Danish Plant Directorate

Genetically modified soya and maize products in animal feed

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Summary

The Danish Plant Directorate has completed a study of genetically modified (GM) feed materials of soya and maize in conventional compound feedingstuffs for pigs, bovines and poultry. The study was based on 102 feed samples collected in the autumn of 2002 from forty feed manufacturers in different parts of Denmark followed by DNA analyses (PCR) in order to determine the content of material from ten GM soya and maize lines.

The objective of the study was to obtain information about the use of GM feed materials before a new EU regulation on approval and labelling of GM feed takes effect from April 2004. Today it is permitted to use processed GM crops as feed without specific measures or product labelling since such crops are regarded as equivalent to the corresponding traditional crops. The study was therefore not part of an official control program.

DNA from Roundup Ready soya (GTS 40-3-2) which is tolerant to the herbicide glyphosate was detected in all the analysed ninety-one samples of compound feedingstuffs with a content of soya feed materials (soya-bean cake). Quantification of the DNA content in thirty-two of the samples suggested that the employed lots of soya-been cake in most cases were derived from pure GM soya crops or crops with a high content of GM soya, since 40-100% of the soya ingredient in twenty-nine of the samples seemed to consist of Roundup Ready soya. In contrast, it was not possible to detect LibertyLink soya (GU262) with tolerance to glufosinate ammonium (nineteen analysed samples). Analyses of three lots of soya-bean cake used for preparation of compound feed gave similar results with respect to the two GM soya.

Nineteen samples of compound feedingstuffs with a content of maize raw materials were analysed for content of GM maize with resistance to herbicides or insects. In five of the samples, GM maize (Mon810, Bt176, T25 or NK603/GA21 maize) were detected, but the levels were below the limit of quantification. The low occurrence of GM maize materials in compound feed was confirmed by analyses of four maize feed ingredients used for preparing compound feed.

The content of the different GM soya and maize materials in compound feedingstuffs was generally as expected from statistical data on the global growth of GM crops and import of soya and maize into Denmark or the EU. If the future use of GM material is unchanged, almost all Danish feedings-stuffs with a content of soya feed materials are likely to be labelled under Regulation EC 1829/2003.

1. Introduction

More than 1 mio. tonnes of soya (soya-bean cake) are each year imported to Denmark for production of animal feed. Most of the soya comes from Argentina, where the production of soya to a large extent is based on genetically modified (GM) plants.

GM crops have so-far been used for production of animal feed without specific measures or product labelling as they from a legal point of view have been regarded as equivalent to the traditional crops. It has therefore been a general assumption that conventionally produced pigs, bovines and poultry in Denmark have eaten feed with a content of GM feed materials, in particular soya-bean cake, but perhaps also products of maize and oilseed rape.

In the summer of 2003, the EU decided on a new regulation on the use of GM food and feed. Regulations are hereby introduced for approval and labelling of feed produced from genetically modified organisms (GMO). As a consequence, GM material without EU approval cannot be used as feed, and feed produced from approved GMOs must be labelled. Exempt from labelling is feed consisting of ingredients with small inevitable and unintended amounts of GM material (less than 0.9%). The new regulation EC 1829/2003 takes effect on April 18, 2004.

In order to obtain information about the use of GM feed in Denmark as a basis for a future official control, samples of conventional feed were collected by the Danish Plant Directorate in the autumn of 2002 for subsequent analyses of GM soya and maize. Since the study was conducted before the new regulation is effective, the detected GM material in feed was not a violation of existing rules.

2. Conduct of study and methods

The samples of feed were collected at forty approved and registered feed manufacturers from different parts of the country from October to December 2002. All samples were from conventional feed, i.e. not from organic or so-called GMO-free feed. In total were taken samples of ninety-five complete or supplementary compound feeds for pigs, bovines and poultry. All compound feeds contained soya and/or maize feed materials. In addition, samples of seven soya or maize products used for production of conventional compound feed were obtained.

From each batch of feed was drawn one sample of 100-200 g. The sample was taken from a limited part of the batch and should therefore not be regarded as representing the complete batch. The sample was handled in a manner which reduced the risk of contamination by feed or DNA.

The homogenised feed samples were analysed for content of GM soya and maize by qualitative and quantitative PCR (1). PCR is based on amplification and determination of specific DNA fragments from the GMO. It is therefore only possible to determine whether a GM plant has been used for the manufacture of a feedingstuff, or to determine the level of the GM material, if the feed contains reasonably intact DNA.

The GMO analyses in this study (see Table 1) included both common GM plants and GM plants which apparently are not marketed (2, 3). Most of the GM plants were modified to allow for the use of the herbicides glyphosate (Roundup) or glufosinate ammonium (Basta, Finale etc.). Many of the GM maize were also made resistant to attack by certain lepidoptera insects, e.g. European Corn Borer.

Table 1. Employed PCR analyses for detection and quantification of GM plant material

Transformation event	Product name	Acquired resistance or tolerance
Soya		
GTS 40-3-2	Roundup Ready	glyphosate
GU262	LibertyLink	glufosinate
Maize		
Mon810	YieldGard	insects
T25	LibertyLink	glufosinate
Bt11	Attribute, YieldGard	insects, (glufosinate)
Bt176	Knockout, Maximizer, NatureGard	glufosinate, insects
DBT418	Bt-Xtra, DeKalBt	glufosinate, insects
CBH-351	StarLink	glufosinate, insects
GA21 and NK603	Roundup Ready	glyphosate
Mon809		glyphosate, insects

The analyses were performed with methods from GeneScan Analytics. All analyses for detection of GM material were carried out by GeneScan (Bremen, Germany) with the exception of two analyses of Roundup Ready soya that were repeated in the laboratory of the Danish Plant Directorate. The quantitative determinations of Roundup Ready soya were in most cases performed by the Danish Plant Directorate, while GeneScan performed the quantitative analyses of GM maize.

3. Results

3.1. GM soya in compound feed

Ninety-one of the compound feedingstuffs contained soya raw materials (different types of soya-bean cake) according to the product specifications. All the samples were analysed by qualitative PCR to detect a possible content of Roundup Ready soja (GTS 40-3-2). Nineteen of the samples were also analysed for content of LibertyLink soja (GU262). Roundup Ready soya is grown commercially in many countries and is also approved for

certain uses in the EU. LibertyLink soya is apparently not a common GM soya and has not been approved in the EU.

The analyses for detection of Roundup Ready soya in compound feed are summarised in Table 2.

Table 2. Detection of Roundup Ready soya GTS 40-3-2 in compound feedingstuffs with a content of soya feed materials

Animal category	Number of analysed feed samples	Number of feed samples in which RR soja was detected
Sows	10	10
Pigs for fattening	29	29
Piglets	12	12
Calves	10	10
Cattle	18	18
Broilers	8	8
Layers	3	3
Ducks	1	1
Total	91	91

As seen from Table 2, DNA from Roundup Ready soya was detected in all the samples of feed with a content of soya.

In contrast, LibertyLink soya could not be detected in any of the investigated feed samples (fourteen feeds for pigs for fattening, three for piglets and two for cattle).

3.2. Quantitative content of Roundup Ready soya in compound feed

As described above, Roundup Ready soya material was detected in all compound feedingstuffs which contained soya ingredients. In order to investigate to which extent the soya ingredients were derived from the transgenic herbicide-resistant plants, we determined the proportion of Roundup Ready soya in the soya ingredient by real-time PCR (as genome/genome %) in thirty-two of the samples. The results are shown in Table 3.

Table 3. Quantification of Roundup Ready soja GTS 40-3-2 by real-time PCR in samples of complete and supplementary compound feedingstuffs with a content of soya (soya-bean cake)

Animal category	Number of analysed feeds	Declared content of soya-bean cake in the feed	Analysed content of Roundup Ready soya (in % of the soya ingredient)[a]
Sows	3	26.2% dehull. toasted, 17.6% toasted 60.7% toasted 10.6% toasted	53 ± 3 25 ± 7 47 ± 9
Pigs for fattening	16	76.4% toasted 16.4% toasted 75.0% toasted, 10.6% dehull. toasted 22.4% toasted toasted 79.3% toasted 19.2% toasted 20.5% toasted 84.0% toasted 84.0% toasted 76.3% toasted 81.0% toasted 77.1% toasted 18.2% toasted 79.5% toasted 66.4% toasted	56 ± 17 89 ± 6 100 ± 20 2 ± 0.4 100 ± 20 47 ± 18 58 ± 12 100 ± 20 100 ± 20 78 ± 11 63 ± 6 48 ± 10 42 ± 7 84 ± 24 76 ± 4 50 ± 13
Piglets	2	24.2% toasted 20.6% toasted	65 ± 12 100 ± 20
Calves	3	32.9% unsieved toasted 30.0% toasted (and 3.0% soja extract)	100 ± 2 53 ± 3 69 ± 13
Cattle	5	8.0% toasted 17.0% toasted 10.5% toasted 30.9% toasted 25.0% toasted	74 ± 17 79 ± 17 68 ± 11 63 ± 3 85 ± 15
Broilers	2	8.3% unsieved, 25.0% dehulled 32.7% dehulled toasted	60 ± 10 7 ± 2
Layers	1	14.0% toasted	75 ± 12

[a] Mean values are given with standard deviation

As seen from Table 3, Roundup Ready soya constituted a large proportion of the soya in most of the compound feeds (40-100%). Only in three feeds were found lower levels (25, 2 and 7%). Most of the compound feeds therefore seemed to contain soya feed materials which were derived from pure or rather pure crops of GM soya. However, it should be noted that the determinations were based on a single sample from a limited part of each feed. The determinations were therefore not necessarily valid for the full batch. In addition, quantitative measurements by PCR are associated with a high degree of uncertainty.

3.3. GM maize in compound feed

Nineteen compound feedingstuffs (sixteen for bovines and three for pigs) with a content of maize feed materials were analysed by PCR for content of the GM maize lines Mon810, T25, Bt11 og Bt176, which are all approved for various purposes in the EU. Twelve of the feeds were also analysed for content of DBT418, CBH-351, GA21/NK603 and Mon809 maize. Of these GM maize, only Mon809 is approved in the EU (3).

The results of the qualitative GMO analyses which showed detectable levels of DNA from GM maize are summarised in Table 4.

Table 4. Detected GM maize lines in compound feed with a declared content of maize products

Animal category	Number of analysed feed samples	Number of feeds showing a content of			
		Mon810	T25	Bt176	NK603/ GA21
Sows	1	0	0	0	0
Piglets	2	0	0	0	0
Calves	3	1	1	0	0
Cattle	13	4	0	2	1
Total	19	5	1	2	1

As the table shows, DNA from Mon810 maize was detected in five compound feedingstuffs for bovines. In addition, one of the feeds was scored as positive for content of T25 maize, two for Bt176, and one for NK603/GA21 maize. Interestingly, the latter three GM maize were all found in feeds which were also scored as positive for content of Mon810 maize. Bt11, DBT418, CBH-351 and Mon809 maize were not detected.

The content of DNA from the observed GM maize lines were in all cases low and could not be quantified.

It should be noted that the five compound feeds with a content of GM maize also contained material from Roundup Ready soya (the analysis in Section 3.1).

3.4. GM material in soya and maize products used for compound feed

Three different soya-bean cake raw materials used for preparation of conventional compound feedingstuffs were analysed for content of Roundup Ready and LibertyLink soya. The results are summarised in Table 5.

Table 5. Detection and quantification of Roundup Ready (RR) and LibertyLink (LL) soya in three soya raw materials

Product	Qualitative PCR analysis of		Measured content of RR soya in the soya product (%)
	RR soya	LL soya	
Soya-bean cake 44/7 unsieved	detected	not detected	64 ± 8
Soya-bean cake toasted	detected	not detected	100 ± 20
Soya-bean cake sieved	detected	not detected	64 ± 23

The results in Table 5 suggest that the three batches of soya-bean cake were derived predominantly or solely from GM soya plants (i.e. up to 100% GM).

Four maize raw materials were analysed for content of the eight GM maize which were the subject of this study. None of the GM maize could be detected.

4. Conclusion

In many countries is the production of soya and maize to some extent based on genetically modified plants. In 2002, the global area for GM crops was about 59 mio. ha located primarily in USA (66% of the global area), Argentina (23%), Canada (6%) and China (4%). Only a small commercial production of GM plants takes place in the EU. The most important GM crops globally were soya beans (37 mio. ha), maize (12 mio. ha), cotton (7 mio ha) and oilseed rape (3 mio. ha). The most important traits were tolerance to herbicides and resistance to attack from insects (4).

The study was performed to obtain information about the use of GM soya and maize feed materials in conventional compound feedingstuffs for Danish production animals with the overall aim of establishing a future official control.

The results with respect to GM soya can be summarised as follows:

- DNA from herbicide-tolerant Roundup Ready soya was detected in all conventional compound feedingstuffs with a content of soya-bean cake (ninety-one analysed feeds for pigs, bovines and poultry).

- Nearly all compound feeds with a content of soya-bean cake (thirty-two feeds analysed) and three lots of soya-bean cake showed a high content of DNA from Roundup Ready soya.

- The high levels of GM soya material (up to 100% GM) suggested that the soya-bean cake in many cases originated from rather pure crops of Roundup Ready soya.

- Herbicide-tolerant LibertyLink soya (GU262) could not be detected in compound feed or pure soya-bean cake.

Feed materials of the common GM soya Roundup Ready soya is therefore very often used in animal feed in Denmark. The frequent use is consistent with import and growth statistics. EU imported in 2001 approximately 16 mio. tons soya, primarily from USA, Argentina and Brazil where a significant part of the soya production is based on Roundup Ready soya. Ten percent of the soya (mainly from Argentina) was imported to Denmark for animal feed, especially pigs (2, 3). It was therefore not surprising that conventional compound feed with a content of soya-bean cake would often be derived from GM soya.

The results with respect to GM maize can be summarised as follows:

- DNA from four types of GM maize was detected in some of the investigated nineteen compound feeds with a content of maize products.

- Mon810 maize was the most common GM maize (detected in 25% of the feeds), but the levels were low and could not be quantified.

- The feeds with a content of Mon810 were in most cases scored as positive for content of one other GM maize (Bt176, T25 or NK603/GA21). The contents were again below the level of quantification.

- Bt11, DBT418, CBH-351 and Mon809 maize were not detected in compound feeds with a content of maize products.

- GM maize lines were not detected in four maize products for the manufacture of compound feedingstuffs.

It therefore appears that feed materials of GM maize are not commonly used for farming purposes in Denmark. The result is again consistent with import and growth statistics. Thus, the import of maize into the EU in 2001 was approximately 10 mio. tons, while the production of maize within the EU was 41 mio. tons (5). Most of the maize was used for animal feed (bovines). The imported maize came primarily from USA and China. In USA, 34% of the area for growth of maize was used for GM maize in 2002, while only very little GM maize is grown within the EU (Spain and Germany) (2).

The four GM maize which were detected in compound feed were all risk-assessed and approved for different uses in many countries. Among the four GM maize which could not be detected in feed was StarLink (CBH-351), which apparently is not commercially grown anymore due to fear of an allergenic potential.

Regulation EC 1829/2003 on genetically modified food and feed takes effect from April 18, 2004. With this regulation, feedingstuffs which contain, consist or is produced from GMOs shall be labelled, unless the content is incidental and technically unavoidable and constitutes less than 0.9% of each ingredient. From the present study it would seem that nearly all Danish compound feedingstuffs with a content of soya raw materials should be

labelled under the new regulation, while labelling of feeds with a content of maize products should be less frequent. The official control of GM feed under the new regulation should probably include sampling and analyses of the pure feed materials as well as the compound feedingstuffs. The present study seems to provide a fine basis for determination of the types of GMO analyses which should be included in the first phases of the control.

References

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