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Genetically modified feeds and their effect on the metabolic parameters of food-producing animals: a review of recent studies

Sylwester Swiatkiewicz[1], Malgorzata Swiatkiewicz[1], Anna Arczewska-Wlosek[1], Damian Jozefiak[2]

[1]National Research Institute of Animal Production, ul. Krakowska 1, 32-083 Balice, Poland

[2] Poznan University of Life Sciences, Department of Animal Nutrition and Feed Management Wolynska 33, 60-637 Poznan, Poland

Corresponding author: sylwester.swiatkiewicz@izoo.krakow.pl

ABSTRACT

The land area devoted to the cultivation of genetically modified (GM) plants has increased in recent years: in 2012 such plants were grown on over 170 million hectares globally, in 28 different countries, and are at present used by 17.3 million farmers worldwide. The majority of GM plants are used as feed material for food-producing farm animals. Despite the facts that GM plants have been used as feed for years and a number of feeding studies have proved their safety for animals, they still give rise to emotional public discussion. This paper reviews and discusses the results of recent experiments on the effects of feeds derived from genetically modified plants (GM feeds) on the physiological and metabolic indices of livestock, poultry and fish. The number of peer-reviewed papers on studies evaluating the influence of feeding food-producing animals with genetically modified materials is high. Most of these studies were carried out with GM plants with improved agronomic traits, i.e. herbicide-tolerant crops and crops protected against common pests; however, in some experiments, GM crops with enhanced nutritional properties were assessed. In the relevant part of these studies, not only production parameters but also different indices of the metabolic status of animals were analysed, and only a few minor differences with no biological relevance were found in livestock or poultry experiments. A greater number of minor effects on selected metabolic parameters were detected in fish studies; however, the causes of these differences were unclear and it is difficult to determine whether they were due to the genetic modification of the GM feed materials used. Since the results presented in the vast majority of experiments did not indicate any negative effects of GM materials, it can be concluded that commercialised transgenic crops can be safely fed to target food-producing animals without affecting metabolic indices or the quality of such products as meat, milk and eggs.

Keywords: genetically modified feeds, livestock, poultry, safety, health status, metabolic response

1. Introduction

Genetically modified (GM) plants, i.e. plants whose genetic material has been altered using genetic engineering techniques (mainly recombinant DNA technology), constitute a significant portion of the crops available on the feed market. In fact, all GM crops grown are transgenic plants, and thus each contains in its genome a DNA construct (transgen) originating from a foreign organism. Since the first GM plants appeared in 1996, the extent of world GM crop cultivation has increased by a factor of 94, reaching over 170 million hectares worldwide in 2012 (James, 2013).

The most common transgenic crops are soybean, maize, cotton and rapeseed (canola), and the most widespread traits introduced to plants by transgenesis are agrotechnic, i.e. herbicide tolerance and insect resistance. Majority (70-90%) of GM plants grown are used as feed material for food-producing farm animals (Flachowsky et al., 2012). Most soybean meal, an important source of protein for farm animals, available in world and European feed markets is produced from GM herbicide-tolerant (HT) plants (Sieradzki et al., 2006). About 35% of the global maize crop is GM, mainly insect resistant Bt maize (James, 2013). Even though GM plants have been grown and used for years, still are many scientists who are not in their favour. The main topics of this debate are the potential unintended and detrimental effects of transgenic DNA and expressed transgenic protein upon ingestion on metabolic processes in animals (the perceived risk of their toxicity or allergenicity or the possibility of inflammatory reaction induction) and, indirectly, through products originating from animals fed with GM crops, in humans (Bertoni and Marsan, 2005; Prescott and Hogan, 2006). As the majorities of farm-animal feeding studies with GM crops have focussed mainly on performance and have been relatively short-term, the scarcity of long-term or multigenerational experiments investigating the metabolic, health and reproduction parameters of target animals has been pointed out. Therefore the aim of this article is to discuss recent peer-reviewed papers on studies with target food- producing animals, where not only the influence of GM feeds on performance parameters but also their effects on the health, physiological and metabolic responses of livestock, poultry and fish were evaluated.

2. Effect of feeding GM feeds to food-producing animals

2.1 Health status, blood parameters and immunological characteristics

Data on the effect of GM feeds on animal health and blood parameters are rather marginally treated and insufficiently published in scientific literature, although concerns regarding potential toxicity of protein expressed by transgenic DNA still exist. Therefore, this knowledge should be deepen not only with theoretical reasons but especially applicative ones, because a reliable introduction of new forms of GM foods must be connected with the lack of any risk for human and animal health. The results of the experiments on the potential effect of GM feeds on health status, blood parameters, and immunological characteristics of food producing animals are summarised in Table 1.

In a series of Polish studies, HT soybean meal and Bt maize included separately or simultaneously in the diet did not negatively affect the health status and cellular immune response in broiler chickens and laying hens (Swiatkiewicz. et al., 2010a, b; Swiatkiewicz et al., 2011b; Bednarek et al., 2013). Kadlec et al. (2009) and Rehout et al. (2009) also found no effect of high dietary level of GM Bt (MON810) maize or HT (RR) soybean meal on blood haematological and biochemical indicators in broilers. Sartowska et al. (2012) too did not observe any negative effect of feeding HT soybean meal and Bt (MON810) maize on health indicators of two generations of Japanese quails. Flachowsky et al. (2005) also reported that a high dietary level of GM Bt 176 maize had no statistically significant effects on health status. In another study feeding of Bt maize did not impair the immune system of Japanese quails measured as specific and nonspecific immune response (Scholtz et al., 2010).

Most studies with feeding pigs with GM feeds have concerned Bt maize. Yonemochi et al. (2010) observed no adverse influence of dietary Bt (event CBH 351, StarLink) maize, as compared to conventional maize, on health status or blood hematological and biochemical values in fatteners. Only differences in blood urea nitrogen and blood glucose level were found between treatments; however, as it was indicated by the authors, the cause of these differences was unknown. Walsh et al. (2011) found no effect of short-term (31 days) feeding of Bt MON810 maize to weanling pigs on growth performance, however, in pigs fed diets containing Bt maize, a tendency toward decreased IL-12 and IFNc production from mitogen-stimulated peripheral mononuclear blood cells and proportion of CD4+ T cells in the spleen, along with increased IL-6 and IL-4 production from isolated splenocytes, was observed. In the ileum, the proportion of B cells and macrophages was reduced, while the proportion of CD4+ T cells was increased, in GM-maize-fed pigs. The authors concluded that dietary GM maize did not affect the growth indices of pigs, and the biological relevance of observed alterations in immune responses is questionable. They also speculated that Bt maize, being protected from insect damage, may contain a lower level of endotoxins, so the exposure to endotoxins from non-GM maize may be responsible for the elevated Th1 profile of cytokines in pigs fed non-GM maize. In a longer-term study Walsh et al. (2012b) indicated, based on the lack of antigen-specific antibody production and the absence of alterations in T-cell populations (i.e. CD3+, CD4+ and CD8+) and inflammatory cytokine production, that there was no evidence that long-term feeding of Bt maize to pigs could elicit an allergic or inflammatory-type peripheral immune response. The goal of study by Buzoianu et al. (2012a) was to investigate the effect of dietary Bt maize on health indices of 40-day-old pigs fed a non-GM or GM maize diet for 110 days, a non-GM maize diet for 30 days followed by a GM maize diet up to day 110, or a GM maize diet for 30 days followed by a non-GM maize diet up to day 110. Feeding with Bt maize did not affect serum biochemistry as of days 60 and 100 or on urine biochemistry as of day 110. There were some treatment × time interactions for serum urea, creatinine and aspartate aminotransferase; however, as indicated by the authors, the obtained values were all within normal reference intervals. Hence, they concluded that long-term feeding of Bt maize to pigs did not negatively affect health indices in pigs. The goal of a multigenerational experiment by the same authors (Buzoianu et al., 2012c) was to investigate the influence of feeding high levels of Bt maize to sows during gestation and lactation on immune function. The authors found that blood monocyte count and percentage were higher, but granulocyte percentage was lower, as of day 110 of gestation, while as of day 28 of lactation monocyte percentage, and as of day 110 of gestation, day 28 of lactation and overall, $CD4^+$ /$CD8^+$ lymphocyte ratios were lower in sows fed a diet with Bt maize. They also reported that the offspring of sows fed GM maize had lower leukocyte and granulocyte counts and percentage, with a higher lymphocyte percentage. There were no differences in cytokine production between experimental treatments. It was concluded that the obtained results regarding the effects of dietary GM Bt maize on immunological blood indices did not indicate inflammation or allergy and are unlikely to be of major biological importance (Buzoianu et al., 2012c). Walsh et al. (2012c) observed a tendency toward some effects of dietary Bt maize on blood biochemistry (serum total protein, urea, creatinine and γ-glutamyltransferase activity) and haematology (platelet count and mean cell Hb concentration) in sows and their offspring, however the authors indicated that the influence of feeding GM was minimal. Swiatkiewicz et al. (2013) found no effects of feeding with Bt (MON810) maize and Ht (RR) soybean meal on haematological indices of sows, which were located in a range typical for healthy adult swine, their reproductive characteristics, and offspring performance. As part of a Polish study with sows and fatteners, the cellular immune response of fatteners and sows fed with GM HT soybean meal and Bt maize was evaluated using the advanced flow cytometry technique (Bednarek et al., 2013). No significant changes in the peripheral leukogram (i.e. the percentage of differentiation of leukocyte subpopulations including LYM, PMNL, and MID) or lymphocyte immunophenotyping with a detailed classification of CD3, CD4, and CD8 (CD8a) positive were found; hence it was concluded that studied GM feed materials did not affect cellular immunity in pigs. Correspondingly, results of an in vitro experiment with porcine lymphocyte cultures indicated that the allergenic properties of globulins derived from GM HT soybeans are similar to those of conventional soybean proteins (Galbas et al., 2011).

In an experiment conducted by Carman et al. (2013) pigs were fed either a mixed GM HT soy (16-26% in the diet) and GM maize (a mixture of different Bt and HT varieties, 70-81%) diet or an equivalent non-GM diet in a long-term toxicology study. There were no differences between pigs fed the GM and non-GM diets regarding routine blood biochemistry measurements (glucose, AST, bilirubin, cholesterol, total protein, albumin, urea nitrogen, creatinine, P, Ca, Na, chloride, bicarbonate, creatine kinase, gamma-glutamyl transferase).

The effect of dietary HT (RR) soybean meal and Bt (MON810) maize on innate and specific immune response in calves was evaluated in a study by Bednarek et al. (2013). Authors indicated that full analysis of WBC components, among others lymphocyte immunophenotyping, indicates a lack of influence by GM feed on cellular immune response in the investigated animals. Similarly, the GM feed materials used in this study did not affect humoral immune response in calves, i.e. specific antibody titres (anti-BRS, PIV-3, BVDV) and acute phase proteins (SAA, Hp) resulted from vaccination against bovine respiratory syncytial virus (BRSV), bovine parainfluenza virus type 3 (PIV-3), and bovine viral diarrhoea virus (BVDV) (Bednarek et al., 2013). Similarly, Shimada et al. (2006) found no adverse effects from feeding with GM Bt11 maize on blood health indicators, i.e. haematological and biochemical parameters, or rumen functions of calves, hence indicating the likelihood that transgenic Bt11 maize is not harmful when fed to cattle. Trabalza-Marinucci et al. (2008) did not observe any effect of dietary GM Bt176 maize on health status of sheep (measured as haematological parameters, antioxidant defenses, lymphocyte proliferative capacity, phagocytosis and intracellular killing of macrophages) in a three-year study. Tripathi et al. (2011) found some effects of dietary Bt cottonseed on blood indicators in lambs; however, all biochemical and haematological values were within the normal range of reported variations for growing lambs and no adverse effect of Bt was noted. In a next study with feeding with Bt cottonseed, used as a replacement for conventional cottonseed or groundnut oil meal, they noted that immune status was not affected (Tripathi et al., 2012). Similar findings were reported by Anilkumar et al. (2010), who observed no adverse effects of GM Bt cottonseed on serum biochemical indices. Similarly, GM Bt cottonseed was nutritionally equivalent to conventional cottonseed and had no influence on health status, measured as blood haematological and biochemical indices, in lactating buffaloes (Singh et al., 2003). In a two-generation experiment with goats, the effects of feeding of GM HT (RR) soybean meal on cell metabolism were evaluated through the determination of several specific enzymes in the sera, hearts, skeletal muscles, livers and kidneys of goats and their offspring (Tudisco et al., 2010). There were no differences in body and organ weights of kids, while feeding goats with GM soybeans increased levels of lactic dehydrogenase in some tissues of kids, suggesting a rise in cell metabolism. However, the serum activities of all enzymes remained unaffected by treatment; hence the authors indicated that it would be overspeculative to claim that the HT soybean meal was responsible for a local increase in LDH metabolism (Tudisco et al., 2010). The dietary treatment had no effect on (gamma-glutamyl transferase) GGT activity in sera, while it had a greater effect in the kidneys and livers of kids from goats fed with GM soybean meal. The authors indicated that the increase of GGT activity in cells from both organs suggests a change in cell metabolism which leads to a higher synthesis. The significance of such an increase is not clear, and, as it was concluded by the authors, these results confirm the feeling that research concerning the effects of GM feeding is still far from over (Mastellone et al., 2013).

The aim of experiment by Sissener et al. (2009b) was to evaluate the effects of HT full-fat soybean meal, used as a feed ingredient at a high dietary inclusion level for Atlantic salmon, on health status (haematological parameters, clinical plasma chemistry, lysozyme levels and differential count of white blood cells). GM soybean meal had no significant effects on the majority of analysed indices, except that plasma triacylglycerol levels were higher, in the

GM-diet-fed fish. In the second part of this study it was observed that the high dietary inclusion level of GM soybean meal had no effect on blood haematological parameters, plasma enzymes, nutrients, or mRNA transcription of heat shock protein 27 in either the liver or distal intestine (Sissener et al., 2009a). Health status of Atlantic salmon fed with increasing dietary levels of Bt (MON810) maize was evaluated by Hemre et al. (2007). The means of mortality, normal ranges of blood parameters (apart from somewhat elevated ASAT values), and minor variations in organ sizes were considered good in all diet groups. Some of the biomarkers indicated minor effects from the GM maize, one being altered glucose transport in the intestine, the other altered maltase enzyme activity; however, the authors concluded that the GM Bt (MON810) maize was utilised well by Atlantic salmon, without profound effects on fish performance. In a subsequent study Sissener et al. (2011) found the effect of dietary Bt maize on regulation of some genes in the liver, combined with the up-regulation of anti-apoptotic protein NR13 and similar tendencies for ferritin heavy chain and MT-A and -B in the distal intestine, suggested some changes in cellular stress/antioxidant status. Since the Bt maize contained 90 μg/kg of deoxynivalenol, while the non-GM maize was below the detection limit, the authors indicated that it was difficult to determine whether the observed effects were caused by the deoxynivalenol level or by some other aspect of the GM maize ingredients.

2.2 Histopathological examination

Histopathological examination is a valid laboratory technique, indispensable in pathomorphological evaluation of side effects of vaccines, drugs, chemical compounds or even nutritional factors. The obtained results from Polish studies (Reichert et al., 2012), indicated that HT soybean meal fed alone or together with Bt maize to broiler chickens, laying hens, fattened pigs and calves do not negatively affect histological features of internal organs and muscles. Results of studies mentioned above and more chosen research dealing with histopathology are specified in Table 2.

Buzoianu et al. (2012a) investigated the effect of feeding non-GM or GM maize Bt on organ weight and intestinal histology of growing pigs fed a diet for 110 days. No significant influence of treatment was observed in organ function and any histological lesions existed. Similarly, no differences between pigs fed the GM and non-GM diets were noticed in stomach erosions or ulcerations in the experiment carried out by Carman et al. (2013) on pigs fed mixed GM HT soy and GM maize diet or an equivalent non-GM diet. However, the GM diet was associated with gastric and uterine differences in pigs. GM-fed pigs had uteri 25% heavier and a higher rate of severe stomach inflammation. The authors speculated that explanation for the inflammation results could be the effect of Cry proteins (Cry 3Bb1 and Cry 1Ab) in the Bt maize used in the experiment, which, being insecticides, induce perforation and disintegration of the gut tissue of certain insects that attack corn plants (Carman et al., 2013). Walsh et al. (2012a) concluded that the short-term feeding of MON810 maize to weaned pigs resulted in a tendency toward a decrease in goblet cells/mm in the duodenal villus, and an increase in kidney weight, without effects on duodenal, jejunal or ileal villus height, crypt death or villus height/crypt ratio, as well as without histopathological indicators of organ dysfunction.

No adverse effect of Bt cottonseed on tissue histopathology and organ weight of lambs was noticed by Tripathi et al. (2011, 2012) and on liver and kidney by Anilkumar et al. (2010). The histological analyses in a study with sheep, regarding the effects of a diet containing GM Bt176 maize, were conducted by Trabalza-Marinucci et al. (2008). Authors observed no negative influences of GM maize on histological features of tissues. Cytochemical analyses of the ruminal epithelium provided evidence of proliferative activation of basal cells, while electron microscopy analyses of the liver and pancreas revealed smaller cell nuclei containing increased amounts of heterochromatin and perichromatin granules in GM maize-fed sheep (Trabalza-Marinucci et al., 2008).

Recently Yang et al. (2014) indicated that feeding transgenic poplar leaves containing a chitinase-BmkIT transgene combination (a new pest-resistant gene source) caused no pathological changes in the histology of internal organs in rabbits. Electron microscopic observation showed that liver and renal cells were normal in rabbits fed transgenic feed and no different from the control group. No feed-derived chitinase, BmkIT or NPTII transgens were detected in small intestines, blood, or leg muscles.

Sissener et al. (2009b) reported that dietary GM (HT) full-fat soybean meal of analysed indices, except that the mid-intestine was smaller in Atlantic salmon, except that the mid-intestine was smaller. In the second part of this study (Sissener et al., 2009a), no effect was observed in the histomorphology of spleens, kidneys and mid-intestines in salmon fed with GM soybean meal, except decreased glycogen deposits in liver and lower mucosal fold height in the distal intestine at one of three sampling points. The authors concluded that although minor differences between the diet groups were found, GM soy did not appear to cause any adverse effects on organ morphology or stress response compared to non-GM soy (Sissener et al., 2009a). These results are supported by the minor effect of a high dietary level of RR soybean had on the abundance of individual proteins in the liver of salmon, indicating that GM soybean does not affect fish liver function. (Sissener et al., 2010).

2.3 Fate of transgenic DNA and proteins

One of the most important parts of the study on genetically modified feeds is the evaluation of transgenic DNA fate in the organism. Results of such experiments (Table 3) provide the answer to the consumers' concerns about the possible transfer of the transgene to products of animal origin or to human tissues. Korwin-Kossakowska et al. (2013) reported the results obtained for four generations of quails fed with GM HT soybean meal or Bt.

Performed analysis showed that no transgenic DNA was detectable in the birds' eggs, breast muscle or internal organs. The results of study carried out on broiler chickens and laying hens by Swiatkiewicz et al. (2010a, b) and Swiatkiewicz et al. (2011b) confirmed that transgenic DNA from GM HT (RR) soybean meal and Bt (MON810) maize is effectively hydrolysed. Results obtained in experiments with roosters and laying hens fed with GM maize containing transgenic Aspergillus niger phytase, warranted that transgenic phyA2 gene and protein are rapidly degraded in the digestive tract and are not detectable in birds' tissues or eggs (Gao et al., 2012; Gao et al., 2013; Ma et al., 2013).

Transgenic DNA and protein were not detectable in blood, liver or muscles of pigs obtaining Bt maize (event CBH 351, StarLink) in the diet (Yonemochi et al., 2010). The experiment by Walsh et al. (2011) determined the effect of 31-days trial carried out on weanling pigs fed with Bt MON810 maize. Transgenic DNA (cry1Ab) and protein were found only in the gastrointestinal digesta, not in the tissues (kidneys, liver, spleen, muscle, heart or blood). The aim of another study by the same authors was to evaluate the fate of the cry1Ab transgene and Bt protein in the digestive system of pigs (Walsh et al., 2012b). Transgenic DNA fragments were detected in gastric digesta, and with low frequency in the ileum, but were not found in the distal part of the gastrointestinal tract, unlike Bt protein fragments, which were present in the colon. However no cry1Ab-transgene or Bt-protein fragments were detected in the animals' organs or blood. Therefore the authors concluded that their findings can offer assurance to regulators and consumers as to the safety of long-term consumption of Bt maize (Walsh et al., 2012b). Neither transgenic DNA nor Cry1Ab-specific antibodies were found in sows or offspring in the experiment by Buzoianu et al. (2012c) who investigated the effect of feeding Bt (MON810) maize to sows during gestation and lactation on the fate of transgenic products in tissues of sows and their offspring. In the experiment by Swiatkiewicz et al. (2011a, 2013), sows and fatteners obtained the diet containing genetically modified HT

(RR) soybean meal and Bt maize (MON810) and the fate of transgenic DNA was evaluated. Transgenic DNA was detectable only in the content of the stomach and duodenum, but not in the intestinal digesta, blood or other examined organs.

Furgal-Dierzuk et al. (2014) observed that transgenic DNA fragments were detectable in rumen contents but not in intestinal digesta, blood or other examined organs of calves. Similarly, Paul et al. (2010) reported that transgenic Cry1Ab protein from Bt (MON810) maize is increasingly degraded during dairy cow digestion, thus its relative amount in feces is markedly reduced, indicating that Cry1Ab protein is no more stable than other feed proteins. Singhal et al. (2011) analysed the transgenic (Bt) protein levels in lactating multiparous cows fed with GM Bt (Bollgard II®) or non-genetically modified isogenic cottonseed. Analyses indicated that Bt proteins were not detected in milk or plasma samples. Similar results, indicating the safety of Bt cottonseed in dairy cows' nutrition, were obtained by Mohanta et al. (2010). Trabalza-Marinucci et al. (2008) concluded that after feeding of Bt176 maize to ewes and their progeny for 3 years, the transgenic DNA was not detectable in tissues, blood, ruminal fluid or ruminal bacteria. In the subsequent study authors evaluated the fate of transgenic DNA and the activity of GGT in blood and chosen organs from kids fed only milk from their mothers, which in turn were fed conventional or GM HT (RR) soybean meal (Mastellone et al., 2013). Small fragments of transgenic genes were found in livers, kidneys and blood (35S promoter) or livers and kidneys (CP4 epsps gene).

None of transgenic DNA fragments were detectable in the animal tissues in the experiment by Tudisco et al. (2006) who evaluated the effect of HT (RR) soybean meal on fate of genetically modified DNA in rabbits.

2.4 Microbiological status of animals

In some experiments additionally, to the molecular analysis of digesta from different parts of the gastrointestinal tract, content of ileum, cecum or/and colon were taken for microbiological tests (Table 4). The aim of the first of these studies, an in vitro batch fermentation model experiment, was to evaluate the possibility of transferring the cry1Ab transgene from GM maize to porcine jejunal microbiota (Buzoianu et al., 2011). A 211-bp fragment of transgene was detected by PCR in the GM maize used to spike the bioreactors with porcine jejunal microbiota, but was not detected in any of the bacteria recovered from the bioreactors. The authors concluded that transgenic DNA from Bt maize is rapidly degraded in an in vitro model of the porcine jejunum and is not transferred to jejunal microbiota (Buzoianu et al., 2011). The aim of another long-term experiment conducted by Buzoianu et al. (2012b) was to evaluate the influence of GM Bt (MON810) maize, fed to pigs for 110 days, on intestinal microbiota. No effects of GM maize on numbers of Enterobacteriaceae, Lactobacillus or total anaerobes in the feces or in the ileal and caecal digesta were found. Also, when high-throughput 16 S rRNA gene sequencing was used, no differences were found in any bacterial taxa between treatments, with the exception of the genus Holdemania. However, the authors indicated that, as the role of Holdemania is still under investigation and no health abnormalities were observed, this change is not likely to be of clinical significance. They concluded that no changes were observed within the caecal microbial community of healthy pigs following long-term exposure to GM Bt maize; hence the obtained results indicate that intestinal microbiota are tolerant to this maize and feeding Bt maize to pigs in the context of intestinal microbiota is safe (Buzoianu et al., 2012b). Similarly, no adverse effects of dietary GM Bt maize on the intestinal microbiota of pigs were found following trans-generational consumption, i.e. feeding Bt maize to sows and their offspring (Buzoianu et al., 2013b).

In a study by Tan et al. (2012), feeding with GM HT (RR) soybean meal did not adversely affect the broilers intestinal microflora population. Nor did GM Bt176 maize silage fed to rumen-cannulated cows influence the dynamics of six ruminal bacterial strains (Wiedemann et al., 2007). Similar results were obtained by Einspanier et al. (2004) and Brusetti et al. (2011) who showed no significant effects of dietary Bt maize on the bacterial diversity of cow rumen in vivo. In a longitudinal study with sheep, the effects of a diet containing GM Bt176 maize was determined (Trabalza-Marinucci et al., 2008). In this experiment no negative influences of treatment on ruminal microbial population characteristics and were noticed. Surprisingly, immune response to Salmonella abortus ovis vaccination was more efficient in GM maize-fed sheep.

2.5 Production performance, digestibility of nutrients, and quality of animal origin products

Since poultry studies are relatively inexpensive, many experiments with birds, especially broiler chickens, fed with GM materials have been carried out during the last 15 years; however, in most of them only nutritional equivalency, production parameters, and/or quality of animal origin products were evaluated. In a series of studies by Swiatkiewicz et al. (2010a), Stadnik et al. (2011b), Swiatkiewicz et al. (2011b), the production and metabolic effects of feeding GM HT (Rondup Ready, RR) soybean meal and Bt (MON810) maize to broiler chickens and laying hens were tested. Authors reported that poultry performance and meat quality indicators were not negatively affect by HT soybean meal and Bt maize included in the diet (Table 5). Surprisingly, it was even found that chickens which had consumed diets containing transgenic materials exhibited improved lipid stability of breast and thigh muscles, as indicated by TBARS values (Stadnik et al., 2011b); however, this effect can hardly be explained by the genetic modification of the GM feeds used. Kadlec et al. (2009) and Rehout et al. (2009) carried out the experiment on broilers fed a diet with high level of GM Bt

(MON810) maize or HT (RR) soybean meal. They reported similar findings which is lack of statistically significant differences in growth performance parameters and slaughter indices between groups. Dela Cruz et al. (2012) found no significant differences in carcass yield or organoleptic properties of the meat of broilers fed conventional and GM Bt or HT maize; however, birds fed a diet containing GM maize were characterised by slightly lower growth performance. In a study by Tan et al. (2012), feeding with GM HT (RR) soybean meal did not adversely affect the growth performance of broilers. There were also no differences in growth indices, mortality, carcass characteristics, or organ weights between chickens fed a diet containing both GM HT soybean meal and HT maize or stacked-trait (Bt + HT) maize and those fed diets without transgenic materials (McNaughton et al., 2011a, 2011b). Corresponding results were found in layers, where a diet containing transgenic HT soybean meal and HT corn, separately or in combination, had no effect on laying performance or egg quality indices (McNaughton et al., 2011c). In a study with laying hens, Rasmussen et al. (2007) found that a diet containing GM Bt maize (StarLink) did not negatively affect production or reproductive performance, i.e. egg production, egg weight, ovary weight, and the number of yolky (yellow) follicles, of laying hens. Similarly, Aeschbacher et al. (2005) found no differences in laying performance, digestibility characteristics, i.e. metabolizability of dietary energy, protein digestibility, and composition of eggs, of hens fed diets containing GM Bt176 or conventional maize. Similar results were obtained in a four-generation study with laying hens fed a diet containing Bt maize: no significant effects on egg production or hatchability were observed (Halle and Flachowsky, 2014). The performance, quality of eggs and meat, and reproductive rate of two generations of Japanese quails fed with HT soybean meal and Bt (MON810) maize was evaluated in study by Sartowska et al. (2012). Authors did not prove any negative effects of GM materials on production (livability, growth and laying performance, carcass characteristics) and reproductive (hatchability) performance. Some differences were reported in the chemical composition of breast muscle and egg yolk, but no clear effect of the experimental diet, i.e. genetic modification, was found; hence the authors concluded that feeding with GM HT soybean meal and Bt maize did not negatively affect the studied parameters of quails (Sartowska et al., 2012). In a subsequent article by the same group of authors, the results obtained for four generations of quails were reported (Korwin-Kossakowska et al., 2013). A high dietary level of GM HT soybean meal or Bt maize made no impact on growth or laying performance. These results are in agreement with the findings of an earlier study by Flachowsky et al. (2005), who reported that a high dietary level of GM Bt 176 maize had no significant effects on growth performance, egg production, slaughter indices in Japanese quails during a ten-generation experiment.

In recent years, experimentation using genetic engineering methods has resulted in the introduction of new biosynthetic pathways to plants and the production of several transgenic crops with substantial changes in chemical composition, referred to as second-generation GM crops. The main objective of such transgenesis was to increase the nutritional value of plants and, thus, of feed materials, by increasing the level of desirable substances, for example limiting amino acids, or decreasing the quantity of harmful compounds, such as phytate, in seeds (Swiatkiewicz and Arczewska-Wlosek, 2011). Clearly, in contrast to first-generation transgenic plants, the use of these GM plants as feed materials may have a positive impact on animal organisms. One of the most important examples of this kind of genetic modification is the improvement of phosphorus availability in crops through transgenesis resulting in the expression of transgenic phytase, the enzyme which hydrolyses phytate bonds, in seeds. Results obtained recently in experiments with roosters and laying hens indicated that feeding with GM maize containing transgenic Aspergillus niger phytase improved P availability and had no adverse effect on performance indices or egg quality (Gao et al., 2012; Gao et al., 2013; Ma et al., 2013). Genetic engineering methods were also used to modify the composition of the fatty acids of lipids in oilseed plants for industrial purposes. Mejia et al. (2010) evaluated the nutritional value of the GM DP-3O5423-1 soybean, containing an increased concentration of oleic acid and a reduced level of linoleic, linolenic and palmitic acid in the seeds' fat, for laying hens. The obtained results proved that hens fed diets containing DP-3O5423-1 GM soybean meal were characterised by egg performance and quality parameters similar to those of hens fed with conventional soybean meal.

Yonemochi et al. (2010) did not observe the negative effect of Bt maize (event CBH 351, StarLink) on growth performance of fatteners. The study by Buzoianu et al. (2012a) considered the effect of Bt maize on selected growth indices of 40-day-old pigs. Feeding with genetically modified maize did not affect growth performance and body composition. In a more recent study Buzoianu et al. (2013a) studied the influence of feeding Bt (MON810) maize to sows during gestation and lactation and to their offspring (from weaning to 115 d post-weaning) on offspring growth and health status. After weaning, offspring pigs were assigned to treatments: non-GM-maize-fed sow/non-GM-maize-fed offspring; non-GM-maize-fed sow/Bt-maize-fed offspring, Bt-maize-fed sow/non-GM-maize-fed offspring; Bt-maize-fed sow/Bt-maize-fed offspring. Offspring from Bt maize-fed sows were heavier than offspring from non-GM-maize-fed sows on d 30, 100, and 115 post-weaning, had higher carcass and lower spleen weights. Bt-maize-fed pigs were characterised by higher dressing percentages when compared to isogenic-maize-fed pigs. The authors concluded that transgenerational feeding of Bt maize diets has no detrimental effects on pig growth performance (Buzoianu et al., 2013a). The influence of a diet containing Bt (MON810) maize on maternal and offspring growth indices was estimated by Walsh et al. (2012c) in a 20-week study. The authors reported that GM-maize-fed sows were heavier on day 56 of gestation; however, their offspring tended to be lighter at weaning. The aim of a short-term study by these authors with male weanling pigs was to evaluate the effects of feeding Bt MON810 maize on animal performance (Walsh et al., 2012a). The pigs were assigned diets containing high levels of GM or non-GM isogenic parent line maize. It was observed that feeding of MON810 maize to weaned pigs caused increased feed consumption and less efficient conversion of feed to gain. Polish experiments with sows and fatteners evaluated the effect of genetically modified HT (RR) soybean meal and Bt maize (MON810), used separately or simultaneously as diet components. In the first of these experiments, Swiatkiewicz et al. (2011a) reported that GM soybean meal and maize grain had nutritional value similar to that of their conventional counterparts, and when fed together to pigs affected neither the growth performance of fatteners weighting from to 30 to 110 kg nor carcass and meat quality. In a study by the same group of authors (Swiatkiewicz et al., 2013), the effects of Bt (MON810) maize and Ht (RR) soybean meal in sows and piglets were evaluated. No differences between reproductive traits of sows fed GM and conventional materials, nor in their offspring's growth performance were observed. On the contrary, Piva et al. (2001) reported improved weight gains in piglets receiving Bt maize, and the authors indicated that the probable reason for improved performance was the higher quality of Bt-maize grain, which was less damaged by European corn borers and less contaminated with Fusarium mycotoxins. Similar results were obtained by Rossi et al. (2011), who found that weaned piglets fed with Bt maize performed better than piglets fed with near-conventional maize and suggested that this better performance was due to the lower FB1 mycotoxin content of Bt maize. Nor did HT soybean meal or Bt maize affect different physicochemical parameters of pork, except that fatteners fed with GM materials exhibited slightly decreased lipid stability in loins as indicated by TBARS values (Stadnik et al., 2011a). Carman et al. (2013) did not notice any differences between pigs fed the GM (a mixture of different Bt and HT varieties) and non-GM diets regarding feed intake, weight gain and mortality.

Based on the results of a long-term study (over 25 months) with dairy cows, Steinke et al. (2010) reported that feeding with GM Bt (MON810) maize did not adversely affect nutrient intake, milk yield, milk composition or body condition, so they indicated the safety and nutritional equivalence of Bt maize to that of its conventional isogenic counterpart (Table 3). In a recent study with calves, Furgal-Dierzuk et al. (2014) reported no adverse effect of feeding with GM HT (RR) soybean meal and/or Bt (MON810) maize on growth performance, chemical composition of meat. Singhal et al. (2011) compared dry matter intake, milk yield and milk composition of lactating multiparous cows fed diets containing GM Bt (Bollgard II®) or non-genetically modified isogenic cottonseed. Most of the analysed indices were not affected by dietary treatment; however, 4% fat-corrected milk production for cows fed with Bt cottonseed was significantly improved as compared with cows fed a conventional diet. The authors concluded that the evaluated Bt cottonseed could replace conventional cottonseed in dairy cattle diets without any adverse effects on milk composition. There were no negative influences of feeding with sheep for three years with diet containing GM Bt176 maize on performance and reproductive traits (Trabalza-Marinucci et al., 2008).

Tripathi et al. (2011) examinated the influence of GM Bt cottonseed containing Cry1Ac protein in lambs feeding but no differences in growth performance were noticed. However, in another trial they even found positive effects of feeding with Bt cottonseed, used as a replacement for conventional cottonseed or groundnut oil meal, on growth performance. In a study by Hartnell et al. (2005), feeding with GM HT (RR) fodder beets, sugar beets, and beet pulp did not adversely affect health status, growth performance or digestibility of nutrients in sheep. Similarly, there were no significant differences in growth indices, carcass characteristics or apparent digestibility of nutrients in lambs fed with GM HT (RR) or conventional canola meal (Stanford et al., 2003).

The goal of the work of Chrenkova et al. (2011) was to determine the effect of dietary GM Bt (MON88017) maize on nutrient digestibility, growth performance and mineral parameters, meat quality indices, and caecal fermentation pattern in rabbits. Based on the obtained results, the authors concluded that feeding with Bt maize had no negative influence on utilisation of nutrients and performance indices of animals or meat quality parameters. Similarly, Tudisco et al. (2006) observed no influence of GM soybeans on body weight of rabbits in the experiment considering the effect of HT (RR) soybean meal on growth performance. In the next trial on rabbits, Yang et al. (2014) concluded that feeding with poplar (Populus cathayana) leaves containing chitinase-BmkIT transgenes did not affect growth performance of animals.

The effects of GM HT (RR) full-fat soybean meal, used for Atlantic salmon diet (25%) on growth performance was tested by Sissener et al. (2009b). As the conclusions of this work, the authors indicated that fish fed a diet containing GM and conventional soybean meal had similar nutrient and health statuses, that the observed effects of a high dietary level of GM soybean were minor, and that such effects might be caused by variations in the soy strains rather than genetic modification per se (Sissener et al., 2009b). The goal of a study by Hemre et al. (2007) was to evaluate whether feeding with increasing dietary levels of GM Bt (MON810) maize (150-300 g/kg) maize, compared with diets containing conventional maize, would show any nutritional adverse effects on postmolt Atlantic salmon. Based on the obtained results, the authors indicated that high rates of growth, feed utilisation and dry matter, protein and lipid apparent digestibility coefficients were found in all diet groups.

3. Conclusions

Summing up, it should be emphasised that the number of published papers on studies which evaluated different effects of feeding with genetically modified materials on food-producing animals is high. Most of them were carried out with GM plants with improved agronomic traits, that is, with herbicide-tolerant crops and crops protected against common pests; however, in some experiments GM crops with enhanced nutritional properties were assessed. In the relevant part of these reviewed studies not only production parameters but also different indices of metabolic status of animals were analysed, and generally only a few minor treatment differences were found, with no biological relevance in livestock or poultry experiments. A greater number of minor effects in selected metabolic parameters were detected in fish studies; however, the causes for these differences were not clear and it is difficult to determine whether they were due to the genetic modification of the GM feed materials used. The results presented in the vast majority of experiments indicated no negative effects from GM materials; thus we conclude that commercialised transgenic crops can be safely used as feed for food-producing animals, without affecting metabolic indices or the properties of such products as meat, milk and eggs.

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Table 1. Effects of feeding genetically modified feeds on the health status, blood parameters, and immunological characteristics of food producing animals – results of selected experiments

Genetically modified feed material	Species Category	Studied characteristics	Results	Authors
HT soybean meal (26-39% in the diet) and Bt maize (54-62%), included individually or in combination to the diet	Broiler chickens, laying hens	Indicators of cellular immune response (immunopheno-typing of peripheral blood lymphocyte subpopulations).	No adverse effect of GM feed.	Bednarek et al. (2013)
Bt maize (30-35 % in the diet)	Broiler chickens	Blood haematology (hemoglobin, erythrocyte and leukocyte count) and biochemistry (total protein, γ-glutamyl transferase, alanine aminotransferase, and aspartate aminotransferase).	No adverse effect of GM feed.	Rehout et al. (2009)
Bt maize (30-35 % in the diet), HT soybean meal (33-39%)	Broiler chickens	Blood haematology (hemoglobin, erythrocyte and leukocyte count) and biochemistry (total protein, γ-glutamyl transferase, alanine aminotransferase, and aspartate aminotransferase).	No adverse effect of GM feed.	Kadlec et al. (2009)
Bt maize (30-35 % in the diet)	Japanese quails	Immune response after immunization using BSA	No adverse effect of GM feed.	Scholtz et al. (2010)
Bt maize (39 % in the diet)	Forty day old pigs in long-term study (110 days)	Peripheral immune response (cytokine and Cry1Ab-specific antibody production, immunophenotyping and haematological analysis).	Lack of antigen-specific antibody production and the absence of alterations in T cell populations and inflammatory cytokine production after feeding of Bt maize.	Walsh et al. (2012b)
Bt maize (87 and 74%, in the gestation and lactation diet, respectively)	Sows during gestation and lactation and their offspring	Blood haematology (number of erythrocytes, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, number of platelets and mean platelet volume) and biochemistry (aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, alkaline phosphatase, creatinine, urea and total protein).	Sows fed Bt maize: tendency to decreased serum total protein, and increased serum creatinine and γ-glutamyl transferase activity on day 28 of lactation, tendency to decreased serum urea on day 110 of gestation and in offspring at birth, tendency to decrease of platelet count and mean cell Hb concentration on day 110 of gestation, but to increased MCHC in offspring. As indicated by the authors, influence of feeding GM maize to sows on maternal and offspring serum blood indices was minimal.	Walsh et al. (2012c)
Bt maize (39-79 % in the diet)	Forty day old pigs in long-term study (110 days)	Biochemistry of serum (aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, alkaline phosphatase, creatinine, urea, total protein) and urine (protein, creatinine, glucose, bilirubin, ketones, urobilinogen, nitrite, leukocytes).	No adverse effect of GM feed.	Buzoianu et al. (2012a)
Bt maize (87 and 74% in the gestation and lactation diet)	Sows during gestation and lactation	Maternal and offspring immunity (leukocytes count, lymphocytes, monocyte, and granulocytes count and percentage, cytokine production).	Sows fed with Bt maize: higher blood monocyte count and percentage, lower granulocyte percentage - on day 110 of gestation, lower monocytes percentage - on day 28 of lactation, lower $CD4^{+}CD8^{+}$ lymphocytes ratio - on day 110 of gestation, day 28 of lactation and overall. Offspring of sows fed GM maize: lower leukocyte and granulocyte counts and percentage, higher lymphocyte percentage. No effect of Bt maize on cytokine production. Transgenic DNA or Cry1Ab-specific antibodies were not found in sows or offspring. Obtained results did not indicate inflammation or allergy and are unlikely to be of major	Buzoianu et al. (2012c)

			biological importance.	
Bt maize in the diets of sows (86% during gestation and 74% during lactation) and their offspring (27-79% during starter-finisher period)	Pigs from weaning to 115 d postweaning (offspring of sows fed or not fed GM Bt maize during gestation and lactation)	Serum biochemistry (aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, alkaline phosphatase, creatinine, urea, total protein).	No pathology indications were observed in serum. Biochemistry values were within normal limits and no overall differences were observed (with the exception of overall γ glutamyltransferase).	Buzoianu et al. (2013a)
HT soybean meal (5-8% in the diet) and Bt maize (4-14%), fed individually or in combination	Sows	Blood haematology (erythrocyte count, leukocyte count, mean cell volume, haemoglobin concentration, mean cell haemoglobin concentration, haematocrit, platelet cell count, mean platelet volume, lymphocyte percentage).	No adverse effect of GM feed.	Swiatkie-wicz et al. (2013)
HT soybean meal (5-8% in the sows diets, 14-18% in the fatteners diets) and Bt maize (4-14% in the sow diets, 10-13% in the fatteners diets), fed individually or in combination	Sows and fatteners	Cellular immune response (peripheral blood leukocyte indices, peripheral lymphocyte subpopulations).	No adverse effect of GM feed.	Bednarek et al. (2013)
Bt maize (70% in the diet)	Fatteners	Blood haematology (erythrocyte count, leukocyte count, mean cell volume, haemoglobin concentration, mean cell haemoglobin concentration, haematocrit), blood biochemistry (lactate dehydrogenase, glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, alkaline phosphatase, urea nitrogen, neutral lipids, cholesterol, protein, albumin, glucose), organs histopathology, necropsy findings.	No effect of GM feed, except lower glucose level in the blood of pigs fed diet containing Bt maize.	Yonemo-chi et al. (2010)
HT soybean meal (25% in the concentrate) and Bt maize (56%), fed individually or in combination	Bull calves (from 10 to 90 day of age)	Cellular (peripheral blood leukocyte indices, peripheral lymphocyte subpopulations) and humoral (anti- BRS, PIV-3, BVDV specific antibody titers and acute phase proteins), phagocytic activity of bovine leukocytes after specific immunization.	No adverse effect of GM feed.	Bednarek et al. (2013)
Bt 11 maize (43% in the diet)	Calves (3-months study)	Health status, blood haematology (erythrocyte and leukocyte counts, haematocrit, haemoglobin) and biochemistry (aspartate transaminase, γ-glutamyl transferase, alkaline phosphatase, bilirubin, protein, urea nitrogen, creatinine, minerals).	No adverse effect of GM feed.	Shimada et al. (2006)
Bt 176 maize (100 to 600 g/day, for dry period and lactation, respectively)	Sheep, i.e. ewes and their progeny (3 year longi-tudinal study)	Blood haematological and biochemical parameters (among others erythrocyte and leukocyte counts, haematocrit, haemoglobin, leukogram, concentrations of minerals, activity of enzymes), antioxidant status, lymphocyte proliferative capacity, phagocytosis and intracellular killing of macrophages.	No adverse effect of GM feed, except higher immune response to Salmonella abortus ovis vaccination in GM maize fed sheep.	Trabalza-Mari-nucci et al. (2008)
Bt cottonseed (22% in the diet)	Growing lambs	Blood haematology (erythrocyte and leukocyte counts, haematocrit, haemoglobin, mean corpuscular volume) and biochemistry (alkaline phosphatase and	Some differences in blood indices between treatments, but all values were within the normal range of reported variations for growing lambs.	Tripathi et al. (2011)

		glutamate pyruvic transaminase activity, protein, globulin albumin, urea, creatinine).		
Bt cottonseed (18% in the diet)	Growing lambs	Blood immunology (serum IgG) and blood cholesterol.	No adverse effect of GM feed.	Tripathi et al. (2012)
Bt cottonseed (1500 g/animal/day or ad libitum)	Sheep	Blood biochemical indices (aspartate transaminase, γ-glutamyl transferase, creatine kinase, urea nitrogen, creatinine).	No adverse effect of GM feed.	Anilku-mar et al. (2010)
HT soybean meal (13% in the concentrate)	Goats (kids fed milk of mother fed conventional or GM soybean meal)	Activity of selected enzymes (aspartate aminotransferase, alanine aminotransferase, creatine kinase, lactic dehydrogenase, γ-glutamyl transferase, alkaline phosphatase) in blood and chosen organs.	No adverse effect of GM feed, but higher lactic dehydrogenase activity in organs of kids from goats fed with GM soybean meal.	Tudisco et al. (2010)
HT soybean meal (13% in the concentrate)	Goats (kids fed milk of mother fed conventional or GM soybean meal)	Activity of γ-glutamyl transferase enzyme in blood and chosen organs.	Higher GGT activity in kidney and liver of kids from goats fed with GM soybean meal.	Mastello-ne et al. (2013)
HT soybean (20% in the diet)	Growing rabbits	Selected metabolic indices (aspartate aminotransferase, alanine aminotransferase, creatine kinase, lactic dehydrogenase, γ-glutamyl transferase, alkaline phosphatase) in blood and chosen organs.	No adverse effect of GM feed, except lower level of lactic dehydrogenase in kidney and heart.	Tudisco et al. (2006)
HT soybean (25% in the diet)	Atlantic salmon (7-months study)	Blood haematology (erythrocyte count, haematocrit, haemoglobin, mean cell volume, mean cell haemoglobin concentration) and plasma biochemistry (lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, glucose, protein, and triacylglycerol content).	No adverse effect of GM feed, except some minor differences, i.e. higher plasma triacylglycerol in GM soybean fed fish. Observed effects were caused by variations in the soy strains rather than the genetic modification per se.	Sissener et al. (2009b)
HT soybean (25% in the diet)	Atlantic salmon (7-months study)	Blood haematology (erythrocyte count, haematocrit, haemoglobin, mean cell volume, mean cell haemoglobin concentration) and biochemistry (lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase activities, glucose, total protein, and triacylglycerol content), cellular stress response measured as heat shock protein (HSP27).	No adverse effect of GM feed, except some minor differences, i.e. glycogen deposits in liver were decreased in the GM soybean fed fish.	Sissener et al. (2009a)
HT soybean (25% in the diet)	Atlantic salmon (7-months study)	Liver metabolism measured as liver protein expression (781 analysed protein spots).	Some minor effects, indicating that high dietary level of GM soybean meal did not affect liver function.	Sissener et al. (2010)
Bt maize (15% or 30% in the diet)	Atlantic salmon postmolt (84-days study)	Health status, among others blood heamatology (erythrocyte count, haematocrit, haemoglobin, mean cell volume, mean cell haemoglobin concentration) and biochemistry (lactate dehydrogenase, alanine aminotransferase, and aspartate aminotransferase activities, glucose, total protein, cholesterol, and triacylglycerol content).	Health status, except somewhat elevated aspartate aminotransferase values, was considered good in all diet groups.	Hemre et al. (2007)
Bt maize (30% in the diet)	Atlantic salmon	Cellular stress indicators.	Some differences in regulation of selected genes in the liver, up-regulation of anti-apoptotic protein NR13 and similar tendencies for ferritin heavy chain and MT-A and -B in the distal intestine.	Sissener et al. (2011)

Table 2. Effects of feeding genetically modified feeds on histopathology and organ weight of food producing animals – results of selected experiments

Genetically modified feed material	Species Category	Studied characteristics	Results	Authors
HT soybean meal (26-39% in poultry diets, 14-18% in the fatteners diets, 25% in the concentrate for bull calves) and Bt maize (54-62%, 10-13%, 56%, respectively), included individually or in combination to the diet	Broiler chickens, laying hens, fatteners, bull calves	Histopathological examination of liver, kidney, spleen, pancreas, duodenum, jejunum, skeletal muscle, and bursa of Fabricius samples, collected from broiler chickens and laying hens.	No adverse effect of GM feed.	Reichert et al. (2012)
HT soybean meal (20-27% in the diet) and HT maize (64-73%), included individually or in combination to the diet	Broiler chickens	Organ weights.	No adverse effect of GM feed.	McNaughton et al. (2011a)
Stacked-trait (Bt + HT) maize (63-74% in the diet)	Broiler chickens	Organ weights.	No adverse effect of GM feed.	McNaughton et al. (2011b)
Bt maize (39 % in the diet)	Weanling pigs	Intestinal histology, weight of organs.	No adverse effect of GM feed, but tendency to increase of kidney weight, and a decrease in goblet cells/mm of duodenal villus.	Walsh et al. (2012a)
Bt maize (39-79 % in the diet)	Forty day old pigs in long-term study (110 days)	Organ weight and histology.	No adverse effect of GM feed.	Buzoianu et al. (2012a)
Bt cottonseed (18% in the diet)	Growing lambs	Organ histopathology.	Tissue histopathology did not indicate any toxic effect of feeding Bt cotton.	Tripathi et al. (2011)
Bt cottonseed (1500 g/animal/day or ad libitum)	Sheep	Histopathology of liver and kidney.	No adverse effect of GM feed.	Anilkumar et al. (2010)
HT soybean meal (13% in the concentrate)	Goats (kids fed only milk of their mother fed conventional or GM soybean meal)	Body and organ weights.	No adverse effect of GM feed.	Tudisco et al. (2010)
HT soybean (25% in the diet)	Atlantic salmon (7-months study)	Intestinal histomorphology, internal organs histomorphology.	No adverse effect of GM feed, except some minor differences, i.e. mucosal fold height in the distal intestine (at one of the three sampling points) was decreased in the GM soybean fed fish.	Sissener et al. (2009a)

Table 3. Effects of feeding genetically modified feeds on fate of transgenic DNA – results of selected experiments

Genetically modified feed material	Species Category	Studied characteristics	Results	Authors
HT soybean meal (26-39% in the diet) and Bt maize (54-62%), included individually or in combination to the diet	Broiler chickens, laying hens	Fate of transgenic DNA in birds' organism.	Transgenic DNA detected in crop, gizzard and duodenum but not in jejunum, ileum, and caecum digesta, excreta, blood, liver, spleen, eggs and breast muscle.	Swiatkiewicz et al. (2010b), Swiatkiewicz et al. (2011b)
HT soybean meal (29-39% in the diet) and Bt maize (25%)	Japanese quails (4 generations)	Fate of transgenic DNA in organism.	Transgenic DNA was not found in breast muscles, eggs and internal organs.	Korwin-Kossakowska et al. (2013)
Bt maize (40-50% in the diet)	Japanese quails (10 generations)	Fate of transgenic DNA in organism.	Transgenic DNA was found in gastrointestinal digesta, but not in eggs and internal organs.	Flachowsky et al. (2005)
Bt maize	In vitro batch fermentation model	Possibility of transfer of the cry1Ab transgene to porcine jejunal microbiota.	Transfer of transgene was not detected.	Buzoianu et al. (2011)
Bt maize (39% in the diet)	Weanling pigs	Fate of transgenic DNA and protein in organism.	The transgenic DNA and protein were found in the gastrointestinal digesta, but not in the tissues (kidneys, liver, spleen, muscle, heart or blood).	Walsh et al. (2011)
Bt maize (39 % in the diet)	Forty day old pigs in long-term study (110 days)	Fate of the Bt transgene.	No cry1Ab transgene or Bt proteins fragments in organs or blood.	Walsh et al. (2012b)
Bt maize (87 and 74% in the gestation and lactation diet)	Sows during gestation and lactation	Fate of transgene.	Transgenic DNA or Cry1Ab-specific antibodies were not found in sows or offspring.	Buzoianu et al. (2012c)
HT soybean meal (14-18% in the fatteners diets) and Bt maize (10-13% in the fatteners diets), fed individually or in combination	Fatteners (30-110 kg of BW)	Fate of transgenic DNA.	Presence of transgenic soybean meal and maize DNA, in the content of stomach and duodenum. The RR or Bt transgenes were not detected in the digesta of the distal intestinal parts, blood, liver, spleen, lung, and muscle.	Swiatkiewicz et al. (2011a)
HT soybean meal (5-8% in the diet) and Bt maize (4-14%), fed individually or in combination	Sows and their offspring	Fate of transgenic DNA.	Transgenic DNA was not detected in blood.	Swiatkiewicz et al. (2013)
Bt maize (70% in the diet)	Fatteners	Fate of transgenic DNA and protein in organism.	Transgenic DNA and protein were not detected in blood, liver or muscles.	Yonemochi et al. (2010)
HT soybean meal (25% in the concentrate) and Bt maize (56%), fed individually or in combination	Bull calves (from 10 to 90 day of age)	Fate of transgenic DNA in organism.	The transgenic DNA and protein were found in the rumen, but not in intestinal digesta, or tissues (kidneys, liver, spleen, lungs pancreas, muscle, blood).	Furgal-Dierzuk et al.(2014)
Bt 176 maize (100 to 600 g//day, for dry period and lactation, respectively)	Sheep, i.e. ewes and their progeny (3 year longitudinal study)	Fate of transgenic DNA in organism.	Transgenic DNA was not detectable in tissues, blood, and ruminal fluid or ruminal bacteria.	Trabalza-Marinucci et al. (2008)
HT soybean meal (13% in the concentrate)	Goats (kids fed only milk of mother fed conventional or GM soybean meal)	Fate of transgenic DNA.	Small fragments of transgenic gene were found in blood and several organs.	Tudisco et al. (2010)

HT soybean meal (13% in the concentrate)	Goats (kids fed only milk of mother fed conventional or GM soybean meal)	Fate of transgenic DNA.	Small fragments of transgenic gene were found in liver, kidney and blood.	Mastellone et al. (2013)
HT soybean (20% in the diet)	Growing rabbits	Detection of transgenic DNA in tissues.	Transgenic DNA was not detectable in tissues.	Tudisco et al. (2006)

Table 4. Effects of feeding genetically modified feeds on microbial population of gastrointestinal tract of food producing animals – results of selected experiments

Genetically modified feed material	Species Category	Studied characteristics	Results	Authors
HT soybean meal (34-36% in the diet)	Broiler chickens	Intestinal microflora population.	No adverse effect of GM feed.	Tan et al. (2012)
Bt maize	In vitro batch fermentation model	Possibility of transfer of the cry1Ab transgene to porcine jejunal microbiota.	Transfer of transgene was not detected.	Buzoianu et al. (2011)
Bt maize (39-79 % in the diet)	Twenty eight day old pigs in long-term study (110 days)	Intestinal microbiota composition (among others Enterobacteriaceae, Lactobacillus and total anaerobes amounts).	No effect of GM feed, with the exception of the genus Holdemania - this effect was however not likely to be of clinical significance.	Buzoianu et al. (2012b)
Bt maize (5.1 kg DM of silage/day)	Rumen-cannulated cows	Ruminal bacterial community	No effect of GM feed.	Wiedemann et al. (2007)
Bt 176 maize (100 to 600 g//day, for dry period and lactation, respectively)	Sheep, i.e. ewes and their progeny (3 year longitudinal study)	Ruminal microbial population characteristics.	No effect of GM feed.	Trabalza-Marinucci et al. (2008)

Table 5. Effects of feeding genetically modified feeds on production performance food producing animals, digestibility of nutrients, and quality of animal origin products – results of selected experiments

Genetically modified feed material	Species Category	Studied characteristics	Results	Authors
HT soybean meal (33-39% in the diet) and Bt maize (54-60%), included individually or in combination to the diet	Broiler chickens	Performance indices, carcass quality, chemical composition of meat.	No adverse effect of GM feed.	Swiatkiewicz et al. (2010a)
HT soybean meal (33-39% in the diet) and Bt maize (54-60%), included individually or in combination to the diet	Broiler chickens	Physico-chemical properties of breast and thigh meat, stability of lipids of muscles.	No adverse effect of GM feed.	Stadnik et al., (2011b)
Bt maize (30-35 % in the diet)	Broiler chickens	Growth performance, slaughter indices.	No adverse effect of GM feed.	Rehout et al. (2009)
Bt maize (30-35 % in the diet), HT soybean meal (33-39%)	Broiler chickens	Growth performance, slaughter indices.	No adverse effect of GM feed.	Kadlec et al. (2009)
HT soybean meal (34-36% in the diet)	Broiler chickens	Growth performance.	No adverse effect of GM feed.	Tan et al. (2012)
Bt maize, or HT maize (50% in the diet)	Broiler chickens	Performance indices, carcass quality, organoleptic properties of meat.	No adverse effect of GM feed, except the slightly lower growth performance.	Dela Cruz et al. (2012)
HT soybean meal (20-27% in the diet) and HT maize (64-73%), included individually or in combination to the diet	Broiler chickens	Performance and carcass indices.	No adverse effect of GM feed.	McNaughton et al. (2011a)
Stacked-trait (Bt + HT) maize (63-74% in the diet)	Broiler chickens	Performance and carcass indices.	No adverse effect of GM feed.	McNaughton et al. (2011b)
HT soybean meal (18-20% in the diet) and HT maize (67-68%), included individually or in combination to the diet	Laying hens	Laying performance, egg quality parameters.	No adverse effect of GM feed.	McNaughton et al. (2011c)
Bt maize (57% in the diet)	Laying hens	Performance and reproductive indices (egg production and weight, ovary weight, the number of yolky follicles).	No adverse effect of GM feed.	Rasmussen et al. (2007)
Bt maize (57% in the diet)	Broiler chickens, laying hens	Laying performance and physiological indices (dietary nutrients and energy utilization, excreta dry matter, egg composition).	No adverse effect of GM feed.	Aeschbacher et al. (2005)
Bt maize (40-50% in the diet)	Laying hens (4 generations)	Laying performance, reproduction indices (hatchability).	No adverse effect of GM feed.	Halle and Flachowsky (2014)
HT soybean meal (29-39% in the diet) and Bt maize (25%)	Japanese quails (2 generations)	Reproductive and performance indices, carcass characteristics, quality of eggs and meat (chemical composition).	No adverse effect of GM feed (reproductive and production indices). Some differences were noticed regarding the chemical composition of breast muscle and egg yolk, however no clear tendency was seen	Sartowska et al. (2012)

			for or against any of the diets used.	
HT soybean meal (29-39% in the diet) and Bt maize (25%)	Japanese quails (4 generations)	Growth and laying performance.	No adverse effect of GM feed.	Korwin-Kossakowska et al. (2013)
Bt maize (40-50% in the diet)	Japanese quails (10 generations)	Growth performance, slaughter indices, laying performance, hatchability.	No adverse effect of GM feed.	Flachowsky et al. (2005)
Bt maize (39% in the diet)	Weanling pigs	Growth performance.	No effect on growth performance.	Walsh et al. (2011)
Bt maize (87 and 74%, in the gestation and lactation diet, respectively)	Sows during gestation and lactation and their offspring	Growth indices.	Sows fed Bt maize: higher body weight of sows on day 56 of gestation but tendency to lower weight of their offspring at weaning.	Walsh et al. (2012c)
Bt maize (39-79% in the diet)	Forty day old pigs in long-term study (110 days)	Growth performance.	No adverse effect of GM feed.	Buzoianu et al. (2012a)
Bt maize (33% in the diet)	Weaned piglets	Growth performance.	Increased performance of Bt fed piglets (probably due to lower content of FB1 mycotoxin).	Rossi et al. (2011)
HT soybean meal (14-18% in the fatteners diets) and Bt maize (10-13% in the fatteners diets), fed individually or in combination	Fatteners (30-110 kg of BW)	Growth performance, carcass quality, meat quality and composition.	No adverse effect of GM feed.	Swiatkiewicz et al. (2011a)
HT soybean meal (5-8% in the diet) and Bt maize (4-14%), fed individually or in combination	Sows and their offspring	Reproductive performance.	No adverse effect of GM feed.	Swiatkiewicz et al. (2013)
HT soybean meal (14-18% in the fatteners diets) and Bt maize (10-13% in the fatteners diets), fed individually or in combination	Fatteners	Physico-chemical properties of breast and thigh meat, stability of lipids of muscles.	No adverse effect of GM feed, except the fact, that fatteners fed with GM materials exhibited slightly decreased lipid stability of loin as indicated by TBARS values.	Stadnik et al., 2011a
Bt maize (70% in the diet)	Fatteners	Performance.	No effect of GM feed.	Yonemochi et al. (2010)
Bt maize (about 71% of maize materials in partial mixed ration + 41 in concentrates)	Dairy cows (over 25 months lasting study)	Nutrients intake, milk yield, milk composition, body condition during 2 lactations.	No adverse effect of GM feed.	Steinke et al. (2010)
Stacked-trait (Bt + HT) maize	Dairy cows	Performance, milk composition	No effect of GM feed.	Brouk et al. (2011)
HT soybean meal (25% in the concentrate) and Bt maize (56%), fed individually or in combination	Bull calves (from 10 to 90 day of age)	Growth performance, chemical composition of meat (basal nutrients, fatty acids).	No adverse effect of GM feed.	Furgal-Dierzuk et al. (2014)
Bt maize (23% of grain and 21% of whole plant silage in the diet)	Calves (3- months study)	Growth performance, rumen metabolic indicators.	No adverse effect of GM feed.	Shimada et al. (2006)
Bt cottonseed (40% in the concentrate)	Lactating multiparous dairy cows	Dry matter intake, milk yield, milk composition.	No adverse effect of GM feed, except improved 4% fat corrected milk production in cows fed diet containing GM cottonseed.	Singhal et al. (2011)
Bt cottonseed (40% in the	Lactating multiparous dairy	Milk production, nutrients intake, nutrients digestibility,	No adverse effect of GM feed.	Mohanta et

concentrate)	cows	body condition score.		al. (2010)
Bt cottonseed (18% in the diet)	Growing lambs	Growth performance, carcass characteristics, digestibility of nutrients, N balance.	Positive effects of feeding of Bt cottonseed (as compared to conventional seeds) on growth indices, no important effects on other parameters.	Tripathi et al. (2012)
HT fodder beet (730 g DM/day), sugar beet (640 g DM/day), beet pulp (750 g DM/day)	Sheep	Digestibility of nutrients.	No adverse effect of GM feed.	Hartnell et al. (2005)
HT canola meal (6.5% in the diet)	Sheep	Growth indices, carcass characteristics, digestibility of nutrients.	No adverse effect of GM feed.	Stanford et al. (2003)
HT soybean (20% in the diet)	Growing rabbits	Growth performance.	No adverse effect of GM feed.	Tudisco et al. (2006)
HT soybean (25% in the diet)	Atlantic salmon (7-months study)	Growth performance.	No adverse effect of GM feed.	Sissener et al. (2009b)
Bt maize (15% or 30% in the diet)	Atlantic salmon postmolt (84-days study)	Growth performance, digestibility of nutrients, selected metabolic indices.	High performance indices and digestibility coefficients in all dietary treatments. Minor effects of GM maize on glucose transport in the intestine, and maltase enzyme activity.	Hemre et al. (2007)