PREVALENCE OF GENETICALLY MODIFIED CROPS IN ANIMAL FEEDINGSTUFFS IN POLAND – THREE YEAR STUDIES

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Abstract

The aim of this work was to detect, identify, and quantify genetically modified crops by DNA analyses for the monitoring of GMO in the Polish feed market. This approach was based on PCR and Real Time PCR techniques. Traditional PCR was used for the detection and identification of GMO. Out of the 232 samples tested within 3 years (2004-2006), GMO was detected in 141 (61%) samples. The highest content of GMO was found in soybean meal samples. This could be the possible result of the import of GM soybeans such as Soya Roundup Ready from the USA, Brazil, and/or Argentina, and its application as a protein sources in animal feedingstuffs. There were only 7 samples that contained GM maize. Within 3 years of this study the tendency of using GMO in animal feeding was stable because about 60% of the samples were GMO positive each year. According to EU law, if the level of GMO content is more than 0.9%, the product must be labelled appropriately.

Key words: feeds, soybean, maize, genetically modified organisms.

Genetically modified organisms (GMO) are one of the major subjects of investigations in biotechnology laboratories all over the world. Genetic engineering allows for modifying genome of all kinds of organisms, also the most common crops e.g. maize, soya, canola, and to receive organisms with demanded features. Moreover, such new GMO are nowadays planted in fields, not only in closed greenhouse facilities, as source of feed and food. The first commercially used genetically modified (GM) plant was Flavr Savr tomato in 1994. Two years later, a commercialisation of GMO in agriculture, with genetically modified soya, maize, canola, cotton, sugar beets, papaya, wheat, rice, and many other plants started (1). In 2006, the global biotech crop area continued to soar as the 100 million hectares barrier was crossed. Most commonly GM crop used was soya (58.6 million hectares, 57% of global biotech area), followed by maize (25.2 million hectares, 25%), cotton (13.4 million hectares, 13%), and canola (4.8 million hectares, 5%). Taking into account the features of GM crops, herbicide tolerance is the major modification (68% or 69.9 million hectares) followed by Bt insect resistance (19% or 19 million hectares) and both previously mentioned traits stacked together occupied 13.1 million hectares (13%). In 2006, 10 million farmers in 22 countries planted 102 million hectares of biotech crops, in both industrial and developing countries (4). Currently planted GM plants are the first generation of GMO, but in the near future, the next generation of GMO will enter the market. This can be the genetically modified maize with increased lysine content and potato with altered starch composition, which are now in registration process in European Food Safety Authority. This is a sign of the trust and confidence in crop biotechnology. On the other hand, genetic engineering of plants used to food and feed production is a serious problem in many countries and evokes apprehension about food and feed safety, mostly in European countries. The European Union issued the law restrictions regarding the usage of GMO. From the legal point of view, there is a requirement to label products as genetically modified when they contain more than 0.9% of GMO or are produced from GMO (12, 13, 15, 16).

The aim of the study was to determine how often GM feed materials and compound feedingstuffs with GM crops are used in the Polish feed market, and to identify the sources of GMO (domestic feed or imported feed) along with kinds of GM organisms. However, the most important was to establish a monitoring programme for GMO in feed and check the correctness of information on GMO in labels on feed batches.

Material and Methods

Samples used for the determination of GM in feedingstuffs were taken by the Veterinary Inspection from 8 provinces of the East and Central Poland in the period from 2004 to 2006. The sampling was done with accordance to the National Control Plan for Feed.
In 2004, altogether 78 samples were examined for GMO, in that 23 samples of soybeans, 26 samples of maize, and 29 compound feed samples.

In the next year, 80 samples of feed were taken, that were 45 soybeans samples, 33 maize samples, and 2 samples of compound feed.

In 2006, the determination of GMO was done on 74 samples, which comprised of 20 soybean samples, 15 samples of maize, 32 samples of compound feed, and 7 samples of canola. The data are summarised in Table 1.

For GMO analyses, qualitative PCR and quantitative Real Time PCR methods were used. Qualitative methods included screening methods for CaMV 35S promoter and NOS terminator (Fig. 2), species specific PCR for invertase gene from maize and lectin gene from soya (Fig. 3), and event-specific PCR methods for the determination of 4 events of GM maize and 1 event of GM soya. The GMO canola was determined by screening for CaMV 35S promoter and NOS terminator in PCR reaction. Methods used in the laboratory were based on scientific publications and EN ISO as regards GMO (2, 3, 7-9, 17).

Species specific methods (invertase and lectin genes) determined the presence of amplifiable maize or soybean DNA in the sample and gave information about the absence of PCR inhibition elements.

It should be pointed out that GMO positive samples were these in which the reference genes, CaMV 35S or/and NOS, and one of GM events were found.

The percentage content of GMO was determined by Real Time PCR in Roche LightCycler 2.0. To determine the quantity of GM maize, commercial kits from CONGEN Biotechnology GmbH (SureFood® GMO MON810 Corn, SureFood® GMO T25 Corn) were used. With regard to Soya Roundup Ready (RR), the method described by Pietsch et al. (10), after our modification of Master kit, was used (14). This method works with TaqMan probes and Roche LightCycler® TaqMan Master kit. The methodology is an event-specific Real Time quantitative TaqMan® procedure for the determination of the relative content of event EPSPS-CP4 DNA to total soybean DNA. The procedure is a simplex system, in which a lectin endogenous assay (reference gene) and the target assay (35S-CTP4) are performed in separate capillaries. Each assay had one pair of PCR primers and one TaqMan® probe, described below.

PCR primers used for the determination of GM maize:

- Invertase:
  - IVR1-F: 5'-CCG CTG TAT CAC AAG GGC TGG TAC C-3'
  - IVR1-R: 5'-GGA GCC CGT GTA GAG CAT GAC GAT C-3'
- 35S CaMV:
  - 35s-cf3: 5'-CCA CGT CTT CAA AGC AAG TGG-3'
  - 35s-cr4: 5'-TCC TCT CCT CCA AAT GAA ATG AAC TTC C-3'
  - Maize MON810:
  - VW01: 5'-TCG AAG GAC GAA GGA CTC TAA CG-3'
  - VW03: 5'-TCC ATC TTT GGG ACC ATG AAC TTC C-3'
  - Maize Bt11:
  - IVS2-2: 5'-CTG GGA GGC CAA GGT ATC TAA T-3'
  - PAT-B: 5'-GCT GCT GTA GCT GGC CTA ATC T-3'
  - Maize T25:
  - T25-F7: 5'-ATG GTG GAT GGC ATG ATG TTG-3'
  - T25-R3: 5'-TGA GCG AAA CCC TAT AAG AAC CC-3'
  - Maize Bt-176:
  - Cry03: 5'-TCG CCG TCC ATC ATC TCC GT-3'
  - Cry04: 5'-GGT CAG GCT CAG GCT GAT GT-3'

PCR primers used for the determination of GM soya:

- Lectin Le1:
  - GM03: 5'-GCC CTC TAC TCC ACC CCC ATC C-3'
  - GM04: 5'-GCC CAT CTG CAA GCC TTT TGG TG-3'
- NOS:
  - NOS 3 (HA-nos118f): 5'-GCA TGA CGT TAT TTA TGA GAT GGG -3'
  - NOS 4 (HA-nos118r): 5'-GAC ACC GCG CGC GAT AAT TTA TCC-3'
- GM event: EPSPS-CP4/CPT:
  - GM07: 5'-ATC CCA CTA TCC TTC TCA AGA-3'
  - GM08: 5'-TGG GTG TTA TGG AAA TTG GAA-3'

Reagents for Soya Roundup Ready Real Time PCR

- Primers and probes

Lectin assay:
  - sole-af1: 5'-GAC GCT ATT GTG ACC TCC TC-3'
  - sole-ar2: 5'-TGT CAG GGG CAT AGA AGG TG-3'
  - Lec-TM1: 5'-FAM-CAA CTC AAT AAG GTT GAC GAA AAC GGC-TAMRA-3' probe 35S-CTP4 assay:
  - p35s-f2: 5'-TGA TGT GAT ATC TCC ACT GAC G-3'
  - petu-r1: 5'-TGT ATG TTA TGA GCC ATG TGG T-3'
  - RRS-TM1: 5'-FAM-CCC ACT ATC CTT CGC AAG ACC CT-TAMRA-3' probe
For every assay, 4 samples of European Reference Material (ERM) in duplicate were used to prepare calibration curves. As samples of ERM, 4 dilutions of 5% Soya Roundup Ready were taken. Each dilution had estimated number of lectin and GM event copies, calculated from DNA concentration and average molecular weight 1C value for soybean genome. The ssDNA concentration was measured in spectrometer Nicolet Evolution 300 ThermoSpectronic after degradation dsDNA to ssDNA by 0.2M NaOH. This was necessary to determine whole DNA in mixture after extraction from samples.

Real Time PCR of samples was carried out in 3 repetitions and dilutions. After Real Time PCR reaction, Roche LightCycler Software 4 calculated the copy numbers of reference gene, and GM event (Fig. 4.). For the determination of GMO percentage, the copy numbers of lectin gene were divided by the copy of GM event and multiplied by 100 (%GMO = lectin/GM event x 100).

### Results

Results of the determination of GMO in animal feedingstuffs from 2004 to 2006 were shown in Table 1. In 2004, out of 78 examined samples of different matrices, 44 (56%) were positive. Out of 44 positive samples of different feeds 43 contained Soya Roundup Ready, and GM maize, event MON810, was present only in 1 positive sample. Among 23 samples of soybean feed materials, 20 (87%) contained Soya Roundup Ready. Similar data were observed in compound feedingstuffs, where out of 29 samples 21 (72%) contained GM soybeans. In 2 maize samples, contamination by Soya Roundup Ready was found.

In 2005, more samples of feed materials than of compound feedingstuffs were examined. There were checked only 2 samples of compound feed and in both of them Soya Roundup Ready was present. In 44 (98%) out of 45 examined samples of soybean meal the results were positive. The GM maize, events MON810 and T25, were found in 2 and 1 samples, respectively. That was 9% of positive GM maize samples (3 out of 33). The percentage of GMO positive samples increased from 56% in 2004 to 61% in 2005.

In the next year, 74 samples were under investigation. For the first time, 7 samples of canola were examined and all of them were GM negative. The same negative results were obtained for maize samples. Genetically modified soya was present in 100% soybeans samples (20 samples) used as feed material. Out of 32 samples of compound feedingstuffs 28 (87%) were GM positive and contained Soya Roundup Ready (28 samples) and GM maize event MON810 (2 samples) and event T25 (1 sample). Additionally, samples with GM maize contained also GM soya.

<table>
<thead>
<tr>
<th>Year</th>
<th>Sample matrix</th>
<th>Number of samples</th>
<th>Positive samples</th>
<th>Negative samples</th>
<th>Found GM events</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>Soybean meal</td>
<td>23</td>
<td>20 (87%)</td>
<td>3 (13%)</td>
<td>Soya Roundup Ready x20</td>
</tr>
<tr>
<td></td>
<td>Maize meal</td>
<td>26</td>
<td>3 (12%)</td>
<td>23 (88%)</td>
<td>Soya Roundup Ready x2</td>
</tr>
<tr>
<td></td>
<td>Compound feed</td>
<td>29</td>
<td>21 (72%)</td>
<td>8 (18%)</td>
<td>Soya Roundup Ready x21</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>78</td>
<td>44 (56%)</td>
<td>40 (44%)</td>
<td>Soya Roundup Ready x43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maize: MON810 x1</td>
</tr>
<tr>
<td>2005</td>
<td>Soybean meal</td>
<td>45</td>
<td>44 (98%)</td>
<td>1 (2%)</td>
<td>Soya Roundup Ready x44</td>
</tr>
<tr>
<td></td>
<td>Maize meal</td>
<td>33</td>
<td>3 (9%)</td>
<td>30 (91%)</td>
<td>Maize: MON810 x2, T25 x1</td>
</tr>
<tr>
<td></td>
<td>Compound feed</td>
<td>2</td>
<td>2 (100%)</td>
<td>0 (0%)</td>
<td>Soya Roundup Ready x2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>80</td>
<td>49 (61%)</td>
<td>31 (39%)</td>
<td>Soya Roundup Ready x46</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maize: MON810 x2, T25 x1</td>
</tr>
<tr>
<td>2006</td>
<td>Soybean meal</td>
<td>20</td>
<td>20 (100%)</td>
<td>0 (0%)</td>
<td>Soya Roundup Ready x20</td>
</tr>
<tr>
<td></td>
<td>Maize meal</td>
<td>15</td>
<td>0 (0%)</td>
<td>15 (100%)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Compound feed</td>
<td>32</td>
<td>28 (87%)</td>
<td>4 (13%)</td>
<td>Soya Roundup Ready x28</td>
</tr>
<tr>
<td></td>
<td>Canola</td>
<td>7</td>
<td>0 (0%)</td>
<td>7 (100%)</td>
<td>Maize: MON810 x2, T25 x1</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>74</td>
<td>48 (65%)</td>
<td>26 (35%)</td>
<td>Soya Roundup Ready x48</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Maize: MON810 x2, T25 x1</td>
</tr>
</tbody>
</table>
Fig. 1. Percentage of GMO positive samples in three-year study.

Fig. 2. PCR for NOS terminator.

Fig. 3. PCR for lectin gen from soya.

Fig. 4. Screen from LightCycler Software.
Discussion

The presented data demonstrate that the main GMO used as a feed material for producing feed in Poland is genetically modified Soya Roundup Ready. That GM crop was present in 141 samples out of 232 examined in three-year period, from 2004 to 2006. The level of GM soya found in soybean meal samples each year was similar and very high, as it was ranging from 87% in 2004 to 100% in 2005. This data is corresponding to the results obtained with compound feed, where all positive samples of that matrix consisted of or contained Soya Roundup Ready. Moreover, in 3 samples of compound feed, GM maize was detected in 2 cases event MON810 and in 1 sample event T25.

Genetically modified maize was identified only in 7 samples; in 5 of them was the event MON810 and in additionally 2 events T25. The usage of GM maize in Poland is rather rare; and about 10% of samples contained that GMO in 2004 and 2005. In 2006, all 15 examined samples of maize were negative. The results obtained have showed that the major part of maize used in Poland for animal feeding is grown in Poland or in Europe. GM maize was not very popular in EU so far, but in many European countries that cultivations started to be commercially used. In the case of canola used for animal feeding, it was not detectable in any of samples examined for GMO in 2006.

In general, in three year period, out of 232 samples 141 (61%) were GMO positive (Fig. 1). That number of GM positive samples is rather stable and there are no great differences among the analysed periods. Similar data on the presence of GM feed on the market in Poland were published by the National Feed Laboratory in Lublin (NFL). They also demonstrated that GM soya is commonly used in animal feeding. During the investigation of the NFL, in 4 samples of maize GM events were detected (11).

Soya Roundup Ready is widely used in animal feeding because of ban for using processed animal protein. Processed animal protein as meat-bone-meals were commonly used as source of protein in animal feeding, but after BSE crisis, they were eliminated from feed market (5, 6). This is the main reason for which so high level of GM soya is used for the production of feeding stuff in Poland. The second reason is an economical aspect, that genetically modified soybean is chipper than traditional variety.

References

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