNA found in the cecal contents of broilers. Furthermore, there were not any significant differences in the relative abundance of the major families under study in the cecal contents of isogenic and *Bacillus thuringiensis* (Bt) rice-fed broilers.¹⁸ In Zou, et al.'s study,⁷ the male group fed with genetically modified (GM) soy beans had different gut microbiota than the non-genetically modified (GM) male group. Meanwhile, with regard to other bacterium types, the trends of abundance were similar between GM and non-GM groups. Furthermore, no significant differences were observed between the male and female or genetically modified (GM) and non-genetically modified (GM) groups. In a study carried out by Buzoianu et al.,¹⁷ there were no significant differences in the relative abundance of bacterial phyla in the cecal contents of pigs fed with *Bacillus thuringiensis* (Bt) and isogenic maize diets. Also, no significant differences were observed between diets regarding the relative abundance of any major families. In addition, Xu et al. concluded that genetically modified (GM) diet had a complicated impact on caecal microflora, and associated this impact with the host's health.¹⁵

In another study by Buzoianu et al.,¹⁴ for many species, there were significant differences ($P<0.05$) between genetically modified (GM) maize-fed piglets and those in the control group during weaning, where genetically modified (GM) maize-fed piglets had a generally higher relative abundance. However, in the case of *Proteobacteria*, there was a lower relative abundance in genetically modified (GM) maize-fed piglets. Overall, authors reported that feeding GM maize to sows and their piglets influenced the intestinal microbiota. However, these were limited effects that had no relationship with any health problems in the sows and their piglets.¹⁴ The patterns of denaturing gradient gel electrophoresis (DGGE) indicated the predominance of bacteria in the ceca of piglets, which suggested that different diets had caused

major changes.¹¹ Analyzing the relative abundance of the bacteria under study revealed small changes in the B/E ratios (*Bifidobacterium/Enterococcus*) during all research stages and in all groups, with the ratios ranging between 1.19-1.33, which shows a proper balance in gastric health.¹¹

Through denaturing gradient gel electrophoresis (DGGE) analysis, Zhao et al.²³ analyzed nine samples over two weeks and found small differences in the fecal samples of both experimental and control groups during the same growth stage. In this regard, they found several common dominant bands, indicating the presence of dominant bacteria in every fecal sample. This finding also showed the low impact of *Bacillus thuringiensis* (Bt) transgenic rice on the dominant microorganisms that are vital for sustaining rats in the long term. A cluster analysis carried out on denaturing gradient gel electrophoresis (DGGE) patterns suggested that the development of rats had a bigger impact on the intestinal microbiota than the *Bacillus thuringiensis* (Bt) rice diet.²³ Espley et al.'s²¹ study showed a significantly lower total bacteria count for the mice in the control group compared with the mice that received genetically modified (GM) diets. For the mice in the control group, a significantly higher total bacteria count was observed after 7 feeding days as compared with after 21 days. Lu et al.²² arrived at the possibility that genetically modified (GM) diet might not have an adverse impact on microflora in the ileum and cecum. Furthermore, no significant differences ($P > 0.05$) were observed between birds fed with genetically modified (GM) corn and those in the control group in terms of aerobic and anaerobic bacteria count. Similar bands were detected in the ileum and cecum of birds in the genetically modified (GM) and non-genetically modified (GM) groups. In addition, there were also similar band numbers in the ileum or cecum ($P > 0.05$), which suggests that the two diets resulted in the presence of similar dominant microflora in the ileum or cecum.

Gender-related Studies

In another related study by Qi et al.,²⁴ in terms of bacterial diversity, a significantly different β -diversity was observed between the genetically modified (GM) and non-genetically modified (GM) groups of male rats on the 30th day and female rats on the 90th day ($P < 0.05$). However, the difference was not significant in the two genders simultaneously, which means that it was not associated with time. As to α -diversity, there were significant differences ($P < 0.05$) between the genetically modified (GM) and non-genetically modified (GM) groups of male rats on the 90th day. The same was also true for the non-genetically modified (GM) and control groups.

As to diet type, there were differences between genders with respect to the relative proportion of phyla

and genera. In the females, there were no any significant differences between the four groups in terms of the abundance of major phyla. Meanwhile, in the male groups, compared with the standard group, there was a significant reduction in the proportion of *Firmicutes* in the genetically modified organism (GMO) group (59.59 vs. 80.25%, $P < 0.05$); also, significant increases were observed in the proportions of *Verrucomicrobia* and *Candidatus Saccharibacteria* in the non-genetically modified organism (GMO) group (5.74% and 1.85% vs. 0.32% and 0.19%, respectively, $P < 0.05$).⁶ In a research by Zhang et al.,²⁵ on the 90th day, there were different gut microbiota structures in 70% of the genetically modified (GM) and 70% of the non-genetically modified (GM) groups of both genders compared with their respective control groups ($P < 0.05$). Furthermore, a significant difference was observed between the 70% of the genetically modified (GM) and the 70% of the non-genetically modified (GM) male groups with respect to the gut microbiota structure ($P < 0.05$), while no significant difference was detected between the two female groups ($P > 0.05$). Since there were no significant differences in the experimental and control groups of both male and female rats on the 90th day, it can be concluded that non-genetically modified (GM) and genetically modified (GM) rice diets did not cause any changes in the gut bacteria ($P > 0.05$). However, there were more obvious changes in the gut microbiota of male rats as compared with the females.²⁵

Discussion

In-vivo and *in-vitro* studies on animals have shown the significant effect of diet, age, gender, culture, lifestyle, and host genotype on the intestinal microbiota.¹⁹ The microbial composition of the gut varies in different parts of the digestive tract. The gut microbiota is composed mainly of anaerobes, which are approximately 2–3 times more than facultative anaerobes and aerobic bacteria, but most of the bacteria in the cecum are aerobic. In the small intestine, the predominant organisms are enterococci and lactobacilli.²⁶ In the studies in this review, all microbial isolates were anaerobic, and the *Lactobacillus* and *Enterococcus* families were common organisms. Similar results have been achieved using the conventional plate count method and the real-time PCR method. Hence, the method of counting would not be the case for discussion in this study.

Nutrition Contents of Food

As some studies have shown that carbohydrate compounds in transgenic and non-transgenic products can be different^{27, 28} and in the study of Buzoianu et al.,¹⁷ carbohydrate was more in transgenic crops, the increase in the population of Enterobacteriaceae and *Bifidobacteria* in the transgenic-fed group can be related to the difference in nutritional content.

In Espley et al.'s research²¹ that used apple as genetically modified (GM) food, there was a significant difference between bacterial species in the control group and the group fed with apples. It may be due to the fiber in apple. An association was made between fiber intake and the composition of gut microbiota by means of a decrease in the gut transit and pH. Fiber is considered the main source of fermentation for microbiota, thereby changing the gut microbiota.²⁹ The action of gut anaerobic bacteria on carbohydrates and their fermentation produces organic acids such as SCFA (acetate, propionate and butyrate), as well as the production of gases such as carbon dioxide and hydrogen, which have a significant impact on the pH of GIT contents. In addition to this change in the pH value, the gastric bacteria composition will also be influenced by the GIT environment. Furthermore, different bacterial compositions will affect the pH value, as well as SCFA characteristics.¹¹ However, in another study, there were significantly higher counts of *Lactobacillus* and *Enterococcus* in the feces of rice-fed animals on the 30th day. *Lactobacillus*, a beneficial bacterium can use glucose and lactose and produce lactic acid to prevent the growth of pathogenic bacteria in the gut and also has an important role in the immune system. Based on studies, it can be claimed that the number and diversity of *Lactobacillus* species vary with the type of food and diet.¹⁵

In the case of transgenic apple, given the genetic alteration in fruit color, coding genes increases anthocyanosides in fruit. In the study of Tugba Ozdal,³⁰ anthocyanosides increased the population of *Bifidobacteria*, *Lactobacilli* and whole intestinal bacteria in humans. As to total bacteria, the results are consistent with those of the study of Espley et al.²¹ However, the results are not the same for *Bifidobacteria* and the *Lactobacilli* family. This variation may be due to differences between humans and animals. On the other hand, gastrointestinal bacteria are decreased by flavonoids augmentation. Therefore, the researcher should carefully analyze the macro- and micro-nutrients of transgenic foods before preparing them.

In Zou, et al.'s research,⁷ the only study that has been done on soybeans as genetically modified (GM) food, soybean had a considerable effect on the increasing frequency of *Biphidiobacterium*, *Clostridium*, *Escherichia coli* (*E. coli*) and *Bordetella* groups compared with the control group.

Duration of Study

In Buzoianu et al.'s research¹⁶ that studied two generations, no significant difference was observed between bacterial species in the parental and newborn groups. Long-term use of Gm could cause changes in the number and species diversity of bacteria in the gut. For example, in Buzoianu et al.'s study,¹⁴ it was shown that a group fed with Gm maize totally

had a higher prevalence of microbiota existing in the gut than the control group (except *Proteobacteria*). Research on the impact of *Bacillus thuringiensis* (Bt) maize diets on the gut microbiota is limited to studies showing that the hosts' gut microbiota has had great tolerance for *Bacillus thuringiensis* (Bt) maize. However, this increasing rate could be due to long-term feeding with Gm.¹⁷

Gender Differences

Although in some animal and human studies such as Mueller et al.'s study³¹ it has been found that microbial flora of the two genders are different, in the research reviewed in a recent study, there was no significant difference between genders, due to non-drug group. For this reason, the use of two genders or each of them can be done with no change in the results of studies. However, according to a series of observations, if the study used both genders, it is recommended that each gender should be compared with itself for data analysis. In the study by Adriana Cabal et al.,³² it was emphasized that nutrition, age, geography, genetics, and lifestyle can have more impact than gender differences.

In Zhang et al.'s research,²⁵ changes in the gut microbiota of male rats compared with female rats have been evident. Totally, more indicators are changing in male rats compared with female rats. This is because male rats have higher sensitivity in the growth period compared with female ones. Several studies have demonstrated that gender influences gut microbiota composition.³³

Body Composition

In a study that discussed bacteria based on their diversity,²⁴ there were some significant differences in microbiota between the genetically modified (GM) group and the control group. These differences in male rats happened on the 30th day, and in female rats it happened on the 90th day. It could be because of the difference in BMI of animals that these two factors are effective for bacterial diversity. Moreover, it seemed that total body fat content also had an impact on the gut microbiota diversity and composition.²⁹ For example, in a study conducted in the USA, it was shown that the BMI in female animals was related to the overall gut microbiota. Also, in another study carried out in Spain, it was reported that the microbiota explained 31.17%.⁸ A study in China found a significantly higher α -variability in the intestinal microbiota of the low-weight group as compared with other weight groups; however, this only applied to women. At the genus level, there was a higher abundance of *Bifidobacterium*, *Coprococcus* and *Dialister*, as well as a significantly higher abundance of *Phascolarctobacterium*, in obese women, while a higher frequency of *Fusobacterium* was observed in obese men.²⁹

Animal Model

Data analysis of different animal models showed that the most changes in the microbial flora were in the chicken and the least in the rat. Since studies by Flemer et al.³⁴ have shown that rats have the highest microbial resemblance to humans, their reproduction would be faster than pigs and chicks, so rats are suggested to be better animal models than other animals in future similar studies.

Conclusion

Based on our literature review, we claim that there is no significant difference in the gut microbiota between the control group and the group fed with the transgenic diet. Due to the low frequency of certain species and the inadequate information indicating gastric health, we had some trouble describing the differences between genetically modified (GM) and non-genetically modified (GM) groups. In addition, most of the bacterial community residing in the rat gut is still unknown, and most of these bacteria are not able to culture. For future research, we recommend that the mechanism of action of GMR should be clarified in animal guts. However, it still provides invaluable data in GMR, whether it is beneficial for human health or hazardous.

Authors' Contribution

All co-authors have contributed significantly to the conception, design and execution of the experiments, analysis of the findings, and preparation and final approval of the manuscript.

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References

- 1 de Vos CJ, Swanenburg M. Health effects of feeding genetically modified (GM) crops to livestock animals: A review. *Food Chem Toxicol.* 2018;117:3-12. doi: 10.1016/j.fct.2017.08.031.

- 2 Yuan Y, Xu W, Luo Y, Liu H, Lu J, Su C, et al. Effects of genetically modified T2A-1 rice on faecal microflora of rats during 90 day supplementation. *J Sci Food Agric.* 2011;91(11):2066-72. doi: 10.1002/jsfa.4421.
- 3 Newell-McGloughlin M. Nutritionally improved agricultural crops. *Plant Physiol.* 2008;147(3):939-53. doi: 10.1104/pp.108.121947.
- 4 Zhang C, Wohlhueter R, Zhang H. Genetically modified foods: A critical review of their promise and problems. *Food Science and Human Wellness.* 2016;5(3):116-23. doi: 10.1016/j.fshw.2016.04.002.
- 5 Brookes G, Barfoot P. Environmental impacts of genetically modified (GM) crop use 1996–2018: impacts on pesticide use and carbon emissions. *GM Crops & Food.* 2020;11(4):215-41. doi: 10.1080/21645698.2020.1773198.
- 6 Ganesan M, Poluektova LY, Kharbanda KK, Osna NA. Liver as a target of human immunodeficiency virus infection. *World J Gastroenterol.* 2018; 24(42): 4728-37. doi: 10.3748/wjg.v24.i42.4728.
- 7 Zou S, Lu J, Luo Y, Qi X, Delaney B, Xu W, et al. The food safety of DP-356043 soybeans on SD rats reflected by physiological variables and fecal microbiota during a 90-day feeding study. *Regulatory Toxicology and Pharmacology.* 2018;97:144-51. doi: 10.1016/j.yrtph.2018.06.016.
- 8 Mohajeri MH, La Fata G, Steinert RE, Weber P. Relationship between the gut microbiome and brain function. *Nutr Rev.* 2018;76(7):481-96. doi: 10.1093/nutrit/nuy009. PMID: 29701810.
- 9 Liang D, Leung RK-K, Guan W, Au WW. Involvement of gut microbiome in human health and disease: brief overview, knowledge gaps and research opportunities. *Gut Pathogens.* 2018;10(1):3. doi: 10.1186/s13099-018-0230-4.
- 10 Liu Q, Yang W, Li M, Wang Y, Zhang H, Wu Y, et al. Effects of Transgenic Bt Rice Containing the Cry1Ab Protein on the Gastrointestinal Health of Highly Inbred Wuzhishan Pigs after Two Generations of Feeding. *J Agric Food Chem.* 2018;66(40):10575-87. doi: 10.1021/acs.jafc.8b02431.
- 11 Yuan Y, Xu W, He X, Liu H, Cao S, Qi X, et al. Effects of genetically modified T2A-1 rice on the GI health of rats after 90-day supplement. *Sci Rep.* 2013;3:1962. doi: 10.1038/srep01962.
- 12 Blandino G, Inturri R, Lazzara F, Di Rosa M, Malaguarnera L. Impact of gut microbiota on diabetes mellitus. *Diabetes Metab.* 2016;42(5):303-15. doi: 10.1016/j.diabet.2016.04.004.
- 13 Al Bander Z, Nitert MD, Mousa A, Naderpoor N. The Gut Microbiota and Inflammation: An Overview. *Int J Environ Res Public Health.* 2020;17(20):7618. doi: 10.3390/ijerph17207618.
- 14 Buzoianu SG, Walsh MC, Rea MC, Quigley L, O'Sullivan O, Cotter PD, et al. Sequence-based analysis of the intestinal Microbiota of sows and their offspring

- fed genetically modified maize expressing a truncated form of *Bacillus thuringiensis* Cry1Ab protein (Bt Maize). *Appl Environ Microbiol.* 2013;79(24):7735-44. doi: 10.1128/AEM.02937-13. PMID: 24096421. PMCID: PMC3837803.
- 15 Xu W, Li L, Lu J, Luo Y, Shang Y, Huang K. Analysis of Caecal Microbiota in Rats Fed with Genetically Modified Rice by Real-Time Quantitative PCR. *J Food Sci.* 2011;76(1):M88-M93. doi: 10.1111/j.1750-3841.2010.01967.x.
- 16 Buzoianu SG, Walsh MC, Rea MC, O'Sullivan O, Crispie F, Cotter PD, et al. The effect of feeding Bt MON810 maize to pigs for 110 days on intestinal microbiota. *PLoS one.* 2012;7(5):e33668. doi: 10.1371/journal.pone.0033668. PMID: 22574106. PMCID: PMC3344822.
- 17 Buzoianu SG, Walsh MC, Rea MC, O'Sullivan O, Cotter PD, Ross RP, et al. High-throughput sequence-based analysis of the intestinal microbiota of weanling pigs fed genetically modified MON810 maize expressing *Bacillus thuringiensis* Cry1Ab (Bt Maize) for 31 Days. *Appl Environ Microbiol.* 2012;78(12):4217-24. doi: 10.1128/AEM.00307-12.
- 18 Lili G, Deng X, Minhong Z, Changlong S, Jinghai F, Fuping S, et al. High-throughput sequencing-based analysis of the intestinal microbiota of broiler chickens fed genetically modified rice expressing Cry1Ac/Cry1Ab chimeric *Bacillus thuringiensis* protein. *J Poult Sci.* 2018;55(1):10-6. doi: 10.2141/jpsa.0170029. PMID: 32055151. PMCID: PMC6756379.
- 19 Lang T, Zou S, Huang K, Guo M, Liu X, He X. Safety assessment of transgenic canola RF3 with bar and barstar gene on Sprague-Dawley (SD) rats by 90-day feeding test. *Regul Toxicol Pharmacol.* 2017;91:226-34. doi: 10.1016/j.yrtph.2017.10.018. PMID: 29074276.
- 20 Buzoianu SG, Walsh MC, Rea MC, O'Sullivan O, Crispie F, Cotter PD, et al. The effect of feeding Bt MON810 maize to pigs for 110 days on intestinal microbiota. *PLoS One.* 2012;7(5):e33668. doi: 10.1371/journal.pone.0033668. PMID: 22574106. PMCID: PMC3344822.
- 21 Espley RV, Butts CA, Laing WA, Martell S, Smith H, McGhie TK, et al. Dietary flavonoids from modified apple reduce inflammation markers and modulate gut microbiota in mice. *J Nutr.* 2014;144(2):146-54. doi: 10.3945/jn.113.182659. PMID: 24353343.
- 22 Lu L, Guo J, Li S, Li A, Zhang L, Liu Z, et al. Influence of Phytase Transgenic Corn on the Intestinal Microflora and the Fate of Transgenic DNA and Protein in Digesta and Tissues of Broilers. *PLoS one.* 2015;10(11):e0143408. doi: 10.1371/journal.pone.0143408. PMID: 26599444. PMCID: PMC4657888.
- 23 Zhao K, Ren F, Han F, Liu Q, Wu G, Xu Y, et al. Edible safety assessment of genetically modified rice TIC-1 for Sprague Dawley rats through horizontal gene transfer, allergenicity and intestinal microbiota. *PLoS one.* 2016;11(10). doi: 10.1371/journal.pone.0163352. PMID: 27706188. PMCID: PMC5051820.
- 24 Qi X, Chen S, Sheng Y, Guo M, Liu Y, He X, et al. Safety assessment of genetically modified rice expressing human serum albumin from urine metabolomics and fecal bacterial profile. *Food Chem Toxicol.* 2015;76:1-10. doi: 10.1016/j.fct.2014.11.022. PMID: 25478734.
- 25 Zhang X, Zhang T, Shi J, Zou S, Guo M, He X, et al. A 90-day subchronic toxicology screen of genetically modified rice Lac-3 and its effects on the gut microbiota in Sprague-Dawley rats. *Regul Toxicol Pharmacol.* 2019;103:292-300. doi: 10.1016/j.yrtph.2019.02.005. PMID: 30738882.
- 26 Walsh CJ, Guinane CM, O'Toole PW, Cotter PD. Beneficial modulation of the gut microbiota. *FEBS letters.* 2014;588(22):4120-30. doi: 10.1016/j.febslet.2014.03.035. PMID: 24681100.
- 27 Warren FJ, Fukuma NM, Mikkelsen D, Flanagan BM, Williams BA, Lisle AT, et al. Food starch structure impacts gut microbiome composition. *MSphere.* 2018;3(3):e00086-18. doi: 10.1128/mSphere.00086-18. PMID: 29769378. PMCID: PMC5956147.
- 28 de Ronde JA, Laurie RN, Caetano T, Greyling MM, Kerepesi I. Comparative study between transgenic and non-transgenic soybean lines proved transgenic lines to be more drought tolerant. *Euphytica.* 2004;138(2):123-32. doi: 10.1023/B:EUPH.0000046806.68554.5b.
- 29 Kim YS, Unno T, Kim BY, Park MS. Sex Differences in Gut Microbiota. *World J Mens Health.* 2020;38(1):48-60. doi: 10.5534/wjmh.190009. PMID: 30929328. PMCID: PMC6920072.
- 30 Ozdal T, Sela DA, Xiao J, Boyacioglu D, Chen F, Capanoglu E. The reciprocal interactions between polyphenols and gut microbiota and effects on bioaccessibility. *Nutrients.* 2016;8(2):78. doi: 10.3390/nu8020078. PMCID: PMC4772042.
- 31 Mueller S, Saunier K, Hanisch C, Norin E, Alm L, Midtvedt T, et al. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. *Appl Environ Microbiol.* 2006;72(2):1027-33. doi: 10.1128/AEM.72.2.1027-1033.2006. PMID: 16461645. PMCID: PMC1392899.
- 32 Cabal A, Wassenaar TM, Ussery DW. Gender Differences in the Gut Microbiome and How These Affect Cardiovascular Diseases. *Gender Differences in the Pathogenesis and Management of Heart Disease.* 2018:89-100. doi: 10.1007/978-3-319-71135-5_7.
- 33 Fransen F, van Beek AA, Borghuis T, Meijer B, Hugenholtz F, van der Gaast-de Jongh C, et al. The impact of gut microbiota on gender-specific differences in immunity. *Front. Immunol.* 2017;8:754. doi: 10.3389/fimmu.2017.00754. PMID: 28713378. PMCID: PMC5491612.
- 34 Flemer B, Gaci N, Borrel G, Sanderson IR, Chaudhary PP, Tottey W, et al. Fecal microbiota variation across the lifespan of the healthy laboratory rat. *Gut microbes.* 2017;8(5):428-39. doi: 10.1080/19490976.2017.1334033. PMID: 28586297. PMCID: PMC5628645.