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Institute of Genetics and Animal Biotechnology
of the Polish Academy of Sciences, Jastrzębiec, Poland

The influence of GMO feed on ecosystem stability of the gastrointestinal tract in different species – a review

Agnieszka Korwin-Kossakowska*, Barbara Gralak, Grażyna Faliszewska, Ewa Karpiniak

Department of Genomics and Biodiversity, Institute of Genetics and Animal Biotechnology, Polish Academy of Sciences, Jastrzębiec, Postępu 36A, 05-552 Magdalenka, Poland

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Research on the human gut microbiota has been rapidly developing in recent years. Microbiota composition is now well recognized as linked to non-communicable chronic diseases and other health problems. However, science has not reached the point of understanding how specific changes in microbiota composition affect health and what represents a "healthy" microbiota. Transgenic plants are plants that have been genetically modified (GM) using recombinant DNA technology. These plants are mainly used as feedstuff. An important issue is connected with the persistence of recombinant DNA and 'novel' protein in the digestive tract and tissues of food-producing animals. The fate of DNA in the gastrointestinal tract of the animals has not been extensively investigated, probably due to the generally held dogma that food DNA could not resist low stomach pH and degradation by pancreatic nucleases and brush-border nucleosidases. The majority of DNA is really degraded within the animal's digestive system; however, this process is incomplete and some remaining small fragments of DNA can appear throughout the gastrointestinal tract. Fragments of DNA were detected in the contents of the small intestine, the cecum, the large intestine, or the feces of mice, also in muscle, liver, spleen, and kidney tissue in chickens. However, there were too small to transfer genetic information.

There is a relatively limited chance that transgenes will be transferred from GM plants to other Eukaryotes. However, with regard to microorganisms, it is theoretically possible. It is well established that bacteria possess sophisticated mechanisms for the acquisition and rearrangement of genetic material and thus the quantitative and qualitative composition of microbiota in different segments

*Corresponding authors: a.kossakowska@ighz.pl

of the digestive tract is changing. Another issue is the horizontal gene transfer with a particular focus on antibiotic resistance genes. Safety of incorporating antibiotic-resistance markers into GM plants has been a matter of public debate. In the past antibiotics were used in genetic modification as markers for the selection of successfully transformed organisms; however, these genes are not currently used.

KEYWORDS: antibiotic resistance / gastrointestinal tract / GMO / Horizontal Gene Transfer / microbiota

An opinion persists that foods derived from genetically modified (GM) crops could adversely alter gut microbiota. Three scenarios may be considered as related to the potential effects of GM food on the gut microbiota: the effect of the transgene product (for example, Bt toxin), unintended alteration of secondary metabolite profiles of GM plants, as well as residue of herbicides and adjuvants e (e.g., glyphosate) and their metabolites in herbicide-resistant crops [Genetically Engineering Crops: Experience and Prospects 2016].

Research on the human gut microbiota (the community of microorganisms that live in the digestive tract) has been developing dynamically. Based on their studies Dethlefsen and Relman [2011] and David *et al*. [2014] suggested that microbiota may undergo rapid changes under the influence of dietary components or antibiotic treatment. Microbiota composition and status are now well recognized to be linked to chronic non-communicable diseases, such as diabetes mellitus, chronic kidney disease as well as other health problems, so factors that cause either beneficial or adverse changes in the microbiota are of interest to researchers and clinicians. However, science has not reached the point of understanding how specific changes in microbiota composition affect health and what represents a "healthy" microbiota [Genetically Engineered Crops: Experiences and Prospects 2016]. The effect of different dietary patterns (e.g. high-fat versus high-carbohydrate diets) on the gut microbiota has been linked to metabolic syndromes [Ley, 2010].

Genetic modifications

Genetic engineering is the alteration of genetic material by direct intervention in genetic processes with the purpose of producing new substances or improving the functions of existing organisms. Transgenic plants are plants that have been genetically modified (GM) using recombinant DNA technology. This may be to express a gene that is not native to the plant or to modify endogenous genes. The protein encoded by this gene will confer a particular trait or characteristic to that plant. The technology may be applied in a number of ways, e.g. to engineer resistance to abiotic stresses, such as drought, extreme temperature or salinity, and biotic stresses, such as insects and pathogens, that would normally prove detrimental to plant growth or survival. The technology may also be used to improve the nutritional value of the plant, an application that could be of particular use in developing countries [Key *et al*. 2008].

GMO in animal nutrition

During the last few years, interest in food safety and the safety of animal production has been growing worldwide, including the EU. Animal feed is a major product of conventional agriculture along with crops developed using GM technology. The use of components and products from genetically modified plants in animal nutrition raises many questions and concerns, such as the role of a nutritional assessment of such modified feeds or feed additives (amino acids, vitamins) as part of safety assessment, the potential influence of genetically modified products on animal health and product quality. Other important issues are connected with the persistence of recombinant DNA and 'novel' protein in the digestive tract and tissues of food-producing animals [Flachowsky *et al.* 2005].

Fate of DNA in animal gastrointestinal tract

In humans the dietary intake of DNA ranges between 0.1-1 g per day and includes more or less degraded fragments of various genes of plant and animal origin, as well as bacterial DNA [Flachovsky *et al*. 2005]. The fate of DNA in the gastrointestinal tract of animals has not been extensively investigated, probably due to a generally held dogma that food DNA could not resist low stomach pH and degradation by pancreatic DNase [Nielsen and Daffonchio 2007]. In fact, the majority of DNA is really degraded within the animal digestive system; however, the process is incomplete and some remaining fragments of DNA can appear throughout the gastrointestinal tract. Also Schubert and co-workers [Schuber *et al*. 1997] showed that food-ingested DNA is not completely degraded in the gastrointestinal tract of mice. Fragments of alimentary DNA were detected in the contents of the small intestine, the cecum, the large intestine or the feces. In some of the investigated animals, DNA fragments of up to 472 bp were found in the blood. In other studies small DNA fragments (<200 bp) have been identified in lymphocytes of cows as well as internal organs of poultry, such as muscle, liver, spleen and kidney [Einspanier *et al.* 2001]. Nevertheless, DNA fragments smaller than 200 base pairs are generally considered to be too small to transmit genetic information. This situation occurs both in conventional and GM plants [Beever and Kemp 2000, Duggan *et al.* 2000 and 2003, Einspanier *et al*. 2001, Klotz *et al*. 2002, Netherwood *et al.* 2002, Aeschbacher *et al*. 2005, Mazza *et al*. 2005, Alexander *et al*. 2007].

In animal production, animals fed on GM crops ingest some amount of intact transgenic DNA. Netherwood and co-workers [2004] published the first study on the fate of transgenic soybean DNA in human volunteers. The volunteers were patients after ileostomy. The study showed that a small proportion of the ESPS (5-enolpyruvylshikimate-3-phosphate synthase) transgene of GM soya, similarly as native soya DNA, survives passage through the human upper gastrointestinal tract, but is completely degraded in the large intestine. The transgene, however, did not survive passage through the intact gastrointestinal tract of healthy human subjects

fed GM soya. Rizzi *et al*. [2012] noted that recombinant plant DNA fragments were detected in the gastrointestinal tract of nonruminant animals, but not detected in blood or other tissues, although some nonrecombinant plant DNA could still be found. The authors concluded that some natural plant DNA fragments persist in the lumen of the gastrointestinal tract and in the bloodstream of animals and humans.

Another aspect is connected with the incorporation of DNA (dietary or transgenic) into the recipient's genome.

Horizontal gene transfer

A major problem connected with the discussion on the "fate" of transgenic DNA in the digestive tract is related to the so-called horizontal (or lateral) gene transfer (HGT - Horizontal Gene Transfer). It is based on the stable transfer of genetic information from one organism to another, where the transferred genes are subject to fixation in the genome and are expressed [Eede *et al.* 2004]. Since GM crops were commercialised, concern has been voiced by some scientists and some members of the public that foreign DNA introduced into plants through genetic engineering technologies might, after ingestion, be transferred to the human gut microbiota and directly or indirectly into human somatic cells. Most of the concerns regarding horizontal gene transfer have been focused on antibiotic-resistance genes used as markers of the transgenesis [Genetically Engineered Crops: Experiences and Prospects 2016].

The horizontal gene transfer is theoretically possible even under natural conditions, but its probability depends on many factors, including the biological ability of a given sequence (necessary in the linear form of DNA), the existence of cells of the recipient organism in a state of competence (standby for adoption of "foreign" DNA) and the occurrence of such a homology of sequence, which facilitates integration of DNA into the recipient organism [Generic Issues Report 2006]. Finally, important is, if the incorporated gene is expressed, and the synthesis of a functional gene product occurs. This problem becomes particularly interesting in the context of genetically modified plants and the potential transfer of the transgene to a foreign organism.

Several studies on this subject have been conducted on different animal species, including chickens. They showed that the use of GMO products is as safe as the use of other foods. Several authors [e.g. Einspanier *et al*. 2001, Jennings *et al.* 2003, Kan and Hartnell 2004, Aeschbacher *et al.* 2005, McNaughton *et al.* 2007, Taylor *et al.* 2007, Świątkiewicz *et al.* 2011, Sieradzki *et al.* 2013] demonstrated the lack of a modified DNA in products derived from chickens fed with GMO feed. The transgenic DNA or its fragments from genetically modified plants have not been identified in any animal products. Similarly, the recombinant protein has never been identified in animal tissues [Einspanier 2013].

One of the studies on this subject was conducted in China and described in an article from 2013 [Ma *et al.* 2013]. The aim of that study was to assess the effects of long-term feeding with transgenic maize (phytase transgenic corn - PTC) on laying

performance and egg quality of hens, as well as investigate the fate of transgenic DNA and protein in digesta, blood, tissues, and eggs. Those authors indicated that the transgenic DNA and protein were rapidly degraded in the digestive tract and were detected neither in blood, tissues nor eggs. The performance of hens fed with diets containing transgenic maize was similar to that of hens fed with nontransgenic isogenic control maize [Ma *et al.* 2013]. Similar results, i.e. absence of the evaluated gene constructs in tissues and eggs of Japanese quails were shown in a study by Korwin-Kossakowska *et al.* [2016]. However, none of the above-mentioned authors performed in-depth studies on the processes occurring in the gastrointestinal tract and focused only on the final effects of feeding poultry with GMOs.

Horizontal gene transfer to bacterial genetic material

Various authors concluded that there is a relatively small chance that transgenes will be transferred from GM plants to other Eukaryotes [Thomson *et al.* 2001, Jennings *et al.* 2003, Mazza *et al*. 2005, Acosta *et al.* 2008, Świątkiewicz *et al.* 2011, Rizzi *et al.* 2012, Kees 2008]. However, with regard to microorganisms it is theoretically possible. It is well established that bacteria possess sophisticated mechanisms for the acquisition and rearrangement of genetic material. The possibility of horizontal gene transfer from transgenic plants to microbiota is a widely recognised risk factor [Mazza *et al.* 2005, EFSA 2006]. Although Netherwood *et al.* [2004] found some evidence of preexisting gene transfer between the GM soya and the human small intestinal microflora, bacteria containing the transgene represented a very small percentage of the microbial population, with no indication that the complete transgene had been transferred to the prokaryotes.

Also the report ”The Decade of EU-Funded GMO Research (2001-2010) [EC, 2010a] described a study that shows that rumen ciliates (a group of protozoans) exposed to Bt176 maize for 2 or 3 years did not incorporate the Bt176 transgene”. There are no reproducible examples of horizontal gene transfer of recombinant plant DNA into the human gastrointestinal microbiota or into human somatic cells. Three independent reviews of the literature on the topic [Van den Eede *et al.* 2004, Keese 2008, Brigulla and Wackernagel 2010] concluded that new gene acquisition by the gut bacteria through horizontal gene transfer would be rare and does not pose a health risk [Genetically Engineered Crops: Experiences and Prospects 2016]. Even if short DNA fragments were incorporated into the genome of bacteria, the fragments carry no valuable genetic information (as mentioned above), but they can change existing genetic information. Even a small genetic modification of bacterial DNA may significantly affect the characteristics of a particular bacterial strain needed to colonise the gastrointestinal tract and compete with other microorganisms for a place in a particular ecosystem.

Effect of GM on microbiota

The microbiota of gastrointestinal tract (GIT) is composed of huge numbers of different bacterial species, e.g. in birds it is about 650, wherein half of them have not yet been characterised [Apajalahti *et al.* 2004]. Microflora in the GIT has developed a number of protective, immune and metabolic functions, which altogether have an enormous impact on the nutrition and health status of the host [Mahabir and Pathak 2014].

Bacteria colonising the digestive tract create a unique ecosystem, in which the development of a single bacterial strain may increase or decrease chances of colonisation by other strains [Metges 2004]. Microbes have particularly quickly adapted to environmental changes. In every microbial population there are individuals called mutators that consistently produce a great degree of variability among their descendants. This variability is usually of no use, but it assumes adaptive value when there is a sudden and severe change in environmental conditions. Bacterial strains tend to adapt by genetic transfer between bacteria more often than by the mutation [Leveque and Mounolou 2003].

The quantitative and qualitative composition of microbiota in different segments of the digestive tract is changing according to the hygienic environmental conditions, the composition of the feed served and its daily consumption [Sawosz *et al.* 2007, Vali 2009].

Several publications have presented experimental studies, in which the influence of GM feed on the composition and activity of gastrointestinal tract (GIT) microbiota was investigated in different species. Tan *et al.* [2012] demonstrated the lack of an effect of 42-day feeding with GM soya on the intestinal microbiota of broilers. However, investigations by Czerwinski *et al*. [2017] indicated an effect of feeding with GM soybean meal and MON810 maize on the number of *Lactobacillales* in the end parts of the gastrointestinal tract of broilers compared to birds fed their conventional equivalents. The diversity of the order *Lactobacillales* in the ileum and caecum of birds fed GM maize was reduced, while that of *Lactobacillales* in the ileum and *Bifidobacteriales* in the caecum of birds fed GM soybean was greater compared with conventional maize and soya. Schroder *et al.* [2007] in their 90-day study showed that feeding of Bt rice (expressing the protein Cry1Ab) to Wistar rats had no effect on the total number of anaerobic and coliform bacteria, and *Lactobacillus* in feces, while the number of coliforms was higher in the jejunum and the number of *Bifidobacteria* was lower in the duodenum of animals fed with genetically modified Bt rice compared to conventional feed/rice. In turn, Yanfang *et al.* [2012] constructed an improved safety assessment animal model using rats and basic subchronic toxicity experiments, measuring a range of parameters including microflora composition, intestinal permeability, epithelial structure, fecal enzymes, bacterial activity and intestinal immunity. Significant differences were found between groups fed GM rice and control groups in terms of several parameters, whereas no differences were observed between genetically modified and non-genetically modified groups regarding the composition

and abundance of the microflora. Buzoianu *et al.* [2012, 2013] studied the effect of Bt maize feeding on microbiota composition in pigs. In their study, 110-day feeding of Bt maize (variety MON810) and of isogenic non-GM maize diets led to no differences in cultured *Enterobacteriaceae, Lactobacillus* and total anaerobes from the gut; 16S rRNA sequencing showed no differences in bacterial taxa, except for the genus Holdemania, with which no health effects are associated [Buzoianu *et al.* 2012]. In the follow-up study, in which the intestinal contents of sows and their offspring were examined with 16S rRNA gene sequencing, the only observed difference for major bacterial phyla was that Proteobacteria were less abundant in sows fed Bt maize before farrowing and in offspring at weaning compared with the controls [Buzoainu *et al.,* 2013]. Fecal Firmicutes were more abundant in offspring fed GM maize. Based on the overall results from these studies the authors concluded that none of the changes seen in the animals was expected to have biologically relevant health effects in the animals [Genetically Engineered Crops: Experiences and Prospects 2016].

Antibiotic resistance genes

Another issue is related to the horizontal gene transfer with a particular focus on antibiotic resistance genes. Safety of incorporating antibiotic-resistance markers into GM plants has been a matter of public debate. Concerns have been expressed that the release of these markers in GM plants may result in an increase in the rate of antibiotic resistance in human pathogens. Genetic modification of plants involves adding a specific stretch of DNA (bacterial also) into the plant's genome, giving it new or different characters. Fears have been expressed in relation to the possibility that antibiotic-resistance genes might be passed from GM plants to bacteria, thus creating bacteria that are resistant to antibiotics such as those used to treat common skin, ear, and eye infections [Guy and Gillespie 2005]. The presence of bacterial DNA in GM plants is unique to GM technology. Antibiotics were used in the past in genetic modification as markers for the selection of successfully transformed organisms in the initial steps of the generation of the genetically modified host organism and may increase the gene transfer risk. In the past, the most frequently used selectable marker in plant cell modification was the nptII gene, which encodes a neomycin phosphotransferase, an enzyme that inactivates the aminoglycoside antibiotics neomycin, kanamycin and paromomycin [Gay and Gillespie 2005]. Transfer of such a gene, if successful, may impair antibiotic therapy. As part of safety assessment of GM plants, a number of expert committees have examined whether the nptII gene in the Calgene FlavrSavr GM tomato or the ampicillin resistance marker in the Novartis Bt176 maize could be transferred from GM plants back to bacteria, thus becoming an additional source of antibiotic-resistant pathogens. Gebhard and Smalla [1998] reported data on marker-nptII gene rescue by *Acinetobacter* in experiments using DNA from GM sugar beet. De Vries *et al.* [2001] reported similar data for transgenic potatoes using *Acinetobacter* and *Pseudomonas stutzer.* Therefore, the nptII gene is no longer used. As Professor

Gillespie [Guy and Gillespie 2005] commented on the subject: "antibiotic-resistance markers do not pose a substantial risk to human health because the contribution that recombinant bacteria might make - should the enormous barriers to transfer be overcome - is so small that any contribution to antibiotic resistance made by GM plants must be overwhelmed by the contribution made by antibiotic prescription in clinical practice."

New era

Selectable marker genes are vital to the research and development of genetically modified crops. Numerous approaches to eliminate antibiotic and herbicide markers have been developed over the last several years and further improvements are now underway. Recently, researchers have described procedures to eliminate residual recognition sequences at recombination sites. Novel marker elimination strategies based on gene targeting and homologous recombination have been reported. Precision genome-editing technologies now facilitate insertion of single or multiple genes into one targeted location in the genome and thereby eliminate variation that is due to position effects. Such precision is expected to reduce unintended effects of gene insertion. With these developments concerns over an uncontrolled spread of antibiotic and herbicide resistance genes in the environment might become irrelevant in the future [ISAAA 2020].

Conclusions

Despite many previous studies, the use of genetically modified organisms is still highly controversial and raises consumers' concerns. The ongoing debate on the safety of products containing GMOs indicates that more research is needed, including the potential impact of GMO feed on processes occurring in animal organisms, especially the ecosystem of the gastrointestinal tract. According to skeptics, this situation is reflected in the quality of animal origin food products and consumer welfare. These concerns have given rise to a proposal of legislative changes concerning GMO feeds in Poland, which (in the case of their implementation) would become the strictest in Europe.

However, changes in the GIT ecosystem caused by the consumption of GMO feeds are much less significant than those caused by quantitative and qualitative changes in animal diets.

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