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Prevalence of genetically modified soybean in animal feedingstuffs in Poland

Zbigniew Sieradzki✉, Małgorzata Mazur, Beata Król, Krzysztof Kwiatek

Department of Hygiene of Animal Feedingstuffs
National Veterinary Research Institute, 24-100 Puławy, Poland
zbigniew.sieradzki@piwet.pulawy.pl

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Abstract

Introduction: Globally, genetically modified (GM) crops were grown on 191.7 million hectares in 2018, which were mostly sown with soybean, maize, cotton, oilseed rape, and rice. The most popular traits introduced through genetic modification include herbicide and pest insect resistance. The aim of this study was to identify and quantify genetically modified soybean used in animal feed in Poland. **Material and methods:** This research was based on the real-time PCR technique. All methods for GM soybean events were adopted from the EURL GMFF database of methods and previously verified to meet the minimum criteria of acceptance. Over 15 years of research, 665 samples were examined in total. **Results:** The most common GM soybean event was MON40-3-2, tested for from the beginning of the investigation. Next, in decreasing order of frequency, were MON89788, MON87701, and A2704-12. In the majority of samples (606; 91%) GM soybeans were identified at a content level above the 0.9% GM content threshold for mandatory labelling. Only 59 soybean samples (9%) were identified as GM negative. GM negative results were mainly identified during the analyses in the last three years of the study, from 2017 to 2019. **Conclusion:** Our data clearly indicate that the majority of soybean used in Poland for animal feeding was genetically modified.

Keywords: GMO, feed, soybean.

Introduction

The use of genetically modified (GM) plant seeds for food and feed production has been continuously increasing in the world. The latest data indicated that GM crops were grown on 191.7 million hectares around the world in 2018 (17). Most of these are commodity crops such as soybean, maize, cotton, oilseed rape, and rice, into which desirable traits are introduced through genetic modification including herbicide and pest insect resistance as the most popular. In 2018, more diverse crop seeds with various enhancements became available on the market. The produce from these seeds includes reduced acrylamide potatoes with non-bruising, non-browning, and late blight-resistant traits; insect-resistant and drought-tolerant sugarcane; non-browning apples; and high oleic acid canola and safflower. Genetically modified soybeans are currently the most important source of feed protein within the European Union and supply a significant proportion of it in other countries around the world. These soybeans are planted on an especially large scale in the USA, Brazil and Argentina, the three main GM crop producers.

In 2018, GM soybean occupied 50% of the global area under modified crops (17). GM soybeans have remained the main such crop since 1996, when the first commercialised genetically modified crop seeds came to notice. Throughout the 23 years since, soybeans have held the top position as regards area covered by GM crop production. The meat and bone meal (MBM) ban in the EU was the starting point of an increasing demand for soybean meal. The unique composition of amino acids and the content of primarily lysine, arginine and tryptophan, especially important in the feeding of poultry and pigs, recommend it. So far, soybean meal has no competition except the banned MBM as a protein source for these animal species in the EU. The other protein source produced in high volume in Europe, rapeseed meal (5), has critical limitations as a feedingstuff for poultry and pigs. Rapeseed oil used to have a poor reputation due to the presence of erucic acid, which has a bitter taste and was later found to cause health problems. Other characteristics recommending against the use of rapeseed meal as animal feed were the content of antinutritional and performance-detrimental glucosinolates and the poorer digestibility of rapeseed protein. Although low-erucic and low-glucosinolate rapeseed varieties are now the main types grown worldwide, it is not used in feed production to the extent it could be.

GMO-free production of food of animal origin forces feed manufacturers to look more closely at rapeseed meal as a means of achieving an appropriate content of protein-containing meals in their feed recipes. However, it is necessary to adhere to species rules for feeding animals with rapeseed. Contemporarily with GM soybean importation, many European countries have started to invest in the development of new non-GMO lines of soya which will have the ability to grow and yield highly in European climates. In countries like Austria and Poland, soybean acclimatisation and commercialisation is encouraged strongly by governments and is very palatable to public opinion. From the European point of view, the argument for the use of GM technology for feed and food production is questioned, and specifically from the continent's citizens point of view, its use is unacceptable (2, 19). The main reason for the lack of enthusiasm is fear concerning GM food safety and the yet-unknown consequences of its cultivation and/or consumption. Voices from within the bioscience professions and many pieces of evidence from scientific GM feed trials on animals have no power to tear down the wall of stereotypes in Europe.

Particular attention has been directed towards herbicide-tolerant crops in recent years, and specifically towards glyphosate, which has been blamed for cancer cases in humans. Glyphosate is widely used around the world, not only for GM plant production but as a good total herbicide for all kinds of plant production. That it is widely used also means that it is widely dispersed into the environment; the most recent data shows that almost all beer and wine contains glyphosate contamination in the USA, as do all popular brands of beer in Germany. Several genes afford resistance to herbicides. The EPSPS gene from the soil bacterium *Agrobacterium tumefaciens* L. determines the synthesis of a glyphosate-impervious protein (CP4EPSPS), and the *pat* gene from *Streptomyces viridochromogenes* imparts tolerance of herbicides containing glufosinate as the active ingredient. In accordance with the law in force in the European Union, a product containing more than 0.9% GMO must be appropriately labelled (7). Authorised EU agencies enforce Union labelling regulations by detecting contraventions. Allied work is the monitoring of the presence of GMOs in food or feed by the appropriate authorities. Molecular analytical techniques have been developed and brought into use for GMO detection such as protein-based and nucleic acid-based methods. In routine analysis of food and feed PCR, and particularly quantitative real-time PCR, has become the method of choice for the determination of the GMO content of samples. Event-specific methods are used in the detection and determination of GMO quantities that depend upon genetic material characteristic only of a specific GMO line. They target a unique site comprising a junction between the transgenic insert and the host genome (7, 14, 21).

The aim of this study was to detect, identify and quantify genetically modified soybean by DNA analyses in animal feed in Poland. Samples were collected under the National Control Plan for Feed. This surveillance research was based on the real-time PCR technique and was applied in GM feed analyses in the National Veterinary Research Institute (NVRI) in Puławy, Poland.

Material and Methods

Samples. Samples of compound feed and animal-feed-derived soybean meal and soybean were gathered from eastern and central Poland by Veterinary Inspectorate officers from January 2004 to July 2019. The material was taken for GM soybean content determination in execution of the National Control Plan for Feed. Certified reference materials (CRM) from the American Oil Chemists' Society (MON89788, MON87705, MON87701 and A2704-12) and the European Commission's Joint Research Centre (MON40-3-2) were used as calibrators to determine GMO amount in %. From 2004 to 2017, only MON40-3-2 was analysed. In 2018, MON 89788 and in January 2019 MON 87701, MON 87705 and A2704-12 were introduced into the investigation.

DNA extraction. The extraction of DNA from samples and certified reference GM soybean materials was carried out by the CTAB method described in ISO 21571 (16). After extraction, the quality and quantity of DNA was measured in a UV spectrometer (Nicolet Evolution 300, Thermo Fisher Scientific, Madison, WI, USA). The purity of the extracted DNA was determined in two steps, by the ratios of the absorbance at 260/280 nm and at 260/230 nm, with compensation for the absorbance at 320 nm.

Real-time PCR. All methods for GM soybean event determination used in this study and enumerated above are listed in the database of the European Union Reference Laboratory for Genetically Modified Food and Feed (EURL GMFF). The sequences of PCR primers and probes used for GM soybean determination are listed in Table 1 with the corresponding EURL GMFF database record. All primers and probes were synthesised by Genomed (Warsaw, Poland), with the HPLC purification step also being performed by that supplier. Detection and determination of GM soybeans were carried out on a 7500 real-time PCR system (Applied Biosystems, Middletown, CT, USA) in a 25 µL volume containing 1x TaqMan Universal Master Mix, 75 nM of each primer, 12.5 nM of TaqMan probe and 5 µL of DNA. The amplification profile comprised a first step at 50°C for 2 min to activate the Uracil N-glycosylase and then initial denaturation at 95°C for 10 min and 45 cycles at 95°C for 10 s and 60°C for 60 s.

Table 1. Primers and probes used in real-time PCR

Target/EURL GMFF reference	Primer/probe	Sequences 5′–3′
Lectin/	Le1F	CCAGCTTCGCCGCTTCCTTC
QT-TAX-GM-002	Le1R	GAAGGCAAGCCCATCTGCAAGCC
	Le1P	FAM-CTTCACCTTCTATGCCCCTGACAC-TAMRA
MON-40-3-2/	RR-F	TTCATTCAAAATAAGATCATACATACAGGTT
QT-EVE-GM-005	RR-R	GGCATTTGTAGGAGCCACCTT
	RR-P	FAM-CCTTTTCCATTTGGG-MGBNFQ
MON-89788/	MON89788F	TCCCGCTCTAGCGCTTCAAT
QT-EVE-GM-006	MON89788R	TCGAGCAGGACCTGCAGAA
	MON89788P	FAM-CTGAAGGCGGGAAACGACAATCTG-TAMRA
MON-87701/	MON87701F	TGGTGATATGAAGATACATGCTTAGCAT
QT-EVE-GM-010	MON87701R	CGTTTCCCGCCTTCAGTTTAAA
	MON87701P	FAM-TCAGTGTTTGACACACACACTAAGCGTGCC-TAMRA
MON-87705/	MON87705F	TTCCCGGACATGAAGCCATTTAC
QT-EVE-GM-003	MON87705R	ACAACGGTGCCTTGGCCCAAAG
	MON87705P	FAM-AAGAGACTCAGGGTGTTGTTATCACTGCGG-TAMRA
A2704-12/	A2704-12F	GCAAAAAAGCGGTTAGCTCCT
QT-EVE-GM-004	A2704-12R	ATTCAGGCTGCGCAACTGTT
	A2704-12P	FAM-CGGTCCTCCGATCGCCCTTCC-TAMRA

In order to assess the GM soybean content, standard curves made in 5 dilutions in two replicates were prepared using the CRM GM soybean DNA. Each dilution had a known number of copies of a reference gene for the soybean genome (lectin) and transgene sequence (a sequence containing part of the soybean genome and transfection cassette). The quantification of GM soybeans was achieved by the amplification of the lectin gene and transgene sequences, and the GM content was a relative measure of the amount of genetically modified material in the total soybean material.

Results

In 2004–2019, as part of the official control plan for GM feed in Poland, 665 samples were examined for the presence and amount of genetically modified soybean. GM soybean DNA was found at a content level above the 0.9% threshold requiring labelling of feed as containing GMO in 606 samples, duly noted as positive, which was 91% of the tested total. Samples totalling 59 (9%) were identified as containing either none or ≤ 0.9% of the analysed GM soybean events, and were therefore recorded as negative. Analysis of the results showed that since 2017, the level of GM soybean-negative samples has been consistently and markedly higher than in the preceding period (Fig. 1). Over the first 12 years of the investigation (2004–2016), 4% samples (22 out of 553 analysed) were negative, but in 2017 that proportion rose to 29.4% (10 out of 34), in 2018 it was 32.5% (13 out of 40), and finally in 2019, 26% (10 out of 38) of samples with soybean ingredients were GM-negative.

As was stated previously, the negative samples were those with GMO amounts ≤ 0.9%. Closer investigation of the amount of GMO in these samples in 2018 and 2019 showed that in 2018, out of 13 negative samples, 4 (31%) did not contain detectable MON40-3-2 or MON89788 events, 6 (46%) had GMO below the limit of quantification (LOQ), and 3 (23%) revealed GMO below 0.9%. In 2019, out of 10 negative samples, 4 (40%) did not give any positive result for the presence of MON40-3-2, MON89788, MON87701, MON87705 or A2704-12 events, 4 (40%) yielded modified soybean content below the LOQ, and the last 2 (20%) harboured GMO at a level lower than 0.9%.

Analysis of positive samples showed that in the majority of them, the GM soybean event MON40-3-2 was present (Fig. 2). In 2004-2017, all positive samples contained this GM event, in 2018 its presence was at the 93% level in positive samples, and in 2019 MON40-3-2 was again identified in all GM samples. Results from 2018 and 2019 showed that the second most commonly used GM event was MON89788, which was present in 100% and 90% of positive samples, respectively. Since 2019, after a major expansion of the methods' event references, we could see that the range of GM events present in animal feedingstuffs in Poland was broader. MON40-3-2 was present in 96% of GM-positive samples, MON89788 in 96%, MON87701 in 79%, and A2704-12 in 54%. The MON87705 soybean event was not detected. Many samples contained more than one GM event, and the most common combination was MON40-3-2/MON89788, followed by MON40-3-2/MON89788/MON87701 and MON 40-3-2/MON89788/MON87701/A2704-12. In contrast to these findings, we also identified two samples containing only the MON40-3-2 GM event.

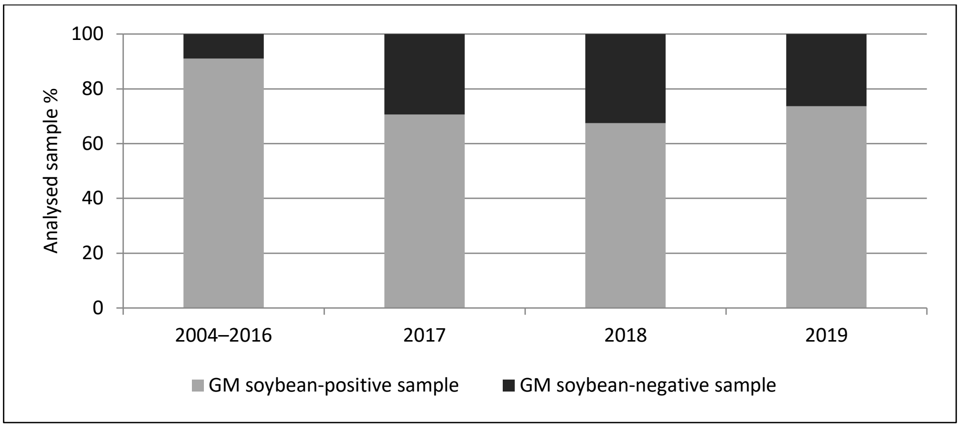

Fig. 1. Percentage of positive and negative samples in the total pool of samples

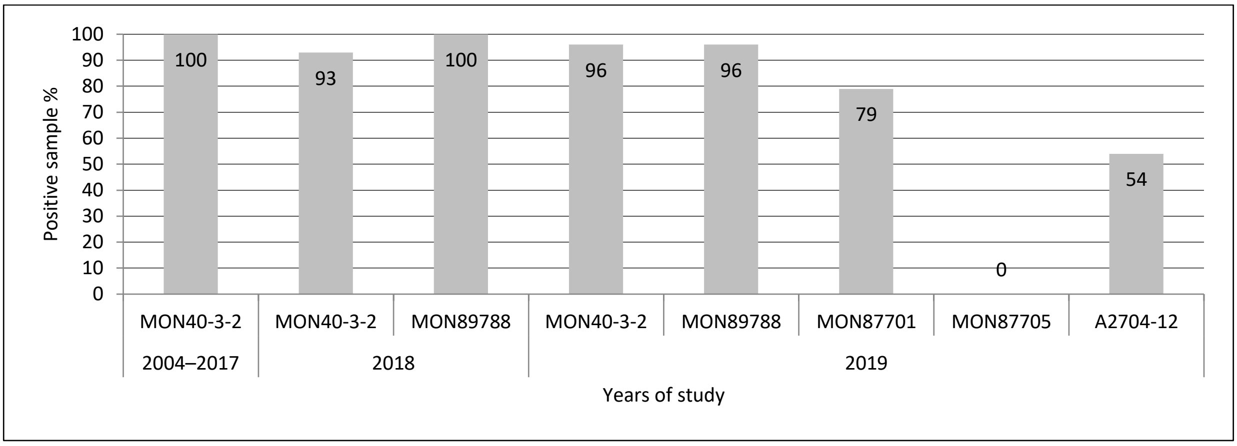

Fig. 2. Percentage of GM events in the total pool of GM soybean-positive samples in three stages of the study

Discussion

Just as many other countries in the EU, Poland depends on GM soybean meal as a major source of feed protein and imports it mainly from South America and the United States. Official data from the European Commission stated that in 2014 the EU was 70% dependent on imports of protein-rich crops (5, 8). Imports of soybean meal between 2000 and 2009 ranged from 1.5 to about 1.8 million tons, and currently Poland imports around 2.3–2.5 million tons of soybean meal per year, a figure that has remained constant over the past few years. For now, relevant EU arable production cannot meet the EU feed protein demand. Production of soybean, rape and sunflower seeds as well as pulses and other legume crops offsets the EU dependence on soybean and soymeal imports to a limited extent (8). A rough estimate derived from the same European Commission data was that around 85% of imported soybean was GM. This was confirmed by a wide interlaboratory study on 135 samples (116 of them containing soybean) providing an insight into the profile of the GM events found across the EU in 2014 (23). A total of 5 soybean GM events were identified, and among them MON40-3-2 (89/116, 77%) was first on the list, followed by MON89788 (46/116, 40%) and A2704-12 (27/116, 23%). More than 10 samples also contained MON87701 and a few samples were positive for the DP-356043 GM soybean event. Kleter *et al.* (18) stated that at the time of writing in 2018, an estimated over 90% of feed materials in EU were labelled as containing GMOs or GMO-derived materials. The widespread use of GM soybean in Poland is confirmed by research results obtained over the last 15 years. In the majority of analysed samples (92%), genetically modified soybean was determined at above the 0.9% level. Since 2004, it has been evident that the MON40-3-2 soybean variety is a major GM crop used in Poland. This should not come as a surprise, taking into account that this variety was and still is the most-grown GM soybean event globally. The presence of MON40-3-2 is common in food and feed in many countries, according to reports of other authors (1, 3, 6, 10, 13, 15, 20, 22, 24, 25, 26, 28, 31, 32). Its presence in feed on the Polish market may also be traced back to member states of the EU, other European countries, and third countries like Ukraine, which export soybean within and to the EU. Deliberate cultivation of GM soybean was identified in Romania after its accession to the EU in 2007, which was likely linked to the pre-accession cultivation of Roundup Ready soybean (MON40-3-2) (22, 31). This same variety was detected in 96 out of 111 soybean samples collected from six administrative regions in Ukraine. The authors concluded that GM crops were grown and sold there (10).

From the documentation provided with samples and from the results from the last three years of the study, it can be clearly seen that the soybean meal submitted for analysis is labelled as GMO-free and is actually free of it. In the majority of these samples, the presence of genetically modified soybean is still detectable, but at very low levels, near or below the limit of quantification of real-time PCR methods. Only 6 out of 645 meal samples were totally free of GM soybean, which is less than 1%. The presence of GM soybean at low levels (usually less than 0.1%) is probably a consequence of contamination of the sample with genetically modified soybean raw material. It bears emphasising that the increasing presence of non-GM soybean on the feed market stems from the necessity to adapt to the requirements of consumers, food producers and retailers. GMO-free claims on labelling nowadays seem to betoken higher quality. Moreover, a general issue with GMO-free labelling is that the label itself may signal to consumers that GMOs are unsafe (4). Due to this, in the last five years many producers of food of animal origin have started to maintain GMO-free systems of production, even though there have been no legal regulations in force to mandate such for the Polish market.

The situation in Poland is very similar to the way the GMO-free market developed in Germany (27). Data gathered from Germany in April 2017 presented 6,170 products labelled as GMO-free, and the Verband Lebensmittel ohne Gentechnik (VLOG), the German Industry Association for Food without Genetic Engineering, estimated revenue of 4.4 million euros was generated with GMO-free-labelled products in the same year (30). Venus *et al.* (29) reported that 76% of those German products were from livestock farming and each of the three main animal-origin product groups (dairy, poultry, and eggs) accounts for about a quarter of the share of the total products carrying a GMO-free mark. Other product categories (comprising the remainder of the total) are pasta and cereal, beverages, honey, and others. Although consumers demand clear GMO-containing and GMO-free labelling, this can lead to new misinterpretations connected with food quality. Even with perfect information, while some consumers gain by having an opportunity to choose GMO-free products, others may lose by paying increased prices through retailers' product differentiation (29). In Poland, where the food market is closely connected to that of Germany, GMO-free labelling also started with eggs, poultry meat and a wide range of dairy products from many manufacturers. The difference is that in Poland GMO-free labelling was started and maintained by food producers without clear applicable legislation in place. Each company implemented it in their own way, taking into account GMO food and feed provisions and the strictures of EU regulations (9). This very substantial industry movement was finally given legislative treatment by the Ministry of Agriculture and Rural Development in the Act on the labelling of products produced without the use of genetically modified organisms as free from those organisms, which was entered law in June 2019 (12).

Article 15 of the Polish Act on animal feedstuffs of 2006 was to forbid the use of genetically modified feed, but it has not come into effect (11). In 2019 a new amendment was introduced, and it once more shifts the date of entry into force of the prohibition on the manufacture, placing on the market and use in animal nutrition of genetically modified feed and genetically modified organisms. According to its provisions, this ban will apply from 1 January, 2021. GM-free soybean may already come from Brazil, Ukraine or domestic Polish cultivation, which is gradually increasing from year to year. Brazil is a good example of a country in which GM and non-GM soybeans are grown (18), the labelling of GM food is mandatory, and the food industry has adjusted to the legislation with respect to consumer requirements (3). Although Brazilian GM soybean is exported to the EU or used for food and feed production on a major commodity scale, non-GMO food in Brazil is available and food is properly labelled for GMO presence (6).

Substitution of genetically modified feedingstuffs in animal nutrition is possible; however, it involves importation of non-GMO materials for higher prices or research into the acquisition of feed protein from domestic sources. Achieving total alleviation of consumer concern and completely liberating the Polish market from GMOs is and will be extremely difficult, because there are no substitutes for soybean flour so far. Conducting monitoring for GMOs is therefore an indispensable element of exercising restrictive control. The production, storage or transport of GM-free feed material must take place under appropriate conditions that prevent contact between it and genetically modified material. In the event of non-compliance with these rules, it is easy for GMO products to contaminate GMO-free ones and for them to be used outside of controlled production and supply processes. In response to these developments in the market's regulatory environment, some retailers and processors have begun to impose GMO-free requirements on the primary stage of production.

Despite the widespread presence of GM soybean in animal feed on the Polish market, there is a lack of publicly accessible data that provide detailed information regarding the trade in and use of GM feed materials counter posed with GMO-free equivalents. A trend fuelled by this towards marking products as GMO-free is possible to observe, which could make the entire GMO labelling system uncongenial and distorted.

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