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OCCURRANCE OF GENETICALLY MODIFIED CROPS IN ANIMAL FEEDINGSTUFFS IN POLAND

POJAVA GENETSKI MODIFICIRANIH USJEVA U KRMIVIMA U POLJSKOJ

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Izvorni znanstveni članak
Primljeno: 16. lipanj 2010.

SUMMARY

The aim of this study was to detect, identify, and quantify genetically modified (GM) crops by DNA analyses in feed used for animal feeding in Poland. This approach was based on PCR and Real Time PCR techniques and was applied to official genetically modified feed control in the National Veterinary Research Institute in Pulawy. Within six years studies (2004-2009) in 231 (66%) samples, out of 350 samples of compound feed and feed materials, genetically modified soybean, maize or rapeseed were found. The highest GMO content was found in soybean meal samples and compound feed which consisted of soybean meal. Among 135 analyzed soybean meal samples in 127 (94%) of them GM soya GTS 40-3-2 was present. As regards compound feed samples, GM soya was identified in 93 (83%) samples out of all 112 samples. The presence of GM maize was rare. Only in 11 (11%) samples, out of all 98 samples of maize, GM maize varieties were found (10 x MON810, 2 x Bt11, 2 x T25). Moreover analyses of 112 compound feed samples indicated that, 16 of them contained Mon810, 4 samples Bt11 and 3 samples T25 GM maize varieties. As regards rapeseed analysis in 5 checked rapeseed samples there were no GM varieties. Another study focused only on GM rapeseed, provided for us independently by official feed control, proved previous results. Out of 100 samples only in 2 of them Libertylink™ rape was present in the amount below limit of quantification (LOQ) of our method. Achieved results of feed samples analyses indicate that the use of GM soya is common in animal feeding in Poland as main source of protein. The usage of GM maize is low, but mainly MON810 maize was found this variety is grown in Europe. As regards rapeseed there were very few GM rape seed positive samples.

Key words: genetically modified crops, DNK analyses, Poland

INTRODUCTION

The commercialization of genetically modified (GM) or transgenic plants started in 1996. Nowadays, around the whole world many farmers are using biotechnology enhanced crops to increase their harvest or to make the production more

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profitable. There are many varieties of maize, soybean, cotton, rapeseed or sugar beet plants which are genetically modified (1). As regards four major GM plants about 70% of soybean, 46% of cotton, 24% of maize and 20% of canola grown in the world is genetically modified. Those GM crop varieties are mainly modified to increase tolerance to herbicides or pests. Nowadays crops which contain two or more genetical modifications have become more popular. That connection of few GM genes in one plant is called stacked-genes (e.g. GM maize MON810xMON863xNK603). Roundup Ready soybeans meals are widely used in Europe as a protein source after the BSE crisis. New generations of transgenic crops have altered their composition to enhance product quality or valuable new traits, which are aimed at satisfying industrial needs and the desires of everyday consumers. On the other hand, genetic engineering of plants used for food or feed production is a serious problem in many countries as regards food and feed natural environment safety, mostly in European countries. The European Union issued law restrictions regarding the usage of GMO. From the legal point of view, there is the requirement to label products as genetically modified when they contain more than 0.9% of GMO or are produced from GMO (6, 7).

The aim of this study was to detect, identify, and quantify genetically modified (GM) crops by DNA analyses in feed used for animal feeding in Poland. It was done mainly in accordance with the National Control Plan for Feed. This control was based on PCR and Real-time PCR techniques and was applied for GM feed analyses in the National Veterinary Research Institute (NVRI) in Pulawy.

MATERIAL AND METHODS

The determination of GM maize, soybean and rapeseed was carried out on samples taken by Veterinary Inspection from East and Central Poland. The sampling was done in accordance with the National Control Plan for Feed for 2004-2009 or by NVRI science project for GM rape seed analyses. Within six years studies conducted as official GM feed control 350 samples of compound feed and feed materials were analysed. The examined material consisted of 135 soybeans meals samples, 112 compound feed samples, 98 maize kernels samples, and 5 samples of rapeseed. As regards NVRI science project for GM rapeseed, 50 rapeseed samples and 50 compound feed samples with rapeseed were analysed.

The detection and identification procedure for genetically modified maize used in our laboratory contained PCR reactions, which amplified reference maize gene: invertase, GM maize screening method for promoter CaMV P35S, event-specific PCR reaction for MON810, and construct-specific PCR reactions for Bt11, Bt176 and T25 GM maize. As regards GM soybean (event GTS 40-3-2), the procedure consisted of soya genome specific PCR for lectin gene, two screening methods (for promoter CaMV P35S and terminator NOS) and event-specific PCR for GTS 40-3-2 event. As regards GM rapeseed the amplified DNA fragment were: *pat(syn)* gene, promoter FMV, EPSPS gene, napine promoter and ACP gene, present in some GM canola varieties. PCR was done at Biometra T1 thermocycler, with dNTP's (Invitrogen), PCR primers (Oligo.pl) and PCR buffer II, MgCl2 and AmpliTaq Gold Polymerase supplied by Applied Biosystems.

The quantification of genetically modified maize was done by the amplification of reference maize genes: hmg (MON810, Bt11), zSSIIb (Bt176), adh1 (T25), and by event-specific PCR's for MON810, Bt11, T25, or unique DNA sequence of genes (cryIAb/IVS9) used for modification of Bt176 GM maize variety. As regards GM soybean event GTS 40-3-2, soya specific lectin gene and GM construct-specific DNA fragment (CaMV35S/ CTP- EPSPS) were amplified. Quantification for GM rapeseed LibertyLink variety was done by GeneScan commercial kit (GMO*Quant* LibertyLink™ Canola). Real-Time PCR reactions were conducted on Roche LightCycler 2.0 by TaqMan probes (labelled by FAM and TAMRA dyes), with Real-Time PCR primers and probes (Oligo.pl) and LightCycler TaqMan Master supplied by Roche Diagnostics.

Methods used in NVRI are based on scientific publications (2, 3, 4, 5, 8) or are mentioned and proposed by Community Reference Laboratory (CRL) for GM food and feed (Joint Research Centre in Ispra, Italy) (9, 10).

RESULTS

Within six years studies of official GM feed control (2004-2009) in 231 (66%) samples, out of 350 samples of compound feed and feed materials, genetically modified crop varieties were found (Table 1). The highest GMO content was found in soybean meal samples and compound feed which consisted of soybean meal. Among 135 analyzed soybean meal samples in 127 (94%) of them GM soya GTS 40-3-2 was present. As regards all analysed 112 compound feed samples, in 93 (83%) of them GM soya was identified. The presence of GM maize was rare, out of 98 samples of maize, only 11 (11%) samples were GM positive (10 x MON810, 2 x Bt11, 2 x T25). Moreover analyses of 112 compound feed samples for GM maize indicated that, 16 of them contained Mon810, 4 samples Bt11 and 3 samples T25 varieties. As regards rapeseed analysis in 5 checked rapeseed samples there were no GM rape varieties.

Our NVRI studies conducted additionally to official GM feed control in Poland focused on genetically modified rapeseed proved those results achieved previously. Only in 2, out of 100 samples of rapeseed and compound feed samples, GM rape Libertylink^{TM} variety was present. Moreover, GM rape quantities were in two cases below the limit of quantification (LOQ) of our method.

DISCUSSION

Results achieved every year from 2004 are very similar, about 60% of samples are GM positive. Every year the major GM crop is genetically modified soya, whose GM variety GTS 40-3-2 is widely used in animal feeding in Poland. As regards GM maize the situation is a little different, more GM maize is used since 2006, due to GM maize MON810 grown in European countries and in Poland. Rapeseed used for animal feeding in Poland is taken from rapeseed oil production for human consumption. Manufactures prefer to use non-GM rapeseed because of the GMO negative campaign in mass-media.

Results achieved indicate that the most common genetically modified plant used for animal feeding in Poland was soya Roundup Ready. About 94% of soybean meals samples contained transgenic soya which is also used for the production of compound

Table 1. Presence of GM crops varieties in part of feed market in Poland in 2004-2009

Tablica 1. Prisutnost GM usjeva u dijelu krmiva u Poljskoj u 2004-2009

Matrix	Number of samples	Positive samples	Negative samples
Soybean meal	135	127 (94%) 127 x RR soya	8 (6%)
Maize	98	11 (11%) 10 x MON810 2 x Bt11 2 x T25	87 (89%)
Rapeseed	5 – official control	0 (0%)	5 (100%)
	100 – NVRI survey	2 (2%)	98 (98%)
Compound feed	112	93 (83%) 93 x RR 16 x MON810 4 x Bt11 3 x T25	19 (17%)
Total	350	182 (63%)	108 (37%)
	450 – with NVRI	184 (41%)	266 (59%)

feed and supplementary feedstuffs. This is connected with the banned use of meat-bone-meals (MBM) in Europe after the BSE crisis. Soya as a rich source of proteins takes the place of MBM in the production of compound feed for animals.

The results obtained show that GM maize is not as popular as GM soya. Only 2 samples contained transgenic maize at low level. In Europe, most of maize used for feed production originates from internal cultivations, the majority of which is not modified so far. In some European Countries GM maize is cultivated commercially. This is becoming more and more popular, because of growing problems with insects such as European Corn Borer. Nowadays that insect has become a serious problem for European farmers, interested in using GM maize varieties.

Similar data as regards the presence of GM feed on the market in Poland have been presented by the National Feed Laboratory in Lublin (NFL). They also demonstrate that GM soya is commonly used in animal feeding. During the investigation of the NFL, only 4 samples of GM maize GM were detected.

Our results correspond to the assumptions of the European Feed Manufacturers Federation which indicates that 8% of the whole feed quantity consumed, but present in 95% of compound feed produced in the EU, potentially concern biotechnology. That feed usually contains or consists of Roundup Ready soya.

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SAŽETAK

Cilj ovog rada bio je otkriti, identificirati i kvantificirati genetski modificirane (GM) usjeve DNK analizama u hrani za životinje u Poljskoj. Pristup se temeljio na tehnikama PCR i Real Time, što se primjenjuju u službenoj kontroli genetski modificirane hrane u Državnom veterinarskom istraživačkom institutu u Pulawyju. U šest godina istraživanja (2004-2009) na

211 (60%) uzoraka, od 350 uzoraka krmnih smjesa i krmnog materijala, pronađeni su genetski modificirana soja, kukuruz i uljna repica. Najviši sadržaj GMO-a nađen je u uzorcima sojinog brašna i krmnih smjesa koje su se sastojale od sojinog brašna. Među 135 analiziranih uzoraka sojinog brašna u 127 (94%) nalazio se GM soje GTS 40-3-2. Što se tiče uzoraka krmnih smjesa u 93 (83%) uzorka GM soje identificiran je u svih 112 uzoraka. Prisutnost GM kukuruza bila je rijetka. Samo u 11 (!!%) od svih 98 uzoraka kukuruza nađene su vrste GM (10 x MON810, 2 x Bt11, 2 x T25). Osim toga analize 112 uzoraka krmnih smjesa pokazale su da ih je 16 sadržavalo Mon810, 4 uzorka Bt11 i 3 uzorka T25 vrste kukuruza. Što se tiče analize uljne repice u 5 pregledanih uzoraka nije bilo GM vrsta. Druge analize usmjerene samo na GM uljne repice, što smo ih obavili neovisno o službenoj kontroli hrane potvrdile su gornje rezultate. U 100 uzoraka samo u 2 uzorka nađen je Libertylink TM repice u iznosu ispod granice kvantifikacije (LOQ) naše metode. Dobiveni rezultati analiza uzoraka krmiva pokazuju da je upotreba GM soje uobičajena u hranidbi životinja u Poljskoj kao glavni izvor bjelančevina. Upotreba GM kukuruza je sada na niskoj razini, a nađen je uglavnom kukuruz MON810 zbog uzgoja te vrste u Europi. Što se tiče uljne repice bilo je vrlo malo pozitivnih GM uzoraka.

Ključne riječi: genetski modificirana krmiva, DNK analiza, Poljska