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Health status and potential uptake of transgenic DNA by Japanese quail fed diets containing genetically modified plant ingredients over 10 generations

A. KORWIN-KOSSAKOWSKA, K. SARTOWSKA, G. TOMCZYK[1], B. PRUSAK, AND G. SENDER

Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, Poland, and [1]Department of Genetics Biodiversity, National Veterinary Research Institute, Pulawy, Poland

Abstract   1. The hypothesis assumes that feed containing GMOs affects animal health and results in the transgene product accumulating in the body. Therefore, the objective of the study was to evaluate the impact of genetically modified (GM) ingredients used in poultry diets on aspects of bird health status and accumulation of transgenic DNA in eggs, breast muscle and internal organs.

2. A total of 10 generations of Japanese quail were fed three types of diets: group A – containing GM soya (Roundup Ready) and non-GM maize, group B – containing GM maize (MON810) and non-GM soya, and group C – containing non-GM soya and maize.

3. Bird performance traits were monitored throughout the trial. In 17-week-old animals of each generation, health examination took place on birds from each group including *post-mortem* necropsy and histological organ evaluation. For the purpose of transgenic DNA detection, samples of selected important tissues were taken. A molecular screening method of PCR amplification was used.

4. The analysis of the sectional examination of birds used in the current experiment did not indicate the existence of the pathological changes caused by pathogens, nutritional factors or of environmental nature. The histopathological changes occurred in all three dietary groups and there were no statistically significant differences between the groups.

5. There was no transgene amplification – neither *CaMV35S* promoter sequence nor *nos* terminator sequence, in the samples derived from breast muscle, selected tissues and germinal discs (eggs).

6. According to the obtained results, it was concluded that there was no negative effect of the use of GM soya or maize with regard to bird health status or to the presence of transgenic DNA in the final consumable product.

INTRODUCTION

Genetically modified (GM) crops have been part of the agricultural landscape for more than 15 years and were grown on 181.5 million hectares in both industrial and developing countries in 2014. The 4 major GM crops are soybean, maize, cotton and canola, most of which express traits of agronomic importance in the various varieties (genotypes), in particular herbicide tolerance and insect resistance (James, 2014). It is envisaged, though, the genetic modification of plants may also have other applications, such as for food security (stress resistance, yield), added nutritional value for humans and animals (e.g. through increased macronutrients or micronutrients) and non-food purposes, such as the production of pharmaceutical preparations (Kramkowska *et al.*, 2013; James, 2014).

At the same time, the usage of GM crops has led to public concern, while worries pertain to the perceived "unnaturalness" of this new technology, its potential impact on the environment, and suspected uncertainties over its risks to humans and animals (Frewer *et al.*, 2004). However, examples of cases showing that their use can be dangerous are scarce (Kramkowska *et al.*, 2013). The safety of GM crops is scientifically assessed before they are

actually allowed in the market as required by legislation in many nations. Although the specific regulations dealing with GM crops may differ from nation to nation, this safety assessment follows the internationally harmonised approach of comparative safety assessment, focusing on the differences between a GM crop and a genetically close, non-GM counterpart with a history of safe use. This harmonised approach has become enshrined into Codex Alimentarius guidelines (Codex Alimentarius, 2008; Kleter and Kok, 2010) and has been elaborated into more detail, for example, by the Panel of experts on genetically modified organisms (GMO$_S$) of the European Food Safety Authority (EFSA, 2011).

Notwithstanding the fact that feeding trials with laboratory animals and livestock animals would not be needed following the internationally harmonised case-by-case approach of GM crop safety assessment, many feeding trials have been reported in which GM-crop-derived foods have been administered to rats and mice for prolonged periods, and parameters, such as body weight (BW), feed consumption, blood chemistry and organ weights have been measured and histopathology done. The majority of these experiments indicated neither clinical effects nor histopathological abnormalities in organs or tissues of exposed animals (EFSA, 2008; Snell *et al.*, 2012; Flachowsky, 2013). Basic data that have been collected using such experiments with laboratory rodents cannot, however, be fully extrapolated to livestock animals such as chickens, pigs or cows (Kolar and Rusche, 2008).

Besides performance and health of livestock, a range of feeding studies with GM crops in such animals have also focused on the potential survival of endogenous crop and transgenic DNA in the gastrointestinal tract, and its potential uptake and transfer to animal tissues and fluids, including those that could be processed into animal-derived edible products. In some of these studies, the possible survival of endogenous and recombinant plant DNA fragments during digestion was reported, particularly for fragments from endogenous multicopy plant genes of crops fed to poultry, in organs, blood and muscle (Rizzi *et al.*, 2012).

Feeding studies with laboratory animals, and even more with livestock, are key elements for the nutritional and safety assessment of feed/food from genetic modified plants (GMP$_S$). Depending on the scientific questions, different types of feeding studies are established. Between them, multigenerational experiments with livestock are rather rare (Ricroch *et al.*, 2013). Numerous publications have stated that it is possible that the process of genetic modification could result in unintended, potentially adverse pleiotropic changes (Cellini *et al.*, 2004) which might not

be detected analytically. Therefore feeding studies in laboratory animals and livestock are considered necessary for nutritional and safety assessment (Flachowsky, 2013).

The objective of the current study was to evaluate the impact of GM ingredients (soya bean meal and maize) used in quail diets in consecutive generations on chosen aspects of bird health status and potential presence of transgenic DNA in breast muscle, eggs and internal organs and thereby on animal product safety for consumers.

MATERIALS AND METHODS

Diets, animals and feeding

The current trial consisted of 10 generations of Japanese quail (*Coturnix cot. japonica*) bred in three parallel feeding groups in the years 2010–2013. The first generation of birds was obtained from chicks of the institute's own flock of Japanese quail and divided randomly into three feeding groups from their first day of life. In the 10 generations, in total 10 947 eggs were incubated and 8438 healthy chicks entered the trial. At the age of 6 weeks, part of the healthy birds of a proper body composition were chosen and randomly divided into the final selection flocks of 17 females and 5 males in each. Each group consisted of 6 repetitions of such flocks, resulting in 102 females and 30 males in each of three groups in each of 10 generations. In total 3960 adult birds were used in the trial. Birds were housed in stainless-steel wire battery cages equipped according to their age with mesh floor, infrared heating lamps and manual drinking and feeding appliance, or later with sloped wire floor and with automated drinking and manual feeding appliance. During the early laying period (from age 7 weeks to age 16 weeks) egg production was evaluated and at the age of 16 weeks eggs were collected for incubation in order to form the next generation.

The following feeds were used: Group A – GM soya bean meal included; Group B – GM maize included, Group C – non-GMO (no genetic modified components) materials. GM soya bean meal used was produced from a Roundup Ready soya bean (also known as GTS 40-3-2); GM maize used in group B was MON810 maize. The development of Roundup Ready soya bean was obtained by recombinant DNA technology. The gene encoding a glyphosate tolerant form of the enzyme 5- enolpyruvylshikimate-3-phosphate synthase (EPSPS), isolated from *Agrobacterium tumefaciens* strain CP4 was introduced into the commercial soya bean. The *epsps* gene is under the regulation of a strong constitutive promoter from *cauliflower mosaic virus* (CaMV 35S) and

nopaline synthase terminator (*nos* terminator) derived from *Agrobacterium tumefaciens*. MON810 maize was developed using recombinant DNA technology and micro projectile bombardment of plant cells, to introduce a gene encoding naturally occurring insecticidal protein (derived from *Bacillus thuringiensis* ssp. *kurstaki*). The protein is active against certain species of *Lepidoptera* insects. More specifically, the protein expressed by MON810 is a truncated form of the insecticidal protein, CRY1A (b) endotoxin and protects the maize plants from leaf and stalk damage caused by corn borer larvae, a lepidopteron insect pest of maize. MON810 was transformed with one copy of *cry1A (b)* gene under the control of the strong constitutive enhanced CaMV 35S promoter and the maize HSP70 intron leader sequence. The *cry1A (b)* coding sequence from *Bacillus thuringiensis* ssp. *kurstaki* was modified to optimise and maximise the expression of the endotoxin CRY1A (b) protein in plants (Querci *et al.,* 2006).

Birds received two types of diet: grower (1–6 weeks) and layer (7–17 weeks). The same pattern was used in all 10 generations. Feeds were prepared by the company Agro-Kocięba (Bogdan Kocięba, Czarnocin, Poland). Details of feed composition are presented in a previous paper (Sartowska *et al.,* 2015). Level of basic nutrients in the above mentioned feeds was analysed in each generation according to AOAC (2005) methods and it was confirmed to be as planned. GM components used in the feed were properly certified. Birds in each cage received the same amount of feed, leftovers were monitored weekly. The content of modified DNA in feeds was examined by a reference GMO laboratory in Plant Breeding and Acclimatization Institute – National Research Institute, (Radzików, Poland). Detection, identification and quantitative determination of the GMO were done by quantitative polymerase chain reaction (PCR) and Real-Time PCR methods. Results confirmed a presence of MON810 maize DNA in group B feed, not in other feeds, as well as a presence of Roundup Ready soya DNA in group A feed and not in other feeds. Group C feed was proven to be free from the above modifications.

Basic production performance was observed in the course of the trial. Results, including the first 4 generations, were published in an earlier article by Korwin-Kossakowska *et al.* (2013), and results including 10 generations were published in an article by Sartowska *et al.* (2015).

Sample collection

In each generation at the end of the laying period (in week 17) experimental dissection took place. Birds were killed by decapitation. After slaughter, sectional examination of the birds was done. The number of animals, from which samples were collected, was as follows: 3 females and 3 males, randomly selected from each of three groups, all together 18 animals in each generation (60 birds per group and 180 all together during the trial). The analysis and examination of the birds used in the experiment was performed by a veterinarian. Veterinary expertise was based on generally accepted principles of subjective evaluation consisting of gradation of the observed changes. There was no numerical scale for the determination of those changes.

Immediately after examination of the animals, their liver, kidney, spleen, duodenum and breast muscle were collected for histological analysis and fixed in 10% neutral buffered formalin. After fixation the tissue sections were processed by routine histological methods. Paraffin sections (5 μm) were stained with haematoxylin and eosin (HE) and examined under a light microscope (Axioskop 2, Carl Zeiss Microscopy GmbH, Jena, Germany). The examinations were performed by the Diagnostic Laboratory of the National Veterinary Research Institute in Puławy according to their proprietary methods.

In each generation the following tissue samples for DNA analysis were collected from 12 females and 3 males from each feeding group: part of the breast muscle, gizzard, liver, spleen, duodenum, kidney and heart (all together 3150 samples). Moreover, during week 17, 6 germinal discs from eggs from each group were collected. All the above mentioned samples were frozen and stored in appropriate conditions –80°C until further analyses.

All procedures were approved by the III Local Ethics Commission in Warsaw number 27/2009 and performed in accordance with the guiding principles for the care and use of experimental animals and with code of ethics of the World Medical Association.

DNA extraction and quantification

The genomic DNA was extracted from the thawed tissues (25 mg) with the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The DNA was quantified by Nano drop 1000 UV/Vis Spectrophotometer (Thermo Scientific, USA) and was checked in ethidium bromide – stained agarose gel.

PCR analyses

Tissue samples and germinal discs from eggs from each of the feeding groups – A, B and C were prepared for analyses of specific gene constructs (*CaMV* 35S promoter and *nos* terminator) with the use of PCR analysis. In order to avoid contamination, PCR reactions were assembled in an

ultraviolet-sterilised hood. Filter tips and sterile disposable tubes were used during pipetting. All PCR amplifications were performed on DNA Engine Tetrad (MJ Research, CA, USA). DNA was amplified using REDTaq ReadyMix Sigma-Aldrich in accordance with the manufacturer's instructions. Sequence and annealing temperature of the primer pair sets (Genomed, Warsaw, Poland) used for PCR are shown in Table 1.

In the first step the DNA obtained from tissues was tested for integrity by amplification of the species-specific systematic affiliation. The *IGF1* (insulin-like growth factor 1) gene fragment specific for avian DNA was amplified using the PCR-restriction fragment length polymorphism (RFLP) method according to Moody *et al.* (2003) and Zhou *et al.* (2005). Specific primers were been used for quail-specific *igf*1 gene (Table 1).

The international standard provides the overall framework of qualitative methods for detection of GMO$_S$ in foodstuffs using the PCR. Qualitative analysis consists of screening and/or specific detection of target nucleic acid sequences in the test samples. The above mentioned method is also used for specific detection and amplification of the "specific construct" of GMP$_S$: *CaMV* 35S promoter or *nos* terminator. Due to the presence of these constructs in many GMP$_S$, this method can be used to screen for the presence of GMP derived components in animal tissue (Querci *et al.*, 2006).

In the second step the PCR amplification of 3330 examined probes (15 animals × 3 feeding groups × 7 tissues × 10 generation and 6 germinal discs × 3 feeding groups × 10 generations) was used for the detection of *CaMV* 35S promoter and *nos* terminator. Specific primers were used for the detection of *CaMV* 35S promoter: *p35S-cf3*, *p35S–cr4* and *nos* terminator: *HA-nos188f*, *HA-nos118r*, according to Lipp *et al.* (2001) and Moody *et al.* (2003) as presented in Table 1. The protocol of the PCR-based method allowing the screening of GMOs in analysed material according to Querci, Jermini and Van den Eede (2006) was used. The following reagents: 0.5 µM of each primer, 0.025 U/µl Red Taq polymerase and nuclease-free water aliquots were mixed in the preparation of MasterMix.

Detection of the PCR fragments

After amplification of the target sequence the PCR products were analysed by 2% agarose gel electrophoresis in the presence of ethidium bromide. The gel was photographed to provide a permanent record of the result of the experiment. As positive control – a reference DNA extracted from a certified European Reference Material, Roundup Ready soya (ERM-BF410gk and ERM-BF410dk) containing 2% and 5% of GM plant ingredients and MON810 maize (ERM-BF413f) containing 5% of GM plant ingredients have been used. The reference material was prepared by the Joint Research Centre Institute for Reference Materials and Measurements (JRC-IRMM) – Institute for Reference Material and Measurements – one of the 7 Institutes of the Joint Research Centre (JRC). The DNA from the reference material was extracted using the NucleoSpin Food Kit (Macherey-Nagel, Duren, Germany). The characteristic fragments were amplified by PCR and detected after separation by agarose gel electrophoresis. The positive control is intended to demonstrate what the result of analyses of test samples containing the target sequence will be. As a negative control, IRMM Roundup Ready soya (ERM-BF410a) and IRMM maize (ERM-BF413a) certified reference material containing no GMP ingredients have been used. Additionally, a "No template" sample was created to confirm purity of the MasterMix, in which water was used instead of DNA.

Statistical analysis

All data regarding clinical changes in organs from the animals from different generations were grouped together. Differences between feeding groups were analysed statistically. Data distribution was evaluated with the UNIVARIATE procedure in SAS and no normal distribution was observed. The results were analysed using NPAR1WAY procedure (nonparametric one way analysis of variance). The differences between experimental groups were evaluated with Wilcoxon and Kruskal–Wallis tests (McDonald, 2014), significance level was set at 5%. Frequency of occurrence of pathological changes was calculated with the FREQ procedure in SAS.

Table 1. *Characteristics of primers for PCR analysis*

Symbol	Primer sequences	Melting point G/C	Reference
IGF 1pF	CATTGCGCAGGCTCTATCTG	53.8	Moody *et al.* (2003)
IGF 1pRr	TCAAGAGAAGCCCTTCAAGC	51.8	Moody *et al.* (2003)
p35S-cf3	CCACGTCTTCAAAGCAAGTGG	57.4	Lipp *et al.* (2001)
p35S–cr4	TCCTCTCCAAATGAAATGAACTTCC	56.3	Lipp *et al.* (2001)
HA-nos188f	GCATGACGTTATTTATGAGATGGG	56.2	Lipp *et al.* (2001)
HA-nos118r	GACACCGCGCGCGATAATTTATCC	61.2	Lipp *et al.* (2001)

RESULTS

Health status of Japanese quail

The health status of the birds was determined on the basis of two studies: non-lethal clinical examination (e.g. whether birds are depressed or lively) and post-mortem studies based on macroscopic pathological changes.

Birds divided into groups were maintained in relevant conditions to meet the welfare requirements. The clinical condition of birds in experimental groups and in the control group did not show signs or symptoms of any previously known disease entities in poultry nor signs of nutritional deficiencies (e.g. vitamin deficiency, deficiencies of micronutrients, etc.). The plumage of the birds showed no change or loss.

Post-mortem necropsy of Japanese quail

Sectional examination

Autopsy was performed to evaluate possible pathological changes in organs and in body cavities. Abdominal organs in all groups remained in proper alignment. The macroscopic evaluation of individual organs and tissues was performed.

Livers of birds in each group were of the colour brown to dark brown, and did not exhibit physiological deviations of liver tissue. In some isolated cases the liver was slightly enlarged, however, there were no necrotic spots or atrophy observed. Spleens of all birds were of a dark cherry colour and of the correct size. The pericardial sac of two birds in group B (GM maize), and of one in group A (GM soya) contained a mediocre amount of straw-coloured clear liquid, however, there were no changes in the heart muscle. Lungs and air sacs showed no changes in any bird. Four birds (respectively two from group A, and one from group B and C) had slightly enlarged kidneys.

The mouth and tongue mucosa showed no changes. In crops and glandular stomachs a pulpy content was observed and in gizzards, a solid-dry fibrous content was found, indicating the correct physiological state. Further sections of the digestive tract of individual birds, in general, did not show changes and physiological deviations. Only in some birds (from all three groups) a slight gasification of *jejunum* and *caecum* was observed. Among these birds, the intestinal mucosa showed a slight thickening in small areas of congestion.

Ovaries of all females were active and in some cases ready-to-lay eggs were present in the oviduct. Males' testes had developed properly, according to current physiological state. Isolated cases had slight enlargement and congestion of the kidney,

but it was impossible to see any regularity of the incidence of this change in birds from different groups.

There were no changes in the skeletal system in its susceptibility to breakage or its fragility among the different groups.

Histopathology of the organs of Japanese quail

There was a slight to moderate degree of congestions of the parenchyma of liver, kidney and spleen and a variable degree of fatty degeneration of the liver observed. In the kidney, liver and duodenum, single, focal lymphoid cell infiltrations were observed. In isolated cases in duodenum also the presence of diffuse infiltration of lymphoid cells in the mucous membrane was noted. These changes occurred in all three dietary groups. In contrast, all breast muscle samples remained unchanged. The frequency of changes, regarding organs where changes were present, is presented in Table 2. Probability of statistically significant differences between the results of the changes in organs of the control group and the results of experimental groups are presented in Table 3. For statistical evaluation of groups with only few observations results were aggregated. Wilcoxon scores and Kruskal–Wallis test showed the lack of statistically significant differences between the control group (C) and the results of the experimental groups (A and B). The level of the *P*-value ranged from 0.12 (infiltration in liver)

Table 2. *Results of histopathological examination of internal organs of control animals (group C) and animals fed genetically modified soybean meal (Group A) or maize (group B) according to the FREQ procedure – summary of 10 generations*

Type of change	Tissue	Group A (N = 60) Changed samples, %	Group B (N = 60) Changed samples, %	Group C (N = 60) Changed samples, %
Congestion of the parenchyma	Liver	96.3	96.2	98.1
	Kidney	100.0	100.0	100.0
	Spleen	97.9	100.0	100.0
	Duodenum	0.0	0.0	0.0
	Breast muscle	0.0	0.0	0.0
Infiltration of lymphoid cells	Liver	63.0	66.0	47.2
	Kidney	25.9	13.0	14.8
	Spleen	0.0	0.0	0.0
	Duodenum	13.0	11.1	13.0
	Breast muscle	0.0	0.0	0.0
Fatty degeneration	Liver	66.7	60.4	67.9
	Kidney	0.0	0.0	0.0
	Spleen	0.0	0.0	0.0
	Duodenum	0.0	0.0	0.0
	Breast muscle	0.0	0.0	0.0

N – number of analysed samples.

Table 3. *Probability of statistically significant differences between the results of control animals (group C) and animals fed genetically modified soybean meal (Group A) or maize (group B) – according to Kruskal–Wallis test*

| Type of change | Tissue | Means of Wilcoxon Scores (Rank Sum) | | | |
		Group A	Group B	Group C	P-value
Congestion of the	Liver	81.5	81.5	81.5	1.00
parenchyma	Kidney	81.5	81.5	81.5	1.00
	Spleen	77.4	79.0	78.0	0.31
Infiltration of	Liver	83.9	86.3	71.2	0.10
lymphoid cells	Kidney	88.0	77.5	79.0	0.19
	Duodenum	82.0	80.5	82.0	1.00
Fatty degradation	Liver	81.8	76.8	82.8	0.73

and 0.19 (infiltration in kidney) to 1.00 (congestion in liver and kidney and infiltration in duodenum.

Detection of transgenic DNA fragments

A fragment of the poultry-specific *IGF1* gene of the size 813 bp was amplified to confirm the integrity of the isolated DNA. A 123 bp DNA fragment from the *CaMV*35S promoter sequence and 118 bp DNA fragment from the *nos* terminator sequence in the reference material were amplified by PCR and detected after separation by agarose gel electrophoresis. In the negative control sample no amplification was observed.

Reference material showed the presence of characteristic bands (410gk, 410dk, 413f), whereas the tested samples and reference material with no GMO (410a, 413a) showed no presence of the evaluated gene constructs. Typical examples of the amplification results are given in Figures 1 and 2. There was no *CaMV*35S promoter sequence or *nos* terminator sequence amplification in any of the 3330 examined samples derived from breast muscle, selected tissues and germinal discs (eggs) from all feeding groups (A, B and C).

DISCUSSION

Studies with feeds from GMP$_S$ in the nutrition of livestock animals have been conducted for years. Among these were, in the years 1997–2007, 18 studies made at the Federal Agricultural Research Centre (FAL) in Braunschweig (Germany) (Flachowsky *et al.*, 2007). The majority of the experiments were undertaken with GMP$_S$ of the so-called first generation (plants with input traits and without substantial changes in composition). Animal studies were carried out for nutritional and safety assessment, such as digestibility, feed intake, health and performance of target
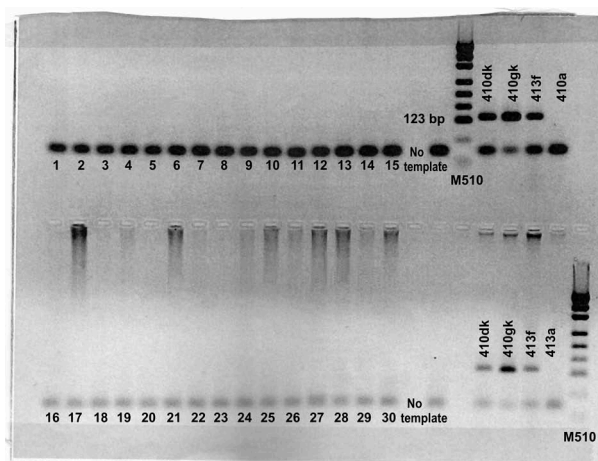
Figure 1. *Screening of animal tissues for the CaMV 35S component (exemplary electrophoretic image, agarose 2% gel). Lines 1–15: PCR product from heart samples, group A; lines 16–30: PCR product from spleen samples, group B; 410a (ERM-BF410a) and 413a (ERM-BF413a) – negative control (DNA extracted from no GM soybean meal and maize);410gk, 410dk, 413f (ERM-BF410gk, ERM-BF410dk, ERM-BF413f) – positive control (DNA extracted from Roundup Ready soybean meal and MON810 maize); No DNA template – negative control of the MasterMix, in which water is used instead of DNA;M 510 – pUC19 DNA/MspI (Blirt SA,Gdansk, Poland) – size marker contains a 501-bp DNA Ladder; 123bp – DNA fragment from the CaMV 35S promoter sequence.*
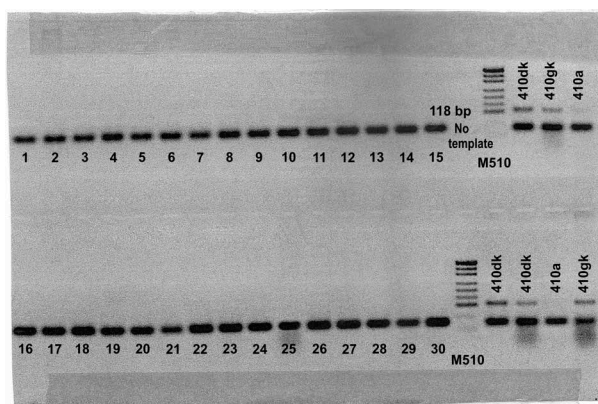
Figure 2. *Screening of animal tissues for the nos terminator component (exemplary electrophoretic image, agarose 2% gel). Lines 1–15: PCR product from liver samples; group A, lines 16–30: PCR product from breast muscle samples, group C; No Template sample – negative control of the MasterMix, in which water is used instead of DNA; M 510 – pUC19 DNA/MspI (Blirt SA,Gdansk, Poland) – size marker contains a 501-bp DNA Ladder; 410a (ERM-BF410a) -negative control (DNA extracted from no GM soybean meal); 410gk, 410dk, (ERM-BF410gk and ERM-BF410dk) – positive control (DNA extracted from Roundup Ready soybean meal); 118 bp – DNA fragment from the nos terminator sequence.*

animal species and quality of food of animal origin. Reproduction was studied in multi-generation experiments with quail and laying hens. Attention was drawn to the fate of DNA during feed

processing in the digestive tract of animals and in the animal body (samples from several organs and tissues (Flachowsky et al., 2007)). The results indicate that routine feeding studies with target animal species add little to nutritional assessment of feed from GMP$_S$ of the first generation, but they are of public interest and important for safety assessment.

Veterinary examination

The analysis of the sectional examination of birds used in the current experiment did not indicate the existence of any pathological changes caused by pathogens, by nutritional factors or of environmental nature. Minor deviations from the physiological condition of single birds in each group could be the result of individual's susceptibility to environmental factors and were not representative of the health status of the group. It is therefore clear that they were random and had no connection to experimental factors.

Histopathological examination is a valid laboratory technique in cases where other diagnostic methods fail. Also, this technique is indispensable in pathomorphological evaluation of side effects of vaccines, drugs and chemical compounds (Reichert et al., 2012). If more advanced methods are needed for histopathology, transmission electron microscopy (TEM) or proteomics could be helpful in disclosing morphological changes in cell structure or proteome alternations. No aetiology has been identified for the histopathological changes observed in the present experiment in the liver and individual samples of kidney, spleen and duodenum. Most likely these changes are a result of intensive feeding, including a high content of protein and energy in the diets. Congestion of the parenchymal organs (from slight to severe degree) could be due to insufficient exsanguination of birds at slaughter. Histopathological lesions of the internal organs are usually associated with disease; however, it is known that fairly often histological changes or "deviations from the physiological state" without any documented negative impact on animal health can be found in clinically healthy animals (Reichert et al., 2012).

Molecular detection of transgenic DNA fragments

Various authors concluded that there is a remote chance of horizontal gene transfer of transgenes from GMP$_S$ to other organisms, particularly to other eukaryotes (Thomson, 2001; Jennings et al., 2003; Mazza et al., 2005; Acosta and Chaparro, 2008; Świątkiewicz et al., 2011). The risks of such a transfer would in the first place depend on the potential exposure to the DNA from the GMP, followed by a series of events leading from cellular uptake to stable integration in the genome of the recipient and its expression. If such a transfer nonetheless occurred, it should be considered in the light of the background presence of the same and similar genes in the environmental gene pool available, as well as the impact that the transfer has on the recipient organism, such as whether it would confer a selective advantage over other individuals of the same species.

The aim of the present multigeneration experiment was to study the fate of fragments of the transgene from Bt MON810 maize grain and herbicide tolerant (Roundup Ready) soybean meal present in the animal feed. In the present experiment, the transgenic DNA was not observed in any of the tested samples of tissues or eggs of quail fed on GMO. Similar results were obtained in many other studies as for example, Yonemochi et al. (2003). There was also no influence, or no significant differences observed on histopathological examination of the major organs and tissues in dairy cows from two groups on diets with or without GMO. Moreover, the cry9C gene and Cry9C protein were not detected by the PCR method and enzyme-linked immunosorbent assay (ELISA) in the milk, blood, liver and muscles of the cows at the end of the experiment.

The chances for a horizontal transfer of such genes to quail tissues, or to that of consumers of quail products, therefore appear be to be infinitesimally small, while no particular risks appear to be linked with a transfer of the cry1Ab or cp4 epsps genes present in GM maize and soybean, based on their safety and function (EFSA, 2009).

What is noteworthy in the current study is the fact that the trial was designed using many (10) generations and the animals from three feeding groups were bred to produce subsequent generations. This allowed repeated observations, improved statistical methodology and produced many valuable results, which is usually not the case in evaluation of animal's health status, and which is so important in the GMOs safety studies.

In conclusion, analysis of clinical status and sectional examination of the birds performed in the experiment did not indicate the existence of any pathological changes induced by pathogens or any factor of nutritional environmental nature. Quail fed with diets containing GM feed ingredients, had no adverse effect on morphology and structure of selected internal organs and muscles, what was assessed under light microscope. Molecular analyses showed no presence of the modified DNA in analysed samples. This points to the fact that there was no transfer of transgenic DNA to animal tissues and eggs, which is in agreement with earlier studies, and on this basis it can be stated that food products thus obtained are safe for consumers.

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DISCLOSURE STATEMENT

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