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Nutritional assessment of feeds from genetically modified organism

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ABSTRACT

In addition to feed safety assessment, including safety for consumers, animals and environment nutritional assessment of feeds produced using recombinant DNA techniques is necessary and should be considered as an essential part of safety assessment. Therefore we need effective systems to assess GMO's in feedstuffs from the nutritional point of view. In 1997 we started a programme to assess GMO's of the so – called first generation including Bt-maize, Pat-maize, Pat-sugar beets and Gt-soyabeans.

Digestion and feeding experiments were carried out with broilers (Bt-maize), layers (Bt-maize, Pat-maize), pigs (Bt-maize, Pat-maize, Pat-sugar beet, soyabeans), sheep (Bt-maize silage, Pat-maize silage), growing bulls (Bt-maize silage) and fistulated cows (Bt-maize silage). Up to now, no significant differences in nutritional assessment between feeds from isogenic and transgenic plants of the first generation were found. The so-called substantial equivalence of transgenic hybrids could be demonstrated. Recombinant plant DNA constructs were not detected in animal tissue samples.

Proposals for nutritional assessment of GMO including search for unexpected (unintended) effects and aspects of safety assessment are discussed in the paper.

KEY WORDS: GMO, ingredients, nutritional assessment, fate of DNA, safety assessment

INTRODUCTION

Genetical modification of plants opens a new period of plant breeding. Enormous efforts were made with respect to the development of crops containing genetically modified traits during the last 10 year. Cultivation of GMO's increased from 2 million ha in 1996 to over 51 million ha in 2000.

Currently herbicide (e.g., Gt-soyabeans equipped with tolerance gene against glyphosate or Pat-maize or sugar beets equipped with tolerance gene against gluphosinate-ammonium) and insecticide tolerant plants (e.g., Bt-maize, equipped with a gene from *Bacillus thuringiensis*) have been successfully commercialised and represent a considerable proportion of the annual crops all over the world.

Presently GMO-research could characterize the start point of a development to preserve natural resources and to improve food supply for human population all over the world. From our understanding objectives of plant breeding including genetic modification should be:

- Feed production which preserves resources (water, nutrients, space etc.), resistance to pests, tolerance of drought, salty soil etc.
- Lessening the content of undesirable (antinutritive) substances in feeds and foods (e.g., secondary plant substances, residues from contaminants, mycotoxins etc.)
- Increasing the content and the availability of value determining substances (e.g., amino acids, fatty acids, vitamins, enzymes), better digestibility, higher utilization of energy and nutrients, less pollution per animal product (milk, meat, eggs).

From the view of animal and human nutrition one can distinguish in GMO's of the first and second generation:

1^{st} Generation: Feed plants are characterized by changed tolerance or resistance to insects, herbicides, pesticides or other influencing factors with minor changes in nutrient content (e.g., Bt-maize, Pat-maize, Gt-soyabeans etc.)

2^{nd} Generation: Feeds are characterized by substantial changes in the content of valuable or undesirable major (e.g., protein, amino acids, fat, fatty acids, starch, sugar, lignin) or minor ingredients (e.g., vitamins, minerals, enzymes, antinutritive ingredients).

This distinction is subjective, but it allows some recommendations for nutritional assessment of feeds. The concept of substantial equivalence introduced by OECD (1993) must be mentioned in connection with safety and nutritional assessment of GMO.

The term substantial equivalence was introduced to compare transgenic foods with their isogenic counterparts. Substantial equivalence embodies the concept that if a new food (or feed) or food (or feed) component is found to be substantially equivalent to an existing food (feed) or food (feed) component, it can be treated in the same manner with respect to safety as its traditional counterpart (OECD, 1993).

The concept of substantial equivalence should be considered as a framework to the safety assessment, but that for genetically modified crops with more complex traits (GMO of 2^{nd} generation) the assessment of nutritional aspects and long-term effects should be investigated. The objective of the present paper is to summarize experiments for nutritional assessment of GMO's of the first generation and to discuss some recommendations for nutritional assessment of GMO. The experiments should contribute to develop a "Novel Feed" regulation (EU, 2000a).

EXPERIMENTS WITH GMO

In 1997 we started at our institute with nutritional assessment of GMO's of the first generation. Composition of feeds, digestibility, feeding experiments, animal health and performance, quality of foods of animal origin and fate of DNA were included in our studies (Table 1). In the mean time studies with Bt-maize (*Bacillus thuringiensis* gene), Pat-maize and Pat-sugar beets (Phosphinoltricinacetyl-transferase gene; Gluphosinate-ammonium-tolerant) and Gt-soyabeans (Glyphosate-tolerant gene) were done at our Institute.

The objective of the experiments was a complex nutritional assessment of isogenic and transgenic hybrids based on measuring of important major and minor ingredients, digestibility and animal performance studies.

In all experiments parental varieties were compared with transgenic hybrids of the first generation of GMO (without substantial changes in composition). The

TABLE 1

Investigations using GMO's of the first generation which have been carried out at the Institute of Animal Nutrition of the FAL Braunschweig, Germany (always compared with parenteral hybrids)

Investigation	Bt-maize		Pat-maize		Pat-sugar beets		Gt-soyabeans
	seeds	silage	seeds	silage	beets	leaf silage	full fat beans
Ingredients							
crude nutrients	x	x	x	x	x	x	x
amino acids	x	-	x	-	-	-	x
fatty acids	x	-	x	-	x	-	-
minerals	x	-	x	-	x	-	x
fibre fractions	x	-	x	-	-	-	x
mycotoxins	x	-	-	-	-	-	-
Animal experiments							
Poultry							
broiler	B/F[2]	-	-	-	-	-	-
layers	B/F[2]	-	B/F	-	-	-	-
Pigs	B/F[1,2]	-	B/F	-	B/F	-	F[1,2]
Ruminants							
sheep	-	B	-	B	B	B	-
growing bulls	-	F[2]	-	-	-	-	-
dairy cows	-	B[2]	-	-	-	-	-

x – measurements, – no data

B – digestion or balance studies

F – feeding trials with performance registration

[1] not yet finished

[2] including studies on the fate of DNA (Einspanier et al., 2001; Reuter et al., 2001)

transgenic and parental cultivars were grown under the same conditions on the experimental fields in the FAL. The gluphosinate tolerant crops were sprayed in the course of the growing season with conventional herbicides, but on some areas a gluphosinate (Basta) was applied. This fact enables a comparison of the parental cultivars with two different treated transgenic variants (variants 1 to 3). Composition of feeds and faeces (digestion experiments) was determined by using of VDL-UFA-methods (Naumann and Bassler, 1993). From some digestion experiments and all feeding trials tissue samples were taken from animals to follow the fate of DNA or DNA-fragments using the PCR-technique (Einspanier et al., 2001). More details of animal experimentation are described by Aulrich et al. (2001) and Böhme et al. (2001).

RESULTS

Most results from our experiments were published by Aulrich et al. (2001; Bt-maize) and Böhme et al. (2001; Pat-maize, Pat-sugar beets). Some informations were also given by Aulrich et al. (1998), Böhme and Aulrich (1999), Daenicke et al. (1999 a,b, 2000) and Halle et al. (1999).

Up to now we did not find significant differences between feeds from isogenic or transgenic plants of the first generation. Tables 2 and 3 show results from experiments with seeds and silages from isogenic and transgenic (Bt) maize. There were no significant differences in composition and digestibility of products from isogenic and transgenic products. Pigs and growing bulls consumed adequate amounts of feed from isogenic or transgenic maize and did not differ in daily weight gain (P>0.05; Tables 2 and 3).

The so-called substantial equivalence (OECD, 1993) could be demonstrated in all experiments done at our Institute (Table 4).

TABLE 2

Influence of isogenic and transgenic (Bt) maize seeds (70% of diet) on digestibility of diet, energy concentration and fattening data of pigs (n=12; initial body weight: 35 kg per animal, duration: 91 days; Reuter et al., 2001)

	Isogenic maize	Transgenic (Bt) maize
Digestibility, %		
crude protein	84.7 ± 2.3	86.0 ± 1.8
N-free extractives	92.7 ± 0.6	93.1 ± 0.6
Metabolizable energy, MJ/kg DM	15.7 ± 0.2	15.7 ± 0.2
Feed intake, kg/d	2.06 ± 0.10	2.04 ± 0.16
Daily weight gain, g	815 ± 93	804 ± 64
Feed efficiency, kg/kg	2.55 ± 0.27	2.59 ± 0.18

TABLE 3

Ingredients, digestibility in sheep (n=4), growing and slaughtering data of bulls (n=20, initial body weight: 188 kg per animal; duration: 246 days) fed with maize silage made from isogenic or transgenic (Bt) hybrids (Daenicke et al., 1999a)

	Silage from isogenic maize	Silage from transgenic (Bt) maize
Dry matter, g/kg	337	321
Crude nutrients, g/kg DM		
crude protein	84	87
crude fibre	186	191
N-free extractives	656	652
Digestibility, %		
organic matter	75.0 ± 3.5	74.5 ± 2.0
crude fibre	66.7 ± 4.4	68.1 ± 3.6
N-free extractives	81.2 ± 2.3	80.8 ± 1.3
Metabolizable energy, MJ/kg DM	10.95 ± 0.03	10.91 ± 0.04
Growing and slaughtering data		
silage intake, kg/d	18.8 ± 1.0	18.7 ± 0.7
energy intake, MJ ME/d	91.2 ± 4.2	88.6 ± 3.2
daily weight gain, g	1487 ± 97	1482 ± 1.21
dressing percentage, %	52.4 ± 1.5	52.8 ± 1.1
belly cavity fat, kg	49.6 ± 5.5	48.7 ± 8.1

Furthermore we followed the fate of DNA in some of our experiments (Table 1; Einspanier et al., 2001; Reuter et al., 2001). In some organs and tissues a short maize chloroplast gene fragment (199 bp) was amplified. Bt-gene specific constructs originating from recombinant Bt-maize were not detectable in any of these animal samples.

Most authors from other research stations agree with our conclusions regarding substantial equivalence (Table 4). In some cases significant differences between transgenic plants and their isogenic counterpart were observed (Table 4). Those data should not be overestimated because of low differences between both varieties (e.g., Brake and Vlachos, 1998), or shortcomings in experimental design (e.g., Hammond et al., 1996). This experiment with dairy cows was critically analysed in one of our previous papers (Flachowsky and Aulrich, 1999).

Normally there is a wide range in composition of feeds and foods of one group as demonstrated in feed and food value tables (Nehring et al., 1971; DLG-Tables, 1997). Therefore small differences in composition of feeds should not be overestimated.

TABLE 4

Summary of experiments on nutritional assessment of transgenic feeds of the first generation in comparison with isogenic hybrids

Authors	Transgenic feed	Ingredients[1]	Feeding to animal species	Nutritional evaluation[1]
Hammond et al. (1996)	Gt-soyabeans	≈	Rats	≈
			Broilers	≈
			Fish	≈
			Dairy cows	≈(↑)[2]
Maertens et al. (1996)	Rape seed	≈	Rabbits	≈
Faust and Miller (1997)	Bt-maize, fresh	≈	Dairy cows	≈
Aulrich et al. (1998)	Bt-maize, seeds	≈	Laying hens	≈
Brake and Vlachos (1998)	Bt-maize, seeds	≈	Broilers	≈(↓)[3]
Böhme and Aulrich (1999)	Pat-maize, seeds	≈	Pigs	≈
Böhme und Aulrich (1999)	Pat-sugar beets	≈	Pigs	≈
Daenicke et al. (1999 a,b)	Bt-maize, silage	≈	Sheep	≈
			Beef cattle	
Halle et al. (1999)	Bt-maize, seeds	≈	Broilers	≈
Rutzmoser et al. (1999)	Bt-maize, silage	≈	Dairy cows	≈
Barriere et al. (2000)	Bt-maize, silage	≈	Dairy cows	≈
			Sheep	
Clarke and Ipharraguere (2000)	Maize varieties (20 experiments)	≈	Dairy cows	
			Beef cattle	
			Poultry	
Daenicke et al. (2000)	Pat-sugar beets, leaf silage	≈	Sheep	≈
Donkin et al. (2000)	Gt-maize, seeds, silage	≈	Dairy cows	≈
Faust (2000)	Bt-maize, silage	≈	Dairy cows	≈
Folmer et al. (2000)	Bt-maize,	≈	Beef cows	≈(↑)[4]
	residues, silage	≈	Beef cows	
Hendrix et al. (2000)	Bt-maize,	≈	Beef cows	≈(↑)[5]
	residues, silage		Steer calves	
Mireles et al. (2000)	Bt-maize, seeds	≈	Broilers	≈
Sidhu et al. (2000)	Gt-maize, seeds	≈	Broilers	≈
Weber et al. (2000)	Bt-maize, seeds	≈	Pigs	≈
Aulrich et al. (2001)	Bt-maize, seeds	≈	Pigs	≈
Reuter et al. (2001)	Bt-maize, seeds	≈	Pigs	≈

[1] assessment of marks: ≈ no significant changes ($P>0.05$)
↑ significant increase, improvement ($P<0.05$)
↓ significant decrease, reduction ($P<0.05$)

[2] higher FCM-performance resulting from mistakes in experimental design

[3] decrease in feed/gain ratio, but in normal range

[4] increase in daily gain, but affected by the hybrid genotype and not the genetic modification

[5] increase in feed/gain ratio, but in normal range

In contrast to main nutrients and the feed value of GMO's of the first generation (Table 4) there exist some reports, which show a significant decrease of mycotoxins in Bt-maize (Table 5). Bt-maize is tolerant against the European maize borer. Therefore maize plants are stronger and better developed. Living conditions for *Fusarium* sp. are worse in comparison with isogenic hybrids resulting in lower contamination with mycotoxins.

Apart from lower content of some mycotoxins in Bt-maize (Table 5) GMO's of the first generation do not offer direct advantages to the consumers presently (Chesson and Flint, 1999). This would be one of the main reasons for unsatisfactory acceptance of green genetic technique in Europe where consumers do not suffer in food deficiency.

More advantages to the consumers, but also to the environment should be communicated to the public in the future (Phipps and Beever, 2000).

There exist already results with GMO of the second generation. For example Molvig et al. (1997) investigated lupins with higher concentration of methionine

TABLE 5

Selected mycotoxins in maize seeds of isogenic and transgenic (Bt) hybrids as reported by various authors (concentration of transgenic hybrid in % of isogenic hybrid)

Authors		Mycotoxins: Deoxynivalenol Isogenic ng/g	Deoxynivalenol Bt %	Zearalenone Isogenic ng/g	Zearalenone Bt %		Fumonisin B_1 Isogenic µg/g	Fumonisin B_1 Bt %
Munkvold et al. (1999)		-	-	-	-	1995	8.8	54
		-	-	-	-	1996	7.0*	24
		-	-	-	-	1997	16.5*	13
Cahaguier and Melcion (2000)	France	350	79	-	-		1.0	20
	Spain	176	11	-	-		6.0	10
Pietri and Piva (2000)		No sign. diff. (very low concentration)		No sign. diff. (very low concentration)		1997 (n=5)	19.8	10
						1998 (n=11)	31.6	17
						1999 (n=30)	3.9	36
Valenta et al. (2001)	Maize borer infested (n=15)	873	18	256	13		-	-
	Not infested (n=15)	77	70	19	15		-	-

* total Fumonisin

(3.9 instead 2.0 g/kg). Digestibility of protein in rats increased from 89.4 to 95.7% ($P<0.05$). Edwards et al. (2000) tested soyabeans with increased crude protein content (from 47.5 to 62.7%). Lower phytate content of seeds may increase availability of phosphorus and reduce phosphorus supplementation as demonstrated by Spencer et al. (2000 a,b). In these cases animal experiments are necessary for nutritional assessment of GMO.

PROPOSALS FOR NUTRITIONAL ASSESSMENT

In the case of GMO's of the first generation and by-products from GMO we do not have to expect significant changes in nutritional value because of the substantial equivalence between isogenic and transgenic plants, but we need some experiments for acceptance of GMO's in the society and safety assessment too.

Presently nutritional and safety assessment includes following main topics:

- Nutritional evaluation (feeding value) on the base of the concept "substantial equivalence"
  - compositional equivalence
  - nutritional equivalence on the base of feeding experiments
  - influence on animal health and quality of products of animal origin (milk, meat, eggs)
- Fate of transgenic protein and transgenic DNA in milk, meat and eggs
- Antibiotic resistance marker genes
- Potential allergenicity
- Attention to unexpected/unintended effects.

A more complex nutritional assessment seems to be necessary with the second generation of GMO. Table 6 shows a proposal to assess GMO's of the 1st and 2nd generations from the standpoint of animal nutrition. Furthermore, the same recombinant DNA construct should not be nutritionaly assessed in each plant, but only in the most important feed plants. *In vitro* studies could be favourable for plants of lower significance, but with the same recombinant DNA. Experimental conditions (species, category and number of animals per group, duration of feeding, diet composition etc.) should be discussed and proposed from expert groups, e.g., from the "Joint Working Group" of the EU for "Novel foods and feeds".

A combination of nutritional and risk assessment in animal experiments is recommended. Long term feeding experiments with important target animal species seem to be necessary for safety studies to find out unexpected (unintended) effects of GMO-consumption.

OECD (2001) recommended only feeding studies with a single fast growing species to detect unexpected effects if not captured by compositional analysis. Spe-

TABLE 6

Proposal for nutritional assessment of GMO's

Parameters	GMO of 1st generation	GMO of 2nd generation
Determination of important ingredients		
crude nutrients	+	++
nutrient content modified (e.g., amino acids, fatty acids, minerals, vitamins, enzymes etc.)	–	++[2]
undesirable ingredients modified (e.g., plant ingredients as lignin, inhibitors, glucosides etc.; or secondary substances as mycotoxins, pesticides etc.)	(+)	++[2]
Digestibility, Balance studies, Availability of modified nutrients in target animal species	(+)	++
In vitro studies to assess nutritional value	(+)	(+)
Long term feeding experiments with target animal species/categories		
animal performances and quality of foods of animal origin	(+)	++
animal health, welfare	(+)	++
fate of modified protein and/or DNA[1]	(+)	(+)

– not necessary + recommendable [1] for scientific reasons
(+) could be favourable ++ necessary [2] for modified ingredient/s

cific attention should be given to the degradation of modified protein and to the fate of DNA in scientific studies (Beever and Kemp, 2000). Increased analytical sensitivity has brought a new dimension to the testing of feeds and animal products for the presence of GM ingredients or residues.

Normally modified protein has been degraded in the digestive tract of animals and cannot be considered as a safety risk up to now (Ash et al., 2000; Aumaitre, 2000; Faust, 2000). *In vitro* techniques could be helpful to assess the degradation of modified protein. The transfer of plant-DNA fragments into the animal body seems to be a normal process (Doerfler and Schubbert, 1997; Doerfler, 2000). This is in opposition with the classical belief according which only simple nutrients can cross the intestinal wall. The interpretation and the physiological significance of these results has not been clearly elucidated. Up to now no recombinant DNA-fragments could be detected in the animal body (Faust, 2000; Einspanier et al., 2001; Reuter et al., 2001), but the fate of DNA should be investigated in scientific studies.

Instead of the proposal for nutritional assessment of GMO's shown in Table 6 we would like to recommend a decision tree for nutritional and risk assessment of GMO's (Figure 1).

Such a decision tree has some advantages as

- More dynamic consideration of studies with GMO's
- No differentiation in GMO's of the first and second generation; composition and nutritive value decide on type of studies
- Actual results decide on the next steps of assessment
- Safety studies could be involved in nutritional assessment.

More discussions seem to be necessary to harmonize nutritional assessments and to include risk assessment in such studies.

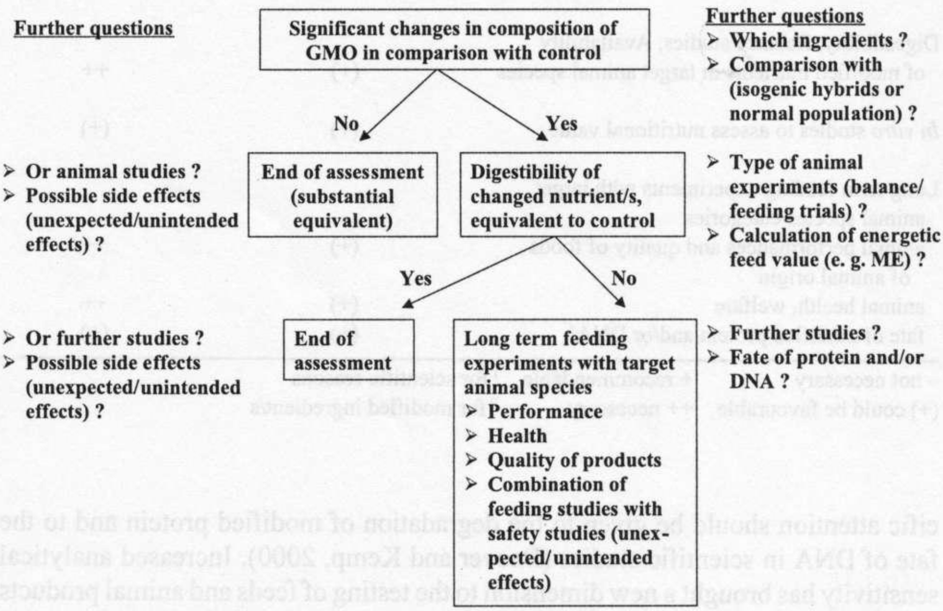

Figure 1. Proposal for a DECISION TREE for the nutritional assessment in combination with safety assessment of GM crops or by-products from GM crops

CONCLUSIONS

Apart from lower mycotoxin-content in Bt-maize no significant differences in nutritional assessment and food quality between feeds from isogenic and transgenic plants and by-products from GMO of the first generation has been reported. The so-called substantial equivalence could be demonstrated.

Apart from nutritional assessment of novel feeds, risk assessment for man, animal and environment is one of the central points of the present discussion. National and international organisations (e.g., EU, 2000 b; OECD, 2001) made proposals for harmonisation of risk assessment procedures.

In the future, nutritional assessment of novel feeds should be combined with the risk assessment. In conclusion a collaboration between scientists has to be encouraged to reduce the amount of time and money spent for studies involving GMO's. Some data of collaborative studies were summarized by Aumaitre et al. (2001) recently.

Furthermore a harmonisation of risk assessments as part of the nutritional assessment and novel feed regulations may help to overcome the present situation.

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STRESZCZENIE

Ocean żywieniowa pasz z roślin modyfikowanych genetycznie (GMO)

Oprócz stwierdzenia bezpieczeństwa pasz wyprodukowanych z zastosowaniem technik rekombinacyjnych DNA, dla konsumentów, zwierząt i środowiska, konieczna jest ich ocena żywieniowa, która powinna stanowić zasadniczą część oceny ich bezpiecznego stosowania. Potrzebny jest więc skuteczny system oceny pasz GMO uwzględniający ich wartość pokarmową. W 1997 r. został zapoczątkowany program mający na celu ocenę GMO tzw. pierwszego pokolenia, w tym Bt-kukurydzy, Pat-kukurydzy, Pat-buraków cukrowych i Gt-soi.

Przeprowadzono doświadczenia strawnościowe i żywieniowe na brojlerach (Bt-kukurydza), nioskach (Bt-kukurydza, Pat-kukurydza), świniach (Bt-kukurydza, Pat-kukurydza, Pat-buraki cukrowe, soja), owcach (kiszonka z Bt-kukurydzy i Pat-kukurydzy), rosnących buhajkach (kiszonka z Bt-kukurydzy) i przetokowanych krowach (kiszonka z Bt-kukurydzy). Do chwili obecnej nie stwierdzono istotnych różnic w ocenie żywieniowej pasz z roślin iso- i transgenicznych pierwszego pokolenia. Wykazano tzw. równoważność żywieniową transgenicznych hybrydów. W próbkach tkanek zwierzęcych nie znaleziono konstruktów rekombinacyjnego DNA roślinnego.

W pracy omówiono propozycje oceny żywieniowej GMO obejmującej poszukiwanie ich nieoczekiwanych (niezidentyfikowanych) działań, oraz aspekty oceny bezpieczeństwa ich stosowania.