Studies on feeds from genetically modified plants (GMP) – Contributions to nutritional and safety assessment

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Abstract

Since 1997, 18 studies with feeds from genetically modified plants (GMP) in the nutrition of dairy cows, growing bulls, growing and finishing pigs, laying hens, chicken for finishing as well as growing and laying quails were conducted at the Federal Agricultural Research Centre (FAL) in Braunschweig (Germany).

The majority of the experiments (16) were undertaken with GMP of the so-called first generation (plants with input traits and without substantial changes in composition) such as Bt-maize, Pat-maize, Pat-sugar beet, Gt-soybean, Gt-potatoes and Bt-potatoes. Two studies were carried out with GMP of the second generation (plants with output traits or with substantial changes in their chemical composition) such as an altered fatty acids profile in rapeseed or inulin potatoes. In all experiments, feeds from GMP were compared with their isogenic counterparts.

The iso- and transgenic feeds were analysed for their composition (proximates, fibre fraction, amino acids, fatty acid pattern, minerals) and undesirable substances (*e.g.*, mycotoxins). Animal studies were carried out for nutritional and safety assessment such as digestibility, feed intake, health and performance of target animal species and quality of food of animal origin. Reproduction was studied in a 10-generation experiment with quails and a 4-generation experiment with laying hens. Duration of experiments and number of animals were limited in some cases due to small amounts of GM-feed available for experimentation.

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Attention was drawn to the fate of DNA during feed processing (silage making, oil extraction), in the digestive tract of animals (slaughtering of animals 0, 4, 8, 12 and 24 h after feeding) and in the animal body (samples from several organs and tissues).

In agreement with more than 100 animal studies available to date, results show no significant differences in the nutritional value of feeds from GMP of the first generation in comparison with non-GMP varieties. To date, no fragments of recombinant DNA have been found in any organ or tissue sample from animals fed GMP. The lower content of mycotoxins in Bt-maize and side effects in GMP of the second generation are of safety concern.

The results indicate that routine feeding studies with target animal species add little to nutritional assessment of feed from GMP of the first generation, but they are of public interest and important for safety assessment. These studies will play a more important role in nutritional and safety assessment of feeds from GMP with output traits. Proposals for such studies were made on the basis of previous experiments.

1. Introduction

The worldwide cultivation of genetically modified plants (GMP) increased from 1.7 million to 90 million ha/year from 1996 to 2005 with soybean, maize, rapeseed and cotton as predominant crops (James, 2005). These plants are characterized by input traits such as tolerance against pesticides or herbicides, or against insects. Correspondingly, these plants do not have substantial changes in their composition and they are termed GMP of the first generation (EFSA, 2004; ILSI, 2004). The coarse framework of substantial equivalence is used to compare transgenic crops of the first generation with their isogenic counterparts (OECD, 1993). Most of these crops are used directly or preserved in animal nutrition or as by-products from the processing industry such as sugar beet pulp, distillers grain, extracted oil meal, *etc*. Therefore, nutritional and safety assessments of feeds from GMP are one of the key questions from the public to nutritionists. Life cycle assessments to compare the environmental impact of production and feeding of GMP in comparison with conventional plants are of future interest (Bennett et al., 2006).

GMP of the so-called second generation (ILSI, 2004) are characterized by output traits as an increased content of valuable components (amino acids, fatty acids, vitamins, *etc*.), an improved availability of nutrients or a lower concentration of undesirable substances (*e.g.*, phytate, lignin, allergenic substances, *etc.*). More details about such plants were described by Flachowsky and Böhme (2005) and ILSI (2004).

Recently, scientific bodies or expert panels proposed guidelines for nutritional and safety assessment of feeds (and food) from GMP of the first generation (EFSA, 2004; ILSI, 2003, 2004; OECD, 2003). They gave recommendations to companies and researches for experimental work with feed and food from GMP. Nutritional studies with feeds from GMP undertaken worldwide and were recently reviewed by Aumaitre et al. (2002), Chesson and Flachowsky (2003), Clark and Ipharraguerre (2001), and Flachowsky et al. (2005a).

The objective of this report was to present advances in nutritional and safety assessments of GMP, considering not only compositional changes but also effects on performance of

farm animals with emphasis on the fate of foreign-DNA in feed processing and in the animal. Since 1997, 18 studies with feeds from GMP in the nutrition of dairy cows, growing bulls, growing and finishing pigs, laying hens, chicken for finishing and growing and laying quails were carried out. Most experiments were performed with GMP of the first generation such as Bt-maize, Pat-maize, Pat-sugar beet, Gt-soybean and Bt-potatoes. Two studies were carried out with GMP of the second generation (plants with output traits or with substantial changes of some ingredients) for example altered fatty acids profile in rapeseed or inulin potatoes.

2. Studies on nutritional assessment of feeds from GMP of the first generation

Sixteen studies with different feeds from GMP of the first generation were carried out with several species/categories at the Institute of Animal Nutrition of the Federal Agricultural Research Centre (FAL). The majority of feeds from GMP under study (Bt-maize, Pat-maize, Pat sugar beet) were grown together with their isogenic counterparts under adequate conditions in the experimental fields, whereas Bt-potatoes and Gt-soybeans were provided by other institutions.

The feeds were analysed for their composition (crude nutrients, constituents of the fibre fraction, amino acids and fatty acids pattern, minerals) and undesirable substances (*e.g.*, mycotoxins), by conventional methods.

Animal studies were conducted for the nutritional assessment of feeds including parameters such as digestibility, feed intake, health and performance of target animal species as well as effects on the quality of food of animal origin. Reproduction was also considered in special studies carried out with quails and laying hens.

2.1. Studies with Bt (Bacillus thuringiensis)-maize

Bt-maize is characterized by the introduction of a gene for a Bt-toxin (Cry 1A(b)-delta-endotoxin gene from *Bacillus thuringiensis*) which protects maize against the European maize borer. Further modifications are described in detail by EC (1997).

The animal experiments with Bt-maize were carried out with beef cattle, sheep, growing and finishing pigs, laying hens, broilers as well as growing and laying quails. Maize silages were fed to ruminants, grains were given to pigs and poultry.

2.1.1. Beef cattle

In 1997, 7 ha each of the non-transgenic line (Cesar) and the transgenic Bt-hybrid were cultivated in the FAL experimental station. Both varieties were harvested at the wax-ripe stage and ensiled in horizontal silos.

No significant compositional differences were detected between the silages made from iso- and transgenic lines (Aulrich et al., 2001).

For the fattening experiment, 40 male calves (German Holsteins) were raised from birth to 188 kg live weight under standard conditions and randomly divided into two groups of 20 animals each. During the fattening period, lasting 246 days, the animals received increasing amounts of concentrate (650 g soybean meal, 290 g wheat,

Table 1
Fattening and slaughter results of bulls ($n = 20$, Aulrich et al., 2001)

Maize silage	Isogenic	Transgenic
Total intake (kg DM/day)	8.0 ± 0.4	7.8 ± 0.3
Concentrate (kg/day)	1.8 ± 0.1	1.8 ± 0.1
Silage (kg/day)	18.8 ± 1.1	18.7 ± 0.9
Body weight gain (g/day)	1487 ± 97	1482 ± 121
Energy conversion ratio (MJ ME/kg gain)	61.5 ± 3.3	60.1 ± 4.1
Final live weight (kg)	537 ± 9	535 ± 9
Hot Carcass weight, warm (kg)	281 ± 11	282 ± 7
Dressing percentage (%)	52.4 ± 1.5	52.8 ± 1.1
Leaf fat[a] (kg)	49.6 ± 5.5	48.7 ± 8.1

[a] Sum of stomach, intestinal, channel and kidney fat.

10 g soya oil, 50 g vitamin and mineral premix/kg) up to three times per day from 1.5 to 2 kg until 550 kg BW. In addition, maize silage of the conventional variety Cesar or prepared from Bt maize was fed *ad libitum*. Daily concentrate and maize silage consumption was individually registered by a computer system. Body weight was monitored automatically. The animals were slaughtered at the end of the fattening period.

The performance and slaughter results from the fattening experiment are shown in Table 1. During the fattening period, the average daily consumption was 18.8 and 18.7 kg fresh matter from the iso- or transgenic silage, respectively. Average daily weight gain was considered to be on a high production level for German Friesian bulls.

The average carcass weight of animals of both groups was nearly identical. The leaf fat including stomach, intestinal, channel and kidney fat was used as a criterion for the fat content in the carcass. Very high amounts of fat were registered amounting 49.6 and 48.7 kg, respectively. However, significant differences between the bulls of both groups were not detected.

2.1.2. Sheep

Each silage used in the fattening experiment with bulls was tested for their digestibility in four weathers. The metabolisable energy (ME) of the silages was calculated following the recommendations of the GfE (1995). No significant differences concerning digestibility and energy content were measured between both silages (Table 2).

Table 2
Coefficient of digestibility and metabolisable energy content of Bt-maize silage in sheep as compared to that of the isogenic line ($n = 4$, Aulrich et al., 2001)

	Isogenic	Transgenic
Organic matter	0.750 ± 0.025	0.745 ± 0.020
Ether extract	0.763 ± 0.032	0.798 ± 0.051
NfE	0.812 ± 0.023	0.808 ± 0.013
ME (MJ/kg DM)	10.95 ± 0.03	10.91 ± 0.04

Table 3
Chemical composition of transgenic maize seeds as compared to that of the parental line (Reuter et al., 2002a)

Item	Isogenic	Transgenic	Data from literature[a]
Dry matter (g/kg)	870.0	872.4	880
Proximates (g/kg DM)			
Crude ash	13.6	14.3	17 ± 6
Crude protein	115.9	110.6	106 ± 12
Ether extract	23.3	23.7	49 ± 11
Crude fibre	22.7	20.1	26 ± 8
N-free-extracts	824.6	831.4	805 ± 23
Starch	720.1	733.5	695 ± 36
Sugar	18.6	19.2	19 ± 5
Total NSP	65.1	63.2	–
Amino acids (g/kg DM)			
Lysine	2.6	2.8	–
Methionine	1.9	1.8	–
Threonine	4.1	3.9	–
Minerals (g/kg DM)			
Ca	0.09	0.08	–
P	3.11	3.13	–
Mg	1.09	1.06	–
K	3.40	3.39	–
Fatty acids (g/100 g total FA)			
$C_{18:1}$	30.4	29.6	–
$C_{18:2}$	50.2	50.9	–
Mycotoxins (μg/kg)			
Zearalenone	3.0	<detection limit	–
Deoxynivalenol	343	<detection limit	–

[a] DLG-Futterwerttabellen (1991, $n = 90$).

2.1.3. Growing and finishing pigs

The objective of the study with Bt-maize was to determine the composition and the nutritional value of the iso- and transgenic maize seeds fed to pigs (Reuter et al., 2002a,b). Both lines were analysed for proximates, starch, sugar, non-starch polysaccharides, amino acids, fatty acids as well as for selected minerals and mycotoxins (Table 3).

The digestibility of the iso- and transgenic maize seed was determined with six pigs per treatment, applying the difference technique. The incorporation rate of maize in the diets was 700 g/kg, to keep the potential for any differences as high as possible. Faeces were collected three times over 8 days each during the growing–finishing period. Compared to the parenteral line the chemical composition, the digestibility of nutrients and the energy content were not significantly ($P > 0.05$) affected by the genetic modification of maize. However, the content of mycotoxins was in agreement with previous studies by Valenta et al. (2001), being higher in the parenteral line (Tables 3 and 4). The reason for lower mycotoxin contamination in Bt-maize is the better resistance against field infections by Fusarium spp. (Dowd, 2000). The lower Fusarium toxin content in Bt-maize as also described by other authors (e.g., Bakan et al., 2002; Cahagnier and Melcion, 2000; Munkvold et al., 1999; Pietri and Piva, 2000) is an important matter of safety concern.

Table 4
Coefficient of digestibility and energetic feeding value of maize for pigs (means ± S.D., Reuter et al., 2002a)

Period, kg LW	39–50		61–72		79–89	
Maize seeds	Isogenic	Transgenic	Isogenic	Transgenic	Isogenic	Transgenic
OM	0.872 ± 0.015	0.889 ± 0.010	0.898 ± 0.009	0.894 ± 0.009	0.901 ± 0.005	0.904 ± 0.008
CP	0.833 ± 0.028	0.862 ± 0.016	0.857 ± 0.022	0.850 ± 0.019	0.858 ± 0.013	0.871 ± 0.015
EE	0.678 ± 0.077	0.723 ± 0.045	0.735 ± 0.065	0.674 ± 0.041	0.772 ± 0.037	0.736 ± 0.045
CF	0.401 ± 0.084	0.470 ± 0.097	0.558 ± 0.065	0.537 ± 0.054	0.552 ± 0.039	0.514 ± 0.063
NfE	0.916 ± 0.008	0.925 ± 0.006	0.932 ± 0.004	0.934 ± 0.005	0.934 ± 0.004	0.938 ± 0.006
ME (MJ/kg)	13.8 ± 0.3	14.0 ± 0.2	14.0 ± 0.2	13.9 ± 0.2	14.1 ± 0.1	14.1 ± 0.1
(MJ/kg DM)	15.4 ± 0.3	15.7 ± 0.2	15.8 ± 0.2	15.6 ± 0.2	15.9 ± 0.1	15.9 ± 0.1

Table 5
Growth performance of growing–finishing pigs fed iso- or transgenic maize diets over a period of 91 days (Reuter et al., 2002b)

	Initial LW		Feed consumption (kg/day)	Daily gain (g/day)	Energy conversion ratio (MJ ME/kg gain)
	n	kg			
Isogenic	12	23.5 ± 2.9	1.95 ± 0.15	815 ± 93	33.4 ± 2.3
Transgenic	35	23.9 ± 3.0	1.94 ± 0.15	804 ± 64	33.7 ± 1.5

Table 6
Chemical composition (g/kg DM) of Bt-maize seeds and the isogenic comparator (Aulrich et al., 2001)

	Isogenic	Transgenic
Crude protein	89.1	98.8
Crude ash	12.5	12.3
Ether extract	34.0	42.9
Starch	757.6	742.8

In addition, a performance trial with 48 pigs was designed to compare the growth performance of pigs fed diets containing either transgenic maize or its iso-line. During the growing–finishing period lasting 91 days, feed intake and daily weight gain (Table 5) were the same for both groups (P>0.05).

Another digestion experiment with Bt-maize was conducted by Aulrich et al. (2001). In these trials, a Bt-cultivar was used and seeds from the variety "Cesar" as a comparator. However, both lines were grown at different locations in Germany. Due to this fact the seeds of both lines showed some small differences in their composition (Table 6).

The digestibility of the two maize varieties was determined according to the difference procedure. The diets contained 500 g maize/kg. Digestibility and the energetic feeding value of both maize varieties were not significantly (P>0.05) influenced by genetic modification (Table 7).

2.1.4. Laying hens

For the experiments with poultry, maize cobs were manually gathered before harvesting the plants, gently dried (40 °C) and the seeds removed.

The chemical composition as analysed illustrates certain differences between iso- and transgenic maize with regard to the contents of crude protein, phosphorus and oleic acid

Table 7
Coefficient of digestibility and energetic feeding value of Bt-maize seeds and the isogenic comparator in pigs (Aulrich et al., 2001)

	Isogenic	Transgenic
Organic matter	0.959 ± 0.012	0.925 ± 0.007
Crude protein	0.905 ± 0.076	0.865 ± 0.061
Starch	0.970 ± 0.005	0.946 ± 0.005
DE (MJ/kg DM)	17.42	17.01
ME (MJ/kg DM)	17.11	16.69

Table 8

Chemical composition of Bt-maize seeds and the isogenic comparator of the growing season 1997 used in the trials with poultry (Aulrich et al., 2001)

	Isogenic	Transgenic
Proximates (g/kg DM)		
Crude protein	108.1	98.2
Crude ash	14.6	15.9
Ether extract	54.4	55.9
N-free extracts	800.2	804.6
Starch	709.9	707.8
NSP (g/kg DM)		
Total NSP	62.1	64.3
Arabinose	15.0	15.3
Xylose	19.8	20.4
Glucose	19.3	20.3
Minerals (g/kg DM)		
P	3.7	3.2
Mg	1.2	1.2
Ca	0.03	0.04
Fatty acids (g/100 g total FA)		
$C_{16:0}$	12.4	12.5
$C_{18:0}$	4.0	4.0
$C_{18:1}$	31.1	28.6
$C_{18:2}$	50.0	51.2
$C_{18:3}$	0.9	1.0

(Table 8). However, these differences were small, and were considered to be within the normal range of biological variation (OECD, 2002b).

Six laying hens (Lohmann LSL, 30-weeks old) were used per group in the balance trials comprising a adaptation and a collection period of 5 days each. The experimental diets contained 500 g maize/kg. The results of the experiment with laying hens showed that digestibility was not significantly influenced by the maize variety (P>0.05, Table 9).

Laying intensity (83.5 and 83.3% at the age of 23–30 weeks) and hatchability (86.8 and 88.0% for isogenic or Bt-maize, respectively, at 500 g maize/kg diet) were not significantly (P>0.05) influenced in a 4-generation study with laying hens (Halle et al., 2006).

Table 9

Coefficient of digestibility and energy content of Bt-maize seed and the isogenic comparator for laying hens ($n = 6$; Aulrich et al., 2001)

Parameter	Isogenic	Transgenic
Digestibility of OM	0.769 ± 0.008	0.772 ± 0.029
Digestibility of CP	0.892 ± 0.011	0.900 ± 0.010
AME_N (MJ/kg DM)	12.31 ± 0.12	12.75 ± 0.13

Table 10

Performance of broilers fed Bt-maize or the isogenic comparator as the principal component in the diet ($n=6$, Aulrich et al., 2001)

Parameter	Isogenic	Transgenic
Feed conversion ratio (g/g)	1.61 ± 0.05	1.63 ± 0.05
Digestibility of CP	0.818 ± 0.014	0.837 ± 0.016
AME_N (MJ/kg DM)	12.82 ± 0.24	13.33 ± 0.24

2.1.5. Broilers

Isogenic and Bt-maize as described in Table 8 were also tested in a digestibility experiment with broilers. Six birds were fed *ad libitum* on diets containing 500 g maize/kg. Excreta were collected from day 30–35 of age. Protein digestibility and energetic feeding value of the diets were also not significantly affected by the maize variety (P>0.05, Table 10).

Another feeding study to compare Bt-maize with the isogenic counterpart was carried out with 35 broilers over the whole growing period of 35 days. The animals were fed a diet containing 740 g iso- or transgenic maize/kg (Tony et al., 2003). The results clearly showed that there were no significant differences (P>0.05) detected concerning feed intake, digestibility, body weight gain and other performance parameters due to feeding of isogenic or Bt-maize.

2.1.6. Growing and laying quails

A 10-generation study was carried out with growing and laying quails (Halle et al., 2004; Flachowsky et al., 2005b). The basal diets were formulated to contain 400 g (starter) or 500 g (growers, hens) isogenic or Bt 176 maize/kg. The starter and grower diets were fed to Japanese quails, on average to 70 male and 75 female chicks of each group (weeks 1–6). The layer diets were fed to 32 hens each from weeks 7 to 12. Rotational crossing was used to avoid in-breeding. Eggs were collected at a hen age of 10 weeks, 210 eggs per group were used for each hatch.

Some results are shown in Fig. 1. Although biological variation existed within and between the groups fed the iso- or transgenic maize, none of the differences were significant at a level of P<0.05. On average, male and female quails (Fig. 1A) fed transgenic maize showed a lower body weight. Mortality of chicks and hens, laying intensity (Fig. 1B), hatchability (Fig. 1C), slaughter results and organ weights did not significantly (P>0.05) differ between both groups and were in the range of biological variation. The results of a 4-generation experiment with laying hens agree with findings obtained with quails by Halle et al. (2006).

In summary, feeding diets containing genetically modified maize (Bt 176) over 10 generations to quails (10 × 12 weeks) and 4 generations to laying hens (4 × 31 weeks) did not significantly influence animal health, feed intake, feed efficiency, laying performance, or hatchability. Meat and egg quality were also not affected.

2.2. Bt-potatoes

Only one study with Bt-potatoes was undertaken at the Institute (Halle et al., 2005). Two genetically modified lines (Cry5-Bt gene, Bt-potatoes) and a non-genetically modified
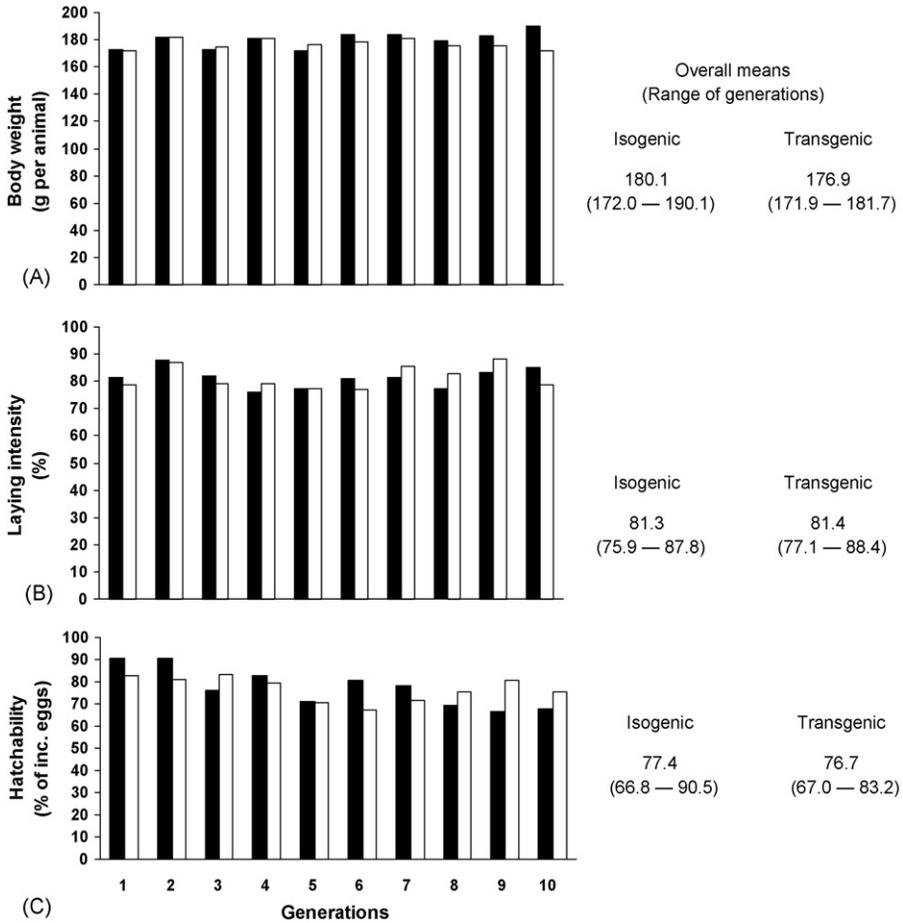
Fig. 1. (A) Body weight of female quails (P>0.05; age: 6 weeks), (B) laying intensity (P>0.05) and (C) hatchability of quails (P>0.05) fed with isogenic (■) and transgenic (Bt, □) maize (Flachowsky et al., 2005b).

control line from *Solanum tuberosum* L., *var. Spunta*, were prepared for analysis and feeding to broilers.

A total of 27 male chicks 14-days old were randomly allotted to three dietary treatments. Feed and water were provided *ad libitum*. The basal diet was formulated to contain 300 g dried non-GM control potatoes or two lines of genetically modified potatoes, G2 or G3/kg. The performance of chicks was measured from day 14 to 28 of age. Summarised results of the experiment are given in Table 11. The composition of the diets, feed intake, body weight and feed conversion were not significantly affected by the potato line (P>0.05). Effects on carcass quality of broilers were also not observed.

Table 11
The influence of non-GM and GM potatoes on feed intake, final body weight and feed conversion of male broilers (from days 14 to 28 of age (Halle et al., 2005)

	Isogenic	Transgenic	
		Spunta G2	Spunta G3
Feed intake (g/broiler)	1124	1172	1158
Body weight gain (g/broiler)	618	637	626
Feed conversion (kg/kg)	1.82	1.84	1.85

Means not significantly different by the Student–Newman–Keuls test, P>0.05.

2.3. Glufosinate tolerant (Pat) maize in pigs

The investigations were conducted with grains of an isogenic maize line and the corresponding transgenic cultivar, into which a synthetically produced phosphinotricin-acetyltransferase-gene (Pat-gene) was inserted. The nucleic acid of the codifying region was chemically synthesized. The nucleotide sequence was derived from the amino acid sequence of the Pat-enzyme, which is produced by the bacterium *Streptomyces viridochromogenes*.

The non-transgenic and the Pat-maize were grown on the experimental fields of the FAL during the summer 1998. In the course of the growing season, crops were sprayed with conventional herbicides. On one half of the area, where the Pat-line was grown, the glufosinate (Basta) was applied. This procedure enabled a comparison of the conventional treated non-transgenic hybrid with two Pat-variants, which had been also conventionally treated or sprayed with Basta.

Maize grain was used as test material for compositional analysis and for digestibility trials with pigs. Harvesting of maize was carried out manually, *i.e.*, the maize ears were taken out of the plants, dehulled and carefully dried at 40 °C before removing the seeds (Böhme et al., 2001). Compositional differences between both maize lines due to the genetic manipulation were not significant (P>0.05, Table 12). The levels of starch ranged from 688 to 701 g/kg DM and protein concentrations ranged from 117 to 120 g/kg DM.

Table 12
Proximate analysis, starch, sugar and NSP-composition of Pat-maize seeds compared with those of the corresponding non-transgenic lines (g/kg DM; Böhme et al., 2001)

Treatment	Control (conventional)	Pat-hybrid (conventional)	Pat-hybrid (Basta)
DM (g/kg fresh weight)	905.6	911.3	919.3
Crude protein	120.4	118.6	117.1
Ether extract	31.4	35.0	32.5
Crude ash	18.9	18.1	17.6
Starch	700.6	692.1	688.0
Sugar	19.8	25.9	20.5
Total NSP	85.9	85.7	88.1
Arabinose	19.7	19.1	19.9
Xylose	27.3	28.1	28.2
Glucose	29.1	28.8	30.2

Table 13
Amino acid analysis of Pat-maize seeds compared with the corresponding non-transgenic controls (Amino acids in g/16 g N, Böhme et al., 2001)

Treatment	Control (conventional)	Pat-hybrid (conventional)	Pat-hybrid (Basta)
Aspartic acid	6.64	6.80	6.74
Threonine	3.68	3.65	3.74
Serine	4.93	4.83	5.00
Glutamic acid	19.89	19.79	20.20
Proline	10.14	9.53	10.26
Glycine	3.89	3.79	3.94
Alanine	7.02	8.11	8.05
Valine	4.70	4.75	4.72
Isoleucine	3.68	3.86	3.80
Leucine	12.71	12.61	12.99
Tyrosine	4.69	4.68	4.71
Phenylalanine	5.22	5.21	5.28
Histidine	3.04	2.92	2.99
Lysine	2.76	2.72	2.75
Arginine	4.28	4.17	4.21
Cysteine	2.46	2.30	2.42
Methionine	2.15	2.57	2.10

Amino acid and fatty acid profiles were determined in the three maize grain samples, to study any effects of the genetic modification on protein and fat composition. The results are given in Tables 13 and 14 and show no significant differences between Pat and non-transgenic grain ($P>0.05$).

The difference technique was used in digestibility trials with pigs. Five pigs of the German Landrace (body weight 40–50 kg) was offered a basal diet containing 410 g barley, 390 g wheat, 130 g potato protein, 30 g soybean oil and 40 g premix/kg, having a CP-content of 215 g/kg and an energy concentration of 15.3 MJ ME/kg DM. In the experimental diets 30 percent of the DM was replaced by the different maize grains. Across the three maize

Table 14
Fatty acid composition of Pat-maize grains compared with the corresponding transgenic controls (percent of total fatty acids, Böhme et al., 2001)

Treatment		Control (conventional)	Pat-hybrid (conventional)	Pat-hybrid (Basta)
Myristic acid	$C_{14:0}$	0.07	0.07	0.05
Palmitic acid	$C_{16:0}$	11.50	11.84	11.53
Palmitoleic acid	$C_{16:1}$	0.07	0.05	0.06
Stearic acid	$C_{18:0}$	1.55	1.58	1.61
Oleic acid	$C_{18:1}$	27.68	27.36	27.52
Linoleic acid	$C_{18:2}$	56.98	56.27	56.74
Linolenic acid	$C_{18:3}$	1.19	1.29	1.20
Arachidic acid	$C_{20:0}$	0.55	0.48	0.53
Eicosenoic acid	$C_{20:1}$	0.34	0.31	0.34
Eicosadienoic acid	$C_{20:2}$	0.04	0.06	0.06
Behenic acid	$C_{22:0}$	0.18	0.19	0.19
Lignoceric acid	$C_{24:0}$	0.18	0.17	0.17

Table 15
Coefficient of digestibility and energy content of Pat-maize grains for pigs as compared with those of the non-transgenic control (means ± S.D., Böhme et al., 2001)

Treatment		Control (conventional)	Pat-hybrid (conventional)	Pat-hybrid (Basta)
Digestibility	OM	0.886 ± 0.041	0.900 ± 0.021	0.893 ± 0.018
	CP	0.811 ± 0.120	0.803 ± 0.099	0.796 ± 0.060
	NfE	0.931 ± 0.026	0.947 ± 0.014	0.953 ± 0.017
Energy (MJ/kg DM)	DE	16.1 ± 0.6	16.4 ± 0.3	16.4 ± 0.3
	ME	15.8 ± 0.6	16.0 ± 0.3	16.1 ± 0.3

grain samples no significant differences in digestibility of nutrients and energy content were observed ($P > 0.05$; Table 15).

2.4. Glufosinate tolerant (Pat) sugar beets (incl. sugar beet top silage)

Similar studies as described in Section 2.3 were carried out with sugar beets (*Beta vulgaris* L. ssp. vulgaris provar. altissima Döll). The non-transformed line was also used as control. The genetic modifying process and the cultivation were the same as described for maize (see Section 2.3 and Böhme et al., 2001).

The sugar beet was harvested manually. The roots were washed and shredded for feeding and analysis. The tops and leaves were chopped and ensiled in 2001 plastic silos. The resulting silages were used for the feeding experiments after a 5-month period. The analyses for crude nutrients indicate that differences between the three sugar-beet cultivars were detected (Table 16). But as they are not significant ($P > 0.05$) and small, they were considered biologically not relevant (OECD, 2002a). The sugar content, which contributes essentially to the nutritive value was analysed to be the same for the non-transgenic and transgenic cultivars.

The sugar-beet top silage did not show differences in DM-composition between the Pat-hybrid and the control. Carbohydrate contents showed a higher variation between agronomic variants of the transgenic line than to the non-transgenic one.

Table 16
Proximate analysis and sugar contents of Pat-sugar-beets and Pat-sugar-beet top silage as compared to those of the corresponding non-transgenic line (g/kg of DM, Böhme et al., 2001)

	DM (g per 1000 g fresh matter)	Crude protein	Ether extract	Crude ash	Sugar
Sugar beets					
Controls (conventional)	232.0	71.5	3.2	29.7	759.6
Pat-hybrid (conventional)	255.3	60.0	3.6	22.9	744.0
Pat-hybrid (Basta)	249.4	63.4	4.4	25.6	738.2
Top silage					
Controls (conventional)	150.4	166.8	17.9	241.2	–
Pat-hybrid (conventional)	152.0	160.0	23.5	262.6	–
Pat-hybrid (Basta)	156.5	153.6	21.8	233.6	–

Table 17
Coefficient of digestibility and energy content of Pat-sugar-beets and Pat-sugar-beet top silage for sheep compared with the non-transgenic control (means ± S.D., Böhme et al., 2001)

Treatment		Control (conventional)	Pat-hybrid (conventional)	Pat-hybrid (Basta)
Digestibility				
Sugar beets	OM	0.919 ± 0.038	0.921 ± 0.002	0.916 ± 0.020
	CP	0.453 ± 0.236	0.499 ± 0.013	0.478 ± 0.078
	CF	0.599 ± 0.370	0.623 ± 0.016	0.640 ± 0.110
	NfE	0.982 ± 0.004	0.969 ± 0.001	0.967 ± 0.012
Sugar beet top silage	OM	0.747 ± 0.011	0.745 ± 0.025	0.770 ± 0.022
	CP	0.662 ± 0.062	0.641 ± 0.051	0.638 ± 0.049
	CF	$0.724^b \pm 0.015$	$0.662^a \pm 0.023$	$0.685^{ab} \pm 0.037$
	NfE	$0.806^a \pm 0.019$	$0.832^{ab} \pm 0.024$	$0.861^b \pm 0.022$
Energy (MJ/kg DM)				
Sugar beets (roots)	DE	15.1 ± 0.6	15.4 ± 0.4	15.4 ± 0.4
	ME	13.2 ± 0.5	13.3 ± 0.3	13.2 ± 0.3
	NEL	8.5 ± 0.5	8.6 ± 0.2	8.5 ± 0.2
Sugar beet top silage	DE	10.4 ± 0.2	9.9 ± 0.3	10.7 ± 0.3
	ME	$8.7^{ab} \pm 0.1$	$8.6^a \pm 0.3$	$9.1^b \pm 0.3$
	NEL	$5.2^{ab} \pm 0.1$	$5.1^a \pm 0.2$	$5.5^b \pm 0.2$

Mean values in the same line not followed by the same superscript letters (a,b) are significantly different (P<0.05).

2.4.1. Sheep

Digestibility experiments with sugar beet roots and top silage were carried out with four wethers of the German blackface breed (about 95 kg BW). The preliminary period lasted 14 days, and faeces were collected over 10 days (Böhme et al., 2001).

Hay was given as basal ration and when sugar-beet roots were included the hay was additionally supplemented with urea. In the experimental diets, 50 or 60 percent of DM was replaced by sugar-beets or top silage. Daily DM consumption was 480 g hay, 470 g sugar-beets and 10 g urea or 515 g hay and 610 g top silage.

The coefficient of digestibility of sugar beets was >0.90 (Table 17). Significant differences between the non-transgenic and the Pat-hybrids were not detected. CF-digestibility seemed to be improved for the Pat-hybrids by 0.033. However, a tendency towards a decline in NFE-digestibility was found. The digestibility and the energy content of sugar-beet top silage for ruminants showed some minor differences between the Pat-silage and controls, which proved to be statistically significant (Table 17). However, as these differences are significant only between the control and the conventionally treated transgenic hybrid, they were considered to be biologically unimportant. This is supported by the fact that the differences in digestibility and energy content are small.

2.4.2. Pigs

Sugar beets were fed in digestibility trials to five pigs of the German Landrace breed (body weight: 40–50 kg). The basal diet corresponded to that of the digestibility trials with Pat-maize (see Section 2.3). In the experimental diets, 30 percent of the DM was replaced by the various sugar beet types.

Table 18
Coefficient of digestibility and energy content of Pat-sugar beets for pigs compared with non-transgenic control (means ± S.D., Böhme et al., 2001)

Treatment	Control (conventional)	Pat-hybrid (conventional)	Pat-hybrid (Basta)
Digestibility			
OM	0.894[b] ± 0.011	0.938[a] ± 0.022	0.925[a] ± 0.026
CP	0.504 ± 0.222	0.559 ± 0.103	0.502 ± 0.245
NFE	0.957 ± 0.016	0.968 ± 0.011	0.964 ± 0.009
Energy (MJ/kg DM)			
DE	14.9 ± 0.5	15.3 ± 0.3	15.0 ± 0.4
ME	13.7 ± 0.4	14.2 ± 0.3	14.0 ± 0.4

Mean values in the same line not followed by the same superscript letters (a,b) are significantly different (P<0.05).

Results showed significant differences in digestibility of the various sugar beet variants but they were minor and recorded for OM only (Table 18). OM-digestibility was improved in the Pat-lines by 0.038 units (P<0.05), but the differences were considered to be in the biological range (OECD, 2002a). The digestibility values of crude nutrients and energy content did not show significant differences (P>0.05; Table 18).

2.5. Roundup ready (RR, Glyphosate tol.) soybeans in pigs

Two varieties from the same isogenic soybean (Glycine max. L. cv A5403) line (40-3-2) were studied. The original line was modified by insertion of the following sequences (EC, 1996): (1) a single copy of the gene coding for CP4 5-enolpyruvylshikimate-3-phosphate synthase (CP4 EPSPS) from *Agrobacterium sp*. strain, (2) the chloroplast transit peptide (CTP) coding sequence from *Petunia hybrida* with the promoter P-E35S from cauliflower mosaic virus and (3) the nopaline synthase gene terminator from *Agrobacterium tumefaciens* (Padgette et al., 1995). The CP4 EPSP synthase is responsible for the glyphosate tolerance of the modified soybeans.

The objective of the experiment was to compare transgenic (RR) full-fat soybeans with the isogenic hybrid in growing–finishing pigs (Aulrich et al., submitted for publication). Full-fat beans were used to avoid DNA-degradation through processing (Berger et al., 2003). The pig diets were formulated to contain as high a content of soybeans as possible (*ca.* 420 g/kg) to attain a high effect of GM-soybeans when studying the fate of DNA-fragments.

Twelve animals were fed the isogenic hybrid and 36 pigs consumed the RR-soybeans containing diets. The finishing period lasted from about 65 to 100 kg body weight. After slaughtering important carcass characteristics were registered and samples from organs and tissues were taken to follow the fate of DNA. RR-soybeans did not differ from the isogenic counterpart in all analysed constituents (Table 19).

Feed intake, daily weight gain (836 and 859 g), feed conversion and slaughter data of pigs fed diets containing conventional or RR soybeans were not significantly different (P>0.05) over the test period (Table 20).

Table 19
Composition (g/kg DM) of iso- and transgenic full-fat roasted soybeans fed to growing–finishing pigs (Aulrich et al., submitted for publication)

Item	Isogenic	Transgenic (RR)
Dry matter (g/kg)	918	915
Organic matter	947	946
Proximates		
Crude protein	432	413
Ether extract	251	255
Sugars	92	95
Crude fibre	33	41
Starch	33	37
Total NSP[a]	8.0	8.9
Arabinose	1.5	1.6
Galactose	3.1	3.1
Glucose	1.7	2.2
Xylose	2.0	1.9
Amino acids		
Lysine	28.3	26.5
Methionine	6.1	6.1
Threonine	17.8	17.2
Minerals		
Calcium	2.0	1.4
Phosphorus	6.5	7.1
Fatty acids (g/100 g total FA)		
$C_{16:0}$	10.3	10.6
$C_{18:0}$	4.2	4.3
$C_{18:1}$	24.7	25.2
$C_{18:2}$	49.8	48.8
$C_{18:3}$	5.8	6.2

[a] NSP: non-starch polysaccharides.

Table 20
Performance[a] of pigs over 42 days of feeding grower-finisher diets containing isogenic or Roundup Ready® full-fat roasted soybeans (Aulrich et al., submitted for publication)

Item	Isogenic	Transgenic
n	12	36
Average daily gain (g)	836 ± 97	859 ± 70
Feed intake (kg/day)	2.44 ± 0.13	2.41 ± 0.15
Feed conversion (kg feed/kg gain)	2.94 ± 0.28	2.81 ± 0.19
Energy efficiency (MJ ME/kg gain)	39.1 ± 4.0	38.0 ± 3.0

[a] Values shown are means ± S.D. Pigs were fed the diets until mean BW of approximately 110 kg, but performance data are compared as at day 42 of feeding, to eliminate effects of feeding interval on calculation of performance indicators.

2.6. Synopsis on feeds from GMP of the first generation

The chemical analyses and the animal studies, which were performed with genetically modified maize, potatoes, sugar beets and soybeans, demonstrate no significant differences as compared to the isogenic counterpart concerning their chemical composition and their physiological production efficiency for the various species of farm animals such as growing bulls, sheep, growing and finishing pigs, laying hens, broilers and growing and laying quails. Thus, these results from a total of 16 investigations confirmed the substantial equivalence between feeds from transgenic plants of the first generation and their isogenic counterpart (OECD, 1993).

3. Studies on nutritional assessment of feeds from GMP of the second generation

GMP of the second generation (output traits, ILSI, 2004) are characterized by:

– Increased contents of desirable substances (*e.g.*, amino acids, vitamins, fatty acids, minerals, enzymes).
– Decreased contents of undesirable substances (*e.g.*, mycotoxins, alkaloids, glucosinolates, lignin, phytate).

At present, detailed standardized test procedures are not available to investigate feeds from the GMP of the second generation. Recently, approaches for testing those feeds were presented by Flachowsky and Böhme (2005). This proposal comprises the following aspects.

Feeds with intended beneficial physiological properties relating to amino acids, fatty acids, minerals, vitamins and other substances may contribute to higher feed intake of animals and/or improved conversion of feed/nutrients into food of animal origin. Furthermore, the excretion of nitrogen, phosphorus and other nutrients may be reduced. Some details of animal experimentation (*e.g.*, number of animals, duration of experiments, composition of diets, measurements) are given by EFSA (2004) and ILSI (2003, 2004). Depending on the claim of changes due to the genetic modification, the experimental designs must be arranged to demonstrate the effects. Different experimental designs are necessary to demonstrate the efficiency of changed nutrient constituents:

– Bioavailability or conversion of nutrient precursors into nutrients (*e.g.*, β-carotene).
– Digestibility/bioavailability of components (*e.g.*, amino acids, fatty acids, vitamins).
– Efficiency of substances which may improve digestibility/availability (*e.g.*, enzymes).
– Utilization of substances with surplus effects (*e.g.*, prebiotics).
– Improvement of sensory properties/palatability of feed (*e.g.*, essential oils, aromas).

However, the genetic modification might not only increase the content of intended desirable substances. There are indications that side effects may occur and cause unfavorable effects as recently discussed by Cellini et al. (2004). Such secondary changes should be considered in the nutritional and safety assessment of GMP of the second generation. Specific animal studies as the basis for comparative approaches seem to be necessary to deal with these questions.

3.1. Increased myristic and palmitic acid in rapeseed for pigs

Rapeseed with modifications in the fatty acid pattern was analysed for its composition and its feeding value for growing–finishing pigs in comparison with the non-modified counterpart (Böhme et al., 2005b).

The acyl-thioesterase gene from *Cuphea lanceolata* was inserted into the genome of the cv. *Drakkar* with the aim, to increase the content of myristic acid and palmitic acid at the expense of oleic acid. The objective of this genetical modification was to produce rapeseed for technical purposes. But as the by-product is intended to be used as feedstuff, the seeds were analysed for composition and energetic feeding value for pigs.

Except for the fatty acids, the GM-rapeseed showed only marginal differences in nutritional composition, but the glucosinolate (GSL) content was increased (Table 21).

The digestibility and the energy content of the diets containing 150 g iso- or transgenic rapeseed/kg fed to five growing and finishing pigs each remained unaffected (Table 22).

The higher concentration of myristic and palmitic acid of the transgenic rapeseed fed to pigs had a negative influence on feed and energy intake and consequently daily weight gain

Table 21
Chemical composition of iso- and transgenic rapeseed (Böhme et al., 2005b)

	Isogenic	Transgenic
DM	937.0	935.3
Proximates (g/kg of DM)		
OM	95.5	952.7
Crude protein	227.9	273.8
Ether extract	440.6	398.6
Starch	29.3	28.1
Sugar	44.1	45.4
Amino acids (g/100 g crude protein)		
Lysine	5.61	5.74
Methionine	1.86	1.97
Cystine	2.26	2.75
Threonine	4.31	3.99
Minerals (g/kg DM)		
Ca	4.39	4.19
P	7.36	8.41
Mg	3.22	3.92
K	6.27	8.29
Na	1.64	1.84
Fatty acids (% of total fatty acids)		
$C_{14:0}$	0	13
$C_{16:0}$	4	20
$C_{18:1}$	67	39
Glucosinolates (μmol/g DM)		
Alkenyl GSL	9.5	16.3
Progoitrin	7.6	12.8
Total GSL	13.2	20.4

Table 22
Coefficient of digestibility and energy content of rapeseed-based diets and performance parameters of pigs ($n = 10$, 32–105 kg body weight, Böhme et al., 2005b)

	Isogenic	Transgenic
Digestibility		
Organic matter		
Grower diet	0.797	0.803
Finisher diet	0.823	0.822
Crude protein		
Grower diet	0.826	0.832
Finisher diet	0.851	0.844
Metabolizable energy (MJ/kg DM)		
Grower diet	14.0	14.1
Finisher diet	14.8	14.6
ME-intake (MJ/day)	32.0	30.6
Live weight gain (g/day)	832	795
Energy conversion ratio (MJ/kg)	38.5	38.5

(Table 22). The reason for this depression was due to the fact that the genetic modification was associated with higher concentrations of undesirable substances (Table 21). These results are an excellent example that genetically modified plants with output traits need a complete compositional and nutritional assessment. This is also supported by results obtained from GM-potatoes.

3.2. Inulin synthesizing potatoes in pigs

The ability to synthesize high molecular weight fructan as inulin was transferred to potato plants via constitutive expression of the sucrose:sucrose 1-fructosyltransferase (1-SST) and the fructan:fructan 1-fructosyltransferase (1-FFT) genes of globe artichoke, *Cynara scolymus* (Heyer et al., 1999). As the fructan pattern of tubers from this transgenic potato plant represents the full inulin spectrum of artichoke roots, the tubers were intended to be used as a prebiotic functional food in human nutrition. The inulin concentration in the dry matter of the transgenic tubers amounted to 50 g/kg (Hellwege et al., 2000). The objective of this study was to analyse the extent to which this modification influenced the content of key nutrients, their availability to pigs as a model for humans, and the concentration of antinutritional substances (Böhme et al., 2005a).

The transgenic potato line and its isogenic counterpart were grown under the same field conditions in Northeast Germany in 2003. The tubers were steamed after harvesting and ensilaged in 200 l plastic silos.

Proximate composition, minerals and amino acids did not show significant differences between lines (Table 23). However, the starch content decreased as inulin was stored, indicating that the storage-capacity of carbohydrates was not affected by genetic modification.

The total alkaloid content of the transgenic tubers was about 25% higher than that of the isogenic potatoes (Table 23). In agreement with the data presented for rapeseed (Table 21), the results confirm that substantial genetic modifications might be associated with altered

Table 23
Selected proximate analysis, starch, macro-elements, amino acids and glycoalkaloids of transgenic inulin synthesising potatoes compared with those of the parenteral line (Böhme et al., 2005a)

	Isogenic	Transgenic
Crude nutrients and starch (g/kg DM)		
Crude protein	107	106
Crude ash	55	58
Starch	674	599
Amino acids (g/100 g crude protein)		
Lysine	4.31	4.00
Methionine	1.18	1.39
Threonine	2.49	2.53
Minerals (g/kg DM)		
Ca	0.51	0.56
P	2.10	2.20
Mg	0.90	0.84
Na	0.30	0.23
Glycoalkaloids (mg/kg DM)		
α-Chaconine	524	652
α-Solanine	204	252
Total alkaloids	728	904

concentrations of undesirable substances, and therefore increased attention should be paid to this fact and in additional safety studies (see Cellini et al., 2004).

The nutritive value of both potato lines was determined in a balance trial (10 days collection period) with four castrated male pigs each (49–78 kg BW) followed by a feeding test lasting 42 days. Duration of the feeding test and the number of animals kept were restricted because of limited amounts of GM-potatoes being available. In the experimental diets 0.40 of the DM of the basal diet, which was based on 370 g wheat, 320 g barley and 180 g potato protein/kg, were replaced by ensiled potatoes.

Digestibility depressions of some nutrients of the inulin-synthesising potatoes were detected and correspondingly a lower energetic feeding value was measured (isogenic: 14.60; transgenic: 14.34 MJ ME/kg DM, P>0.05). The lower production potential of the silage from transgenic potatoes was also confirmed in the feeding test. The average daily liveweight gain of the pigs fed transgenic silage was 43 g lower as compared to controls (P>0.05). The results show the reduced energy and prebiotic potential of the GM-potatoes, but they are not significantly different from those of the control (P>0.05) because of the low number of animals.

4. Fate of DNA

The fate of DNA especially transgenic DNA during feed processing and in the animal received attention after studies by Schubbert et al. (1994, 1997, 1998) who found that DNA-fragments (up to 1000 base pairs; bp) after feeding of phage DNA to mice were absorbed and detected in blood, liver, spleen and other organs and tissues. Based on these findings

Table 24
Methods of processing of rapeseed (Berger et al., 2003)

	Treatment			
	1	2	3	4
Processing	Crushing	Crushing	Crushing	Crushing
	–	–	Conditioning (96 °C, 20 min)	Conditioning (103–111 °C, 30 min)
	Pressing (69 °C)	Pressing (95 °C)	Pressing (95 °C)	Pressing (95 °C)
	–	Extraction	Extraction	Extraction
	–	Desolventizing–toasting (105 °C)	Desolventizing–toasting (105 °C)	Desolventizing–toasting (105 °C)

one of the objective of the experiments at the FAL was to follow the fate of DNA during processing and feeding of GM-plants.

4.1. Feed processing and ensiling

In the first study, iso- and transgenic herbicide resistant rapeseed, in which the phosphinotricinacetyltransferase gene (Pat-gene) was inserted, was processed in different ways (Berger et al., 2003). Both rapeseed varieties were treated under industrial processing conditions in four ways (see Table 24). Samples were taken from the rapeseed after every processing step. The DNA was extracted by a commercial kit and different fragments (isogenic: 248 bp, 970 bp of the phosphoenolpyruvatcarboxylase-gene; transgenic: 194, 680 and 1003 bp of the Pat-gene) were determined by a PCR based method.

The main results are summarized in Table 25. In the final product of the first treatment, the rapeseed cake, intact DNA and fragments of all sizes could be detected. The extracted and toasted meal of the second treatment did not show intact DNA. Only fragments of small sizes were determined. After additional conditioning of the seeds (treatment 3) only an isogenic fragment of 248 bp was detected. Increasing conditioning temperature and time (treatment 4) led to a transgenic DNA-fragment of 194 bp only. Isogenic DNA-fragments were not found.

Table 25
Determined DNA-fragments in final products of isogenic (i) and transgenic (t) rapeseed (Berger et al., 2003)

		Treatment			
		1	2	3	4
Rapeseed—final products		Cake	Toasted meal	Toasted meal	Toasted meal
Determined DNA-fragments (bp)					
21,000 bp (intact DNA)	i	+	−	−	−
	t	+	−	−	−
248 bp	i	+	+	+	−
970 bp	i	+	−	−	−
194 bp	t	+	+	−	+
680 bp	t	+	−	−	−
1003 bp	t	+	−	−	−

+, detected; −, not detected.

Table 26
Determined DNA-fragments in transgenic corn cob mix (CCM) and whole plant silage (WPS) as influenced by the duration of the ensiling period (Aulrich et al., 2004)

Duration of ensiling (days)	Fragment 1016 bp		Fragment 680 bp		Fragment 194 bp	
	CCM	WPS	CCM	WPS	CCM	WPS
0	+	+	+	+	+	+
2	+	+	+	+	+	+
5	+	+	+	+	+	+
7	−	+	+	+	+	+
14	−	+	+	+	+	+
21	−	+	+	+	+	+
28	−	+	−	+	+	+
35	−	−	−	+	+	+
70	−	−	−	−	+	+
100	−	−	−	−	+	+
200	−	−	−	−	+	+

The study showed that mechanical treatments had no influence on the stability of DNA, while the processes of extraction and toasting caused highly fragmented DNA.

The influence of ensiling on DNA fragmentation was the objective of the second study conducted with maize (Aulrich et al., 2004). The iso- and transgenic herbicide resistance (Pat-gene) varieties were grown on experimental fields of the FAL. After harvesting the iso- and transgenic maize were ensiled under laboratory conditions as corn cob mix (CCM) and as whole plant silages (WPS). Samples were taken during the ensiling process (Table 26). DNA was extracted with a commercial kit and different fragments of the Pat-gene (1016, 680, 194 bp) were determined applying PCR based methods as already described.

At the beginning of the ensiling process large fragments of 1016 bp were detected up to 5 days in CCM and up to 28 days in WPS (Table 26; data only shown for fragments of transgenic corn). Fragments of 680 bp were detected up to 21 days in CCM and up to 35 days in WPS. From day 35 up to the end of ensiling the DNA was degraded to fragments smaller than 680 bp. It was only possible to detect fragments of 194 bp.

The results indicate that forage conservation by ensiling causes a degradation of DNA to small fragments of about 200 bp. The degradation proved to be faster in CCM as compared to WPS, probably due to different in fermentation conditions.

4.2. Fate of DNA in animals

Schubbert et al. (1994, 1997) detected foreign DNA-fragments in various organs and tissues of mice a few hours after feeding. This makes it necessary to investigate the fate of plant DNA in farm animals, especially as far as recombinant feed plants are of concern. Beginning with the first experiments the fate of DNA in the animal body was studied (Einspanier et al., 2001). Meanwhile the fate of DNA was studied in dairy cows, beef cattle, growing–finishing pigs, laying hens, broilers and quails fed Bt-maize, Bt-potatoes, RR-soybeans, or inulin potatoes.

Table 27
Studies of the transfer of "foreign" DNA fragments into farm animals

DNA source	Animal species categories	Results		References
		Detection of transgenic DNA	Detection of "foreign" non-transgenic DNA	
Bt-maize, grain and silage	Broilers, Layers, Fattening cattle, Dairy cows	No transgenic DNA in animal tissues	Plant DNA fragments in muscle, liver, spleen, kidneys of broilers and layers, not in blood, muscle, liver, spleen, kidneys of finishing bulls, in eggs and feces of broilers and layers and in feces of dairy cows	Einspanier et al. (2001)
Bt-maize, grain	Pigs	Transgenic DNA fragments up to 48 h in the rectum, not in blood, organs and tissues	Plant DNA fragments in the gastrointestinal tract, in blood, organs and tissues	Reuter and Aulrich (2003)
Bt-maize, grain	Broilers	Transgenic DNA in the gastrointestinal tract. No transgenic DNA in blood, organs and tissues	Plant DNA fragments in the gastrointestinal tract, in blood, organs and tissues	Tony et al. (2003)
Bt-maize, grain	Quails (10 generations)	Transgenic DNA-fragments (211 bp) in the stomach and whole gastrointestinal tract. No transgenic DNA fragments in muscle, liver, stomach, spleen, kidney, heart and eggs	Plant DNA fragments in the gastrointestinal tract	Flachowsky et al. (2005b)
Bt-potato	Broilers	No transgenic DNA in muscle, liver, kidney and spleen	Plant DNA fragments in muscle, liver, kidney and spleen till 8 h after feeding	El Sanhoty (2004)
Gt-soybeans, fullfat seeds	Pigs	No transgenic DNA in muscle, liver, kidney and spleen	Plant DNA fragments in the gastrointestinal tract	Aulrich et al. (2002)
Inulin potato, silage	Pigs	Transgenic DNA-fragments (104 bp) in the stomach, no transgenic DNA-fragments in animal tissues	Plant DNA fragments in the gastrointestinal tract, no plant DNA fragments in animal tissues	Broll et al. (2005)

Table 28
Summary of studies undertaken with feeds from GMP of the first generation conducted of the Federal Agricultural Research Centre

GMP	Analytical measurements	Animal species/categories	Type of study	Animal number (isogen/transgen)	Duration (days)	Results (comparison to isogenic counterpart)				
						Composition of GM-feeds	Digestibility	Zootechnical parameters	Further measurements	References
Bt- maize										
Grain	Crude nutrients, amino acids, fatty acids, NSP, minerals, mycotoxins	Growing and finishing pigs	Digestibility	Three times: 6/6	14	No significant differences	No significant differences	–	–	Reuter et al. (2002a)
			Growing/finishing	12/36	91	No significant differences	–	No significant differences	Slaughtering data, fate of DNA	Reuter et al. (2002b)
	Crude nutrients	Growing pigs	Digestibility	5/5	14	No significant differences	No significant differences	–	–	Aulrich et al. (2001)
	Crude nutrients, starch, NSP, amino acids, fatty acids, minerals	Laying hens	Digestibility, measurement of performance	6/6	10	No significant differences	No significant differences	No significant differences	Fate of DNA	Aulrich et al. (2001)
	Crude nutrients	Broilers	Digestibility	6/6	5	No significant differences	No significant differences	–	–	Aulrich et al. (2001)
	Crude nutrients	Broilers	Growing	9/27	35	No significant differences	–	No significant differences	Fate of DNA	Tony et al. (2003)
	Crude nutrients, starch, amino acids, fatty acids	Growing and laying quails	10 generations (growing, laying)	Growers 140/140	10 times: 42	No significant differences	–	No significant differences	Reproduction	Halle et al. (2004)
				Layers 32/32	42	No significant differences	–	No significant differences	Fate of DNA	Flachowsky et al. (2005b)
	Crude nutrients, starch, amino acids, fatty acids	Growing and laying hens	4 generations (growing, laying)	Growers 200/200	Four times: 126	No significant differences	–	No significant differences	Reproduction	Halle et al. (2006)
				Layers 32/32	91	No significant differences	–	No significant differences		
Silage	Crude nutrients	Growing and finishing bulls	Growing/finishing	20/20	246	No significant differences	–	No significant differences	Slaughtering data, fate of DNA	Aulrich et al. (2001)
	Crude nutrients	Sheep	Digestibility	4/4	24	No significant differences	No significant differences	–	–	Aulrich et al. (2001)

Table 28 (*Continued*)

GMP	Analytical measurements	Animal species/categories	Type of study	Animal number (isogen/transgen)	Duration (days)	Composition of GM-feeds	Digestibility	Zootechnical parameters	Further measurements	References
Bt-potatoes	Crude nutrients	Broilers	Growing	9/18	21	No significant differences	–	No significant differences	Fate of DNA	Halle et al. (2005)
Pat-maize	Crude nutrients, starch, sugar, NSP, amino acids, fatty acids	Pigs	Digestibility	5/5	14	No significant differences	No significant differences	–	–	Böhme et al. (2001)
Pat sugar beets										
Roots	Crude nutrients, sugar	Sheep	Digestibility	4/4	24	No significant differences	No significant differences	–	–	Böhme et al. (2001)
	Crude nutrients sugar	Pigs	Digestibility	5/5	14	No significant differences	No significant differences	–	–	Böhme et al. (2001)
Top silage	Crude nutrients	Sheep	Digestibility	4/4	24	No significant differences	No significant differences	–	–	Böhme et al. (2001)
Roundup Ready soybeans	Crude nutrients, starch, amino acids, minerals, fatty acids	Pigs	Growing/fattening	12/36	40	No significant differences	–	No significant differences	Slaughtering data, fate of DNA	Aulrich et al. (2001)

The most important results are summarized in Table 27. In agreement with other findings (see Flachowsky et al., 2005a) most DNA is degraded in the gastrointestinal tract (Reuter et al., 2002b; Wiedemann et al., 2006), but some fragments have been found in animal tissues. However, no residues of recombinant DNA were detected in any organ or tissue samples including eggs and milk obtained from animals fed with feeds from GMP (Table 27).

However, in the case that plant DNA-fragments should be absorbed, it might be that transgenic DNA-fragments are also absorbed. If this occurs, the frequency is likely to be extremely low, as shown by Mazza et al. (2005).

5. Conclusions

The results presented in Table 28 are in agreement with more than 100 international experiments, recently reviewed by Flachowsky et al. (2005a). The following conclusions can be drawn:

– Genetically modified plants without substantial changes in their composition (those of the first generation) do not significantly differ in their nutritional value from those of the isogenic variety.
– A transfer of recombinant DNA from feed into the animal body was not detected. To date, no residues of recombinant DNA have been found in any organ or tissue.
– Correspondingly, routine feeding studies with the target animal species generally add little to the nutritional and safety assessment (EFSA, 2004) but they are of public concern.
– As an approach for nutritional and safety assessment of feeds from GMP of the first generation a decision tree has been proposed (Flachowsky and Aulrich, 2001).
– Strategies for nutritional and safety assessment which are developed for those of the first generation cannot directly applied for GMP with substantial changes of the constituents (second generation).
– Case by case studies with animals will gain much more importance. Proposals for nutritional assessment of feeds from GMP with output traits were submitted (Flachowsky and Böhme, 2005). Such feeding studies should be combined with safety studies in target animal species.

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