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REVIEW ARTICLE

Animal nutrition with feeds from genetically modified plants

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Abstract
Plant breeders have made and will continue to make important contributions toward meeting the need for more and better feed and food. The use of new techniques to modify the genetic makeup of plants to improve their properties has led to a new generation of crops, grains and their by-products for feed. The use of ingredients and products from genetically modified plants (GMP) in animal nutrition properly raises many questions and issues, such as the role of a nutritional assessment of the modified feed or feed additive as part of safety assessment, the possible influence of genetically modified (GM) products on animal health and product quality and the persistence of the recombinant DNA and of the 'novel' protein in the digestive tract and tissues of food-producing animals. During the last few years many studies have determined the nutrient value of GM feeds compared to their conventional counterparts and some have additionally followed the fate of DNA and novel protein. The results available to date are reassuring and reveal no significant differences in the safety and nutritional value of feedstuffs containing material derived from the so-called 1st generation of genetically modified plants (those with unchanged gross composition) in comparison with non-GM varieties. In addition, no residues of recombinant DNA or novel proteins have been found in any organ or tissue samples obtained from animals fed with GMP. These results indicate that for compositionally equivalent GMP routine-feeding studies with target species generally add little to nutritional and safety assessment. However, the strategies devised for the nutritional and safety assessment of the 1st generation products will be much more difficult to apply to 2nd generation GMP in which significant changes in constituents have been deliberately introduced (e.g., increased fatty acids or amino acids content or a reduced concentration of undesirable constituents). It is suggested that studies made with animals will play a much more important role in insuring the safety of these 2nd generation constructs.

Keywords: *Animal nutrition, feed, genetically modified plants, nutritional assessment, DNA, protein degradation*

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1. Introduction

Beginning with the domestication of plants mankind tried to improve the characteristics of the wild varieties to make better use of the available natural resources. The successes of plant breeders in part made possible the dramatic increase of world population seen in the last few decades and which is set to further increase during the next 25 to 30 years from 6 to 8 billion people and to about 10 billion people in 2050 (Garza & Stover, 2003; UN, 2002).

It is inevitable that this growing population pressures will exacerbate the already existing problems of food insecurity and nutrient deficiencies (FAO, 2002). The global demand for food, especially for food of animal origin, will need to be doubled by the year 2025 (McCalla, 1999) and almost tripled by the year 2050 (Vasil, 1998). The reason for this disproportionate growth of population and demand for animal protein is a shift in eating habits toward over more food of animal origin in developing countries, as a consequence of higher incomes and increased ''standard of living''.

Given the shrinking resources of land per inhabitant, water, energy and other raw materials, the need for frugality in the resources used to produce this food is evident. This applies particularly to the provision of feedstuffs, because more than half of the plant mass produced by farmers is used in animal nutrition and the conversion of feeds into foods of animal origin is associated with considerable losses in energy, protein and further nutrients (Flachowsky, 2002). From the viewpoint of animal nutrition, the requirements to be met by plant breeding can be regarded as:

- Adequate production of high-quality feed with minimum use of resources, such water, fossil energy, nutrients and land area;
- Increased resistance to pests, tolerance to drought and salt levels in soils etc;
- Production of plants with a low content of undesirable (anti-nutritional) constituents in feedstuffs;
- An increase in the content and availability of plant constituents, which determine nutritional value (such as amino acids, fatty acids and vitamins), greater digestibility and thus higher energy and nutrient utilisation, and reduced environmental pollution by animal excrements.

Many of these requirements could be met in the long term by traditional plant breeding methods. However, in genetic engineering a tool is available which enables changes to be made to the genetic material of plants in the short term and with relatively high accuracy (CAST, 2003). Although it would be foolish to see 'green genetic engineering' as providing the solution to all matters of nutrition in the future, it can be expected to contribute to an improvement in the global food situation (Qaim & Virchow, 1999). During the eight year period 1996 – 2004, global area of transgenic crops increased 48 fold, from 1.7 to 81 million hectares in 2004 with increasing proportion grown by developing countries (James, 2004).

Genetic engineering as currently practised can be considered as an early technology with problems, but with a potential to contribute to improve food security and food safety (Avery, 2004; Bouis et al., 2003; CAST, 2003; Hartnell, 2004; Krawinkel & Mahr, 2004; Qaim 2000). Unfortunately, few studies have sort to demonstrate the potential advantages for consumers or a more efficient utilization of natural resources (Flachowsky, 2003). Results presented for a number of environmental and human health impact categories suggest that growing the genetically modified (GM) herbicide-tolerant crop would be less harmful to the environment and human health than the conventional crop, largely due to lower emissions from herbicide manufacture, transport and field operations (Phipps & Park, 2002). Similar

studies, which critically analyse inputs and outputs in relation to the commercialization of GM technology and comparative ecological risk assessments, seem to be urgently needed (Peterson & Hulting 2004).

Genetically modified plants can be used in a wide variety to feed animals (Chesson & Flint, 1999):

- Vegetative and generative plants or parts of plants (green forage, seeds, roots, tubers, etc.);
- Conserved products from genetically modified plants (GMP) (silage, hay);
- By-products of agriculture and food production, obtained from the processing of GMP (e.g., straw, by-products of milling, of the starch, oil, sugar and brewing industries);
- Feed additives consisting of or derived from genetically modified organisms (e.g., micro-organisms, amino acids, vitamins, enzymes).

The questions, considered in this review arise from research on novel GM constructs, the role and value of GM crops in agricultural practice and the value (or perceived lack of value) for the consumer of foods of animal origin produced using GM feeds and feed ingredients. The public debate has given rise to serious concerns in the minds of consumers, and therefore comprehensive risk assessments are necessary in addition to nutritional studies, if 'green genetic engineering' is to be accepted by European consumers (see EFSA, 2004; Garza & Stover, 2003; Hepple, 2004; ILSI, 2003b, 2004). Most of the experiments described in the paper were done with GMP of the so-called 1st generation. These are plants with unchanged cross composition and they are mostly registered. GMP from the 2nd generation in which significant changes in constituents have been introduced, are mostly not yet registered and open a wide field of possibilities for variable characteristics. In the second part of the paper some examples for nutritional assessment of GMP from the 2nd generation are given, which are not substantial equivalent (OECD 1993) to their isogenic counterparts. In such cases other types of studies for nutritional assessment are recommended (EFSA 2004, Flachowsky and Aulrich 2001a, see Figure 1; ILSI 2004).

2. Explanation and definitions

Some definitions are given below which may contribute to an understanding of this text.

2.1. Genetically modified organism (GMO) and products from GMOs

Genetically modified organisms are understood to be plants, micro-organisms or animals into which foreign deoxyribonucleic acid (DNA) coding one or more new genes has been integrated.

Foundation lines/hybrids are the conventional or unmodified parental or isogenic line/hybrids used in transformation events and the resultant GMO line/hybrids are referred to as the transgenic line/hybrids. Both the products of genetically modified organisms and the genetically modified organisms themselves are potentially available for human and/or animal nutrition.

Feed additives produced from genetically modified micro-organisms are already of considerable importance in animal nutrition (see Schwarz & Meyer, 1996; von Wright & Bruce, 2003). They are added to feedstuffs not merely to provide domesticated animals with essential nutrients to meet their needs (e.g., amino acids, vitamins), and are therefore of primary importance for animal health, performance and the effective conversion of feed constituents into food of animal origin, but products of GMO are also used in animal nutrition as non-essential feed additives (e.g., enzymes). As various surveys have been published in recent years on the action of such substances (e.g., phytase: Düngelhof &

Rodehutscord, 1995; NSP-degrading enzymes in pigs: Haberer & Schulz, 1998; in broilers: Daenicke, 1999), the present article will not deal with the products of GMO in animal nutrition. The paper deals only with GMP or parts of those plants.

2.2. 1st and 2nd generation of GMP

A working distinction is often made between the 1st and 2nd generation of GMP. This distinction is purely pragmatic or historical, and does not reflect any particular scientific principle or technological development.

The first generation of GMP are generally considered to be those crops carrying simple input traits such as increased resistance to pests or tolerance of herbicides. The proteins produced, which confer these benefits, occur in very low concentrations in the modified crops and so do not significantly change either the composition or feed value when compared to the foundation lines (isogenic lines). In contrast, the 2nd generation of GMP include crops in which the nutrient composition or availability has been deliberately changed by genetic engineering (Harlander, 2002; ILSI, 2004). Consequently, effects on the nutritional value of the feed are to be expected. These changes may have the objective of both increasing/changing the content of constituents which determine feed value or are desired (e.g., protein, amino acids, fat, fatty acids, minerals, vitamins, enzymes) as well as reducing the content of constituents which are undesirable or which are detrimental to digestibility (e.g., lignin, phytate, various secondary plant constituents) or feed safety (e.g., mycotoxins, further undesirable substances, see Table XXI).

2.3. Substantial equivalence

The concept of substantial equivalence is based on the idea that an existing plant used as feed with a history of safe use and known feed value, can serve as a comparator when assessing the safety and the feed value of a genetically modified plant (OECD, 1993; EC, 1997). Substantial equivalence is the starting point of the nutritional and safety assessment of GM material and can be described as a comparative approach to the assessment of safety (EFSA, 2004). Compositional analysis is a cornerstone for the nutritional assessment of new crop varieties whether they are bred conventionally or are derived from modern biotechnology. It should be noted that there are significant differences in composition of conventionally bred varieties within crops and therefore the compositional analysis of GM crops must be assessed against the background of the natural variability in their conventional counterpart(s). Although the term substantial equivalence was introduced for the assessment of foods, it is equally relevant to the safety assessment of those plants and their products used as feedstuffs.

According to the OECD (1993), a ‘new’ food or a new food ingredient is regarded as substantially equivalent if no significant differences occur in comparison with an appropriate traditional source. A food/feed plant is thus substantially equivalent if it corresponds to a conventional variety in its agronomy, composition, metabolic processes and its content of undesirable substances. If substantial equivalence can be established then by analogy, the novel food can be assumed to be as safe as the material to which it was compared. The provisions of the former ‘‘Novel Food’’ Regulation (Regulation No. 258/97 of 15.05.1997, EC 1997) were based on these definitions.

In practise, substantial equivalence is assessed mainly by comparing the agronomic characteristics of the plant and its composition. However, in determining the degree of

equivalence, it should not be overlooked that conventional feedstuffs also exhibit a considerable biological variability in their growth characteristics and constituents. The consensus documents prepared by OECD (2001a, b; 2002a, b, c) on the compositional analyses proposed for new varieties of soybean, maize, potatoes, rapeseed and sugar beet provide excellent guidance for the analyses need as part of the nutritional assessment of GM crops modified for agronomic traits and improved nutritional characteristics.

The OECD (2001c) regards substantial equivalence as a suitable robust framework for the nutritional and safety assessment of 1st generation GMP. Although it is not a statutory requirement, various bodies have also undertaken the nutritional assessment of 1st generation GM crops in a series of extensive digestion and feeding experiments with various species and categories of animals, in addition to laboratory studies.

The concept of substantial equivalence is much more difficult to apply to 2nd generation GMP (Clark & Lehmann, 2001). In such plants, modifications may be intended to change composition and biological value. With such plants, nutritional studies, such as conversion and feeding experiments with laboratory or farm animals, *in vitro* or *in sacco* measurements may assume a much more important role in confirming that the changes produced were those, and only those, intended. With some constructs, studies of this sort could replace the concept of substantial equivalence with one of nutritional equivalence.

2.4. Which questions are asked to animal nutritionists?

The following are some of the questions relating to animal nutrition that arise from the use of genetically modified plants:

- Is the comparison of the concentration of the important constituents of feedstuffs from GMP with its isogenic foundation lines sufficient to establish safety and nutritional equivalence when only minimal changes to composition are introduced by genetic engineering (1st generation crops)?
- Should the analysis of the constituents and nutritional assessment of GMP be done in comparison with isogenic foundation lines when there are significant intended changes to the concentration and/or nature of its constituents (2nd generation crops)?
- Are long-term feeding experiments with GM crops with the most important agricultural animals necessary to establish the absence of adverse effects on animal health, performance and quality of food of animal origin (Chesson, 2001; EFSA, 2004; Flachowsky & Aulrich, 2001a, b; ILSI, 2003b, 2004)?
- Should studies of the conversion (retention, metabolism) of the ingredients of the feedstuffs modified by genetic engineering (DNA, protein, amino acids or other constituents) in animals be an integral part of the safety assessment?

Attention should be paid in all these studies to unintended (unexpected) consequences of the transformation process (EC, 2000; EC, 2003; EFSA, 2004; ILSI, 2003b, 2004; OECD, 2002c). Unintended effects are not unique to genetic engineering and are found as a consequence of conventional breeding where they probably occur with similar or greater frequency (ILSI 2004, Ridley et al., 2002). Apart from reports by Malatesta et al. (2002a, b) about modifications of some nuclear features and cellular constituents in GMP fed mice, no unintended effects have been observed in commercial varieties of GMP to date. Other questions relate to the effects of

genetically modified micro-organisms as feed additives and the use of genetically modified organisms (GMO) and their products such as amino acids, vitamins and enzymes in animal production. One question, which could be of interest in the future in animal nutrition, concerns the energy and nutrient requirements of transgenic domesticated animals. If such animals have an altered performance, then they are likely to have different requirements for energy and for both macro- and micro-nutrients. The expression of novel enzymes not previously released in the digestive tract (e.g., plant or microbial phytase and non-starch polysaccharidases) may also have repercussions on animals and their nutrition.

3. Nutritional assessment of GMP

3.1. First generation of GMP

To date the feeding studies have been run almost exclusively with the 'first generation' of GMP – most studies with genetically modified maize and soybean, but some with cotton, rapeseed, wheat, rice, potato and sugar beets or by-products from these GM crops.

In the case of Bt maize, the most common construct studied, the expression of one of a family of Bt proteins is transferred from the soil bacterium *Bacillus thuringiensis* (Bt) into the maize, which results in the death of the larvae of the European corn borer when it feeds on this maize without the use of an insecticide (Fearing et al., 1997; Zellner, 1999). Insertion of the Pat gene (phosphinothricin acetyl transferase gene) endows the plant with tolerance to the herbicide glufosinate ammonium (''Basta'') while the *epsps* gene (5-Enolpyruvylshikimate-3-phosphate synthase gene) confers resistance to glyphosate (''Roundup Ready''). In all the studies the transgenic lines were compared with the corresponding isogenic foundation lines or commercial conventional lines (see summaries by Aumaitre, 2004; Aumaitre et al., 2002; Chesson & Flachowsky, 2003; Clark & Ipharraguerre, 2001; Faust, 2002; Flachowsky & Aulrich, 2001a, b).

3.1.1. Composition. In establishing the degree of equivalence the composition of feeds from isogenic and GMP were determined in many studies. Tables I and II show results from different studies. The comparison of modified maize and sugar beet (Table I) as well as wheat (Table II) and their conventional counterparts showed no significant differences between the respective pairs. Such numerical differences that were detected lie within the range of variation for feedstuffs of this variety (see consensus documents of OECD 2001a, b; 2002a, b, c, or ILSI, 2003a). Analogous results are reported in the literature (e.g., Padgette et al., 1996; Obert et al., 2004; see Tables IV, V, VII, XI and XVII). As these results show, compositional analysis has to be interpreted with some care. Exactly the same composition is not to be expected as plants were grown at different geographical locations and on different dates. Under these circumstances a considerable biological variability is normal. Differences in the content of certain constituents, such as those described by Masoero et al. (1999), should not be overestimated on account of the diverse factors exerting their production. In addition as more parameters are studied then a number of differences that arise purely by chance will increase. Using the 95% confidence intervals implies a 5% random difference.

When Bt maize is grown, secondary consequences may be observed (Dowd, 2000). Maize plants less severely weakened by the corn borer, might be expected to show better resistance to field infections, particularly by Fusarium infection. As a consequence of the lower level of fungal infection in the field, reduced mycotoxin contamination is to be expected, as was

demonstrated in respect of various mycotoxins, but not in all cases (see Table III). In studies made over several years Dowd (2000) investigated the influence of various levels of infestation with corn borer on isogenic and Bt hybrids in respect of mycotoxin contamination and came to the conclusion that overall a lower level of mycotoxin contamination was observed in the transgenic hybrids despite the considerable geographical and temporal variation observed.

3.1.2. Comparative digestion and feeding studies. In the meantime about 100 digestion and feeding studies with GM feed ingredients with various food producing animal species have been reported in the literature. Some results will be shown in detail, others are summarized (see Tables VII, XI, XVII and XVIII).

3.1.3. Poultry. The first experiment with feeds from GMP in poultry nutrition was published by Hammond et al. (1996). They found no significant influence on fattening and slaughter performance of broilers when soybean meal of isogenic origin (control) was replaced by a similar material from two transgenic hybrids.

Brake and Vlachos (1998) reported similar results when high percentages of isogenic vs. transgenic maize were fed in diets to broilers (Table IV). The significant reduction in feed conversion when Bt maize was used (from 1.75 to 1.72 kg per kg weight gain) should not be overestimated. It results from a somewhat lower fd intake and a final weight 23 g higher (Table IV), and is within the normal physiological range. Recently Taylor et al. (2004c) tested high portions of Gt canola meal (25%) in broiler diets and did not find any significant effect on slaughtering data and body composition (see Table V).

In experiments with layers and broilers where Bt maize (50% of the diet) was fed in comparison with the conventional variety Cesar (Aulrich et al., 2001) no significant differences in digestibility and in energy content were observed ($P > 0.05$; Table VI).

Table I. Selected constituents of transgenic insect resistant (Bt) and herbicide tolerant (Pat) maize grains and herbicide tolerant (Pat) sugar beet in comparison with isogenic lines (from Aulrich et al., 2001; Böhme et al., 2001).

Constituents	Isogenic maize	Insect resistant maize	Isogenic maize	Herbicide tolerant maize	Isogenic sugar beet	Herbicide tolerant sugar beet
Nutrients [g/kg DM]						
Crude ash	15	16	19	18	30	30
Crude protein	108	98	120	119	72	60
Ether extract	54	56	31	35	3	4
Crude fibre	23	25	34	30	56	46
NFE	800	805	796	798	839	860
Starch	710	708	692	701	n.a.	n.a.
Sugar	n.a.	n.a.	n.a.	n.a.	736	744
Amino acids [g/kg DM]						
Lysine	2.9	3.0	3.3	3.2	n.a.	n.a.
Methionine	2.2	2.1	2.6	2.5	n.a.	n.a.
Fatty acids [% of total fatty acids]						
Palmitic acid	12.4	12.5	11.5	11.8	n.a.	n.a.
Oleic acid	31.1	28.6	27.7	27.4	n.a.	n.a.
Linoleic acid	50.0	51.2	57.0	56.3	n.a.	n.a.

n.a. = no analysis.

Table II. Constituents of isogenic (control) and glyphosate-tolerant (Gt) wheat harvested in 2000 ($n = 20$) (from Obert et al., 2004).

Constituents	Control		Gt Wheat		Literature Range
	Mean	Range	Mean	Range	
Nutrients [g/kg DM]					
Crude protein	169	148 – 203	167	151 – 197	83 – 193
Ether extract	12.4	9.6 – 18.6	12.5	9.9 – 15.6	19 – 28.6
Total dietary fibre	172	140 – 256	168	143 – 209	
Crude ash	19.1	15.9 – 22.4	19.9	16.0 – 24.8	11.7 – 29.6
Amino acids [g/kg DM]					
Lysine	28.2	24.9 – 30.1	28.4	26.1 – 30.1	23 – 34
Methionine	16.7	14.2 – 19.9	16.8	14.8 – 18.5	12 – 21
Threonine	26.9	24.5 – 29.5	26.7	25.0 – 29.1	24.0 – 29.3
Tryptophan	9.3	8.1 – 11.1	9.3	8.2 – 10.8	12.8 – 15
Fatty acids [% of total fatty acids]					
Palmitic acid	18.7	17.6 – 19.6	18.5	17.6 – 19.2	11 – 32
Oleic acid	19.4	17.1 – 21.0	20.1	18.8 – 22.1	11 – 29
Linoleic acid	54.8	52.4 – 56.8	54.2	52.2 – 55.9	37.9 – 74
Linolenic acid	4.00	3.54 – 4.96	3.96	3.74 – 4.37	0.71 – 4.84

In addition to the studies summarized above, other feeding studies have been made with poultry (Table VII). The authors included various lines of insect resistant (Bt) maize and glyphosate-resistant maize, soybean, wheat, canola or potatoes. In each case, diets were formulated to allow a high proportion of the test material to be incorporated (e.g., 50 – 78% maize or 27% soybean) and comparison were made with nontransgenic or near isogenic lines.

In each study, the chemical composition of the GM feed ingredients proved to be essential indistinguishable from its conventional counterpart. Consequently, and not surprisingly, comparative feeding studies with broilers and layers also failed to show differences of any consequence in the various production parameters monitored. There were some studies (see Table VII) in which significant differences were observed, but these were not considered cause for concern. Piva et al. (2001b) observed a higher live weight gain in the test group compared to the control group, but ascribed this to a lower mycotoxin content in the Bt maize compared to the conventional maize used in the diet of the control group (see Table III). Halle et al. (2004) did not describe significant differences in growth, laying performance and reproduction of quails in a multi-generation experiment (Table VII).

3.1.4. Pigs. The replacement of 70% isogenic with Bt maize in studies by Reuter et al. (2002a, b) in pigs had no significant influence on the digestibility of selected crude nutrients, on the energy content of the mixtures and on the fattening and slaughtering performance of the animals (see Table VIII).

Apart from the digestibility of organic matter of sugar beets nutritional equivalence was also found with isogenic and herbicide-tolerant (Pat) maize grain or sugar beet (Table IX). Concurrent ecological research (e.g., different crop protection measures) was conducted in which isogenic and transgenic products were obtained from different growing conditions. The grain maize was dried gently (40°C), the sugar beet was chopped and fed fresh to growing pigs (40 – 60 kg live weight). The proportion of chopped beet had to be limited to 30% of the dry

Table III. Concentration of selected *Fusarium* toxins in isogenic and transgenic (*Bt*) maize grains (concentration in the transgenic hybrids expressed as % of the isogenic foundation hybrid).

		Deoxynivalenol		Zearalenone		Fumonisin B_1	
Author	Growing season/region	Isogenic [ng/g]	Bt [%]	Isogenic [ng/g]	Bt [%]	Isogenic [μg/g]	Bt [%]
Munkvold et al. (1999)	1995	n.r.	n.r.	n.r.	n.r.	8.8	54
	1996	n.r.	n.r.	n.r.	n.r.	7.0[§]	24
	1997	n.r.	n.r.	n.r.	n.r.	16.5[§]	13
Cahagnier and Melcion (2000)	France	350	79	n.r.	n.r.	1.0	20
	Spain	176	11	n.r.	n.r.	6.0	10
Pietri and Piva (2000)	1997 ($n=5$)	n.s.d.		n.s.d.		19.8	10
	1998 ($n=11$)					31.6	17
	1999 ($n=30$)					3.9	36
Valenta et al. (2001)	Corn borer infested ($n=15$)	873	18	256	13	n.r.	n.r.
	not infested ($n=15$)	77	70	19	15	n.r.	n.r.
Bakan et al. (2002)	France	472	154	3	< d.l.	n.r.	n.r.
	France	751	44	33	12	n.r.	n.r.
	France	179	101	3	133	n.r.	n.r.
	Spain	82	20	7	43	n.r.	n.r.
	Spain	271	7.4	4	75	n.r.	n.r.
Reuter et al. (2002b)	1999 Germany	343	< d.l.[¶]	3	< d.l.	n.r.	n.r.

n.r. = Not reported. [§]Total fumonisin. n.s.d = No significant difference (very low concentration). [¶]Below the detection

Table IV. Constituents of maize and fattening and slaughtering results after feeding isogenic and Bt maize to broilers (from Brake and Vlachos, 1998).

Parameter	Control maize	Bt maize
Constituents of maize [g/kg]		
Crude protein	88.7	84.3
Ether extract	30.0	31.9
Crude fibre	21.0	22.0
Crude ash	9.3	10.2
Lysine	2.5	2.6
Methionine and Cysteine	4.4	4.4
Maize in grower diet [%]	64.4	67.4
Metabolizable energy [MJ/kg]	13.4	13.4
Feed intake [kg/bird]	3.15	3.14
Final weight [g/bird]	1802	1825
Feed conversion ratio [kg feed/kg BWG]	1.75[a]	1.72[b]
Losses [%]	2.2	3.9
Slaughtering data [% of final weight][#]		
Thighs	12.4	12.5
Breast	16.8	17.2
Depot fat	1.36	1.42

[a, b] $P<0.05$. *Duration of experiment: 38 days; 640 birds/group. [#]At 41 days of age.

matter in the total rations for reasons of feed intake, and as the same basic rations were to be used in both series of experiments, the proportion of maize was also restricted to 30%. The variations which were observed, mainly in the sugar beet, (DM content, crude protein, crude fibre, NFE, digestibility of the organic matter) were within the normal range for investigations

of this type. Therefore differences in digestibility of organic matter should not be overestimated (see Table IX).

Armstrong et al. (2001), in a large-scale study (100 pigs, 24 – 111 kg BW), compared the influence of glyphosate-tolerant soybean meal with that of isogenic products and determined the effects on sensory qualities, loss in cooking and shear forces of pork. No effect of the GM feed was found on any of the criteria investigated. Hyun et al. (2004) conducted two studies with growing/finishing pigs (22 – 116 kg; 30 – 120 kg) fed diets containing Gt maize (68 – 82%) or conventional maize lines. Authors did not measure any significant effect of Gt maize on feed intake, growing performance, carcass yield or fatness parameters. No influence of feeds from GMP on slaughtering results and carcass measurements is also reported by Bressner et al. (2003), Peterson et al. (2003), Reuter et al. (2002b) and Fischer et al. (2003) (see Table XI).

Piva et al. (2001a) compared the feeding value of isogenic with Bt maize in piglets. Both maize lines were cultivated under similar conditions, but the non-GM hybrid was more extensively contaminated with *Fusarium* toxins. In what the authors suggest was a consequence, weight gain of the piglets fed the Bt-maize was significantly higher than those fed the non-GM line (see Table X).

The reported digestion and feeding experiments made with pigs are summarized in Table XI. Apart from experiments by Piva et al. (2001a), Weber and Richert (2001) and Custodio et al. (2004), comparative feeding studies with various pig categories failed to show differences of any consequences in any of the parameters monitored.

Table V. Selected data of composition of nontransgenic control and glyphosate tolerant canola meal and slaughtering results of broilers fed with diets containing canola meal (from Taylor et al., 2004c).

Parameter	Control canola meal (46A65)*	Gt canola meal (RT73)*
Constituents of canola meal [g/kg]		
Crude protein	428	427
Crude fibre	117	119
Final body weight [kg]	1.84	1.93
Feed conversion [kg/kg]	1.61	1.61
Carcass yield		
Chill weight [kg/bird]	1.55	1.60
Breast meat [% of chill weight]	25.2	24.9
Breast meat analysis [% of meat]		
Protein	23.7	23.7
Fat	0.86	0.82

*Duration of experiment: 42 days; 100 birds per treatment; 25% canola meal in starter diet, 20% canola meal in grower/finisher diet.

Table VI. Digestibility and energy content of diets for layers and broilers, containing 50% isogenic or Bt maize (from Aulrich et al., 2001)

Parameter	Control maize*	Bt maize*
Digestibility od organic matter (Layers) [%]	76.9 ± 0.8	77.2 ± 2.9
Metabolizable energy [MJ AME_N/kg DM]		
Layers	12.31 ± 0.12	12.75 ± 0.13
Broilers	12.82 ± 0.24	13.33 ± 0.24

*Six birds per treatment.

Table VII. Comparison of chemical composition and nutritional value to poultry of feeds from GMP and conventional parenteral or near isogenic lines.

Authors	Transgenic feed ingredient*	Results of compositional analysis#	Poultry categories	Results of nutritional assessment#
Hammond et al. (1996)	Gt soybeans	≈	Broilers	≈
Brake and Vlachos (1998)	Bt maize	≈	Broilers	≈(↓)§
Mireles et al. (2000)	Bt maize	≈	Broilers	≈
Sidhu et al. (2000)	Gt maize	≈	Broilers	≈
Aulrich et al. (2001)	Bt maize	≈	Layers/Broilers	≈
Aeschbacher et al. (2002)	Bt maize	≈	Broilers	≈
Gaines et al. (2001a)	Bt maize	≈	Broilers	≈
	Gt maize	≈	Broilers	≈
Kan et al. (2001)	Bt soybean	≈	Broilers	≈
Piva et al. (2001b)	Bt maize	≈	Broilers	≈(↑)¶
Taylor et al. (2001a, b)	Bt maize	≈	Broilers	≈
	Gt maize	≈	Broilers	≈
Stanisiewski et al. (2002)	Gt rapeseed	≈	Broilers	≈
Taylor et al. (2002)	Bt maize	≈	Broilers	≈
	Gt maize	≈	Broilers	≈
Yonemochi et al. (2002)	Bt maize	No data	Broilers	≈(↑)†
Kan and Hartnell (2003)	Gt wheat	≈	Broilers	≈
Tony et al. (2003)	Bt maize	≈	Broilers	≈
Brake et al. (2003)	Bt maize	≈	Broilers	≈
Taylor et al. (2003a, b, c)	Gt maize	≈	Broilers	≈(↓)‡
	Bt maize	≈	Broilers	≈
	Bt/Gt maize	≈	Broilers	≈
	Bt (wild-type) maize	≈	Broilers	≈
El Sanhoty (2004)	Bt potato	≈	Broilers	≈
Halle et al. (2004)	Bt maize	≈	Quails‖	≈
Taylor et al. (2004a, b, c)	Gt canola meal	≈	Broilers	≈
	Bt/Gt maize	≈	Broilers	≈
	Bt maize	≈	Broilers	≈
Kan and Hartnell (2004)	Gt wheat	≈	Broilers	≈ (↑)**

*Bt: *Bacillus thuringiensis*; Gt: Glyphosate-tolerant; Bt/Gt: combined traits in maize; Bt (wild-type Cry3Bb1 protein from *Bt*): maize protected against corn rootworm larvae. #Meaning of symbols: ≈ no significant changes ($P > 0.05$); ↑ significant increase, improvement ($P < 0.05$); ↓ significant decrease, reduction ($P < 0.05$). §Slight reduction in feed efficiency, which is within the physiological range. ¶Higher BWG as a consequence of lower mycotoxin content in Bt-maize. †Significantly higher BWG and FCR of Bt fed broilers during starter phase, but disappearence of this difference during the finisher phase. ‡Significantly lower fat pad weight of birds fed diets containing Gt maize. ‖Multi-generation experiment, results up to 4th generation. **Lower carcass yield for birds fed the nontransgenic control.

3.1.5. Ruminants. Feeds incorporating GMP have been fed to dairy cows, growing cattle and sheep. As with poultry, the first study was published by Hammond et al. (1996, see Table XVII). The higher milk performance of cows fed with glyphosate-tolerant soybeans reported by the authors was probably a result of weaknesses in the experimental design (critically discussed by Flachowsky & Aulrich, 1999). Subsequent studies have not shown significant differences between groups fed from isogenic or transgenic plants. Isogenic maize (variety Cesar) and transgenic maize (Bt maize) with a DM content of ≈ 33% were made into silage and used in digestion experiments with wethers and in a long-term feeding experiment with growing and fattening bulls. There were no significant differences between the two maize silages either in the constituents or in the digestibility of the crude nutrients or energy content

Table VIII. Influence of a high percentages of isogenic versus Bt maize (70% of diet) on the digestibility, energetic feeding value and selected fattening data of pigs (from Reuter et al., 2002a, b).

Parameter	Control maize	Bt maize
Digestibility [%]		
Crude protein	84.9 ± 2.1	86.1 ± 1.7
NFE	92.7 ± 0.5	93.2 ± 0.6
Metabolizable energy [MJ/kg DM]	15.7 ± 0.2	15.7 ± 0.2
Fattening performance*		
Feed intake [kg/d]	1.95 ± 0.15	1.94 ± 0.15
Body weight gain [g/d]	815 ± 93	804 ± 64
Feed conversion ratio [kg feed/kg BWG]	2.39 ± 0.17	2.41 ± 0.15
Energy efficiency [MJ ME/kg BWG]	33.4 ± 2.3	33.7 ± 1.5

*Duration of the experiment: 91 days; 12 pigs in the control group, 36 pigs in the Bt maize group.

Table IX. Apparent digestibility and energy content of isogenic and transgenic herbicide-tolerant (Pat) maize and sugar beets in pigs* (from Böhme et al., 2001).

Parameter	Isogenic lines Conventional herbicides	Transgenic lines (Pat)	
		Conventional herbicides	Treatment with 'Basta'
Digestibility of organic matter [%]			
Maize	89.6 ± 4.1	90.0 ± 2.1	89.3 ± 1.8
Sugar beets	$89.4^{b} \pm 1.1$	$93.8^{a} \pm 2.2$	$92.5^{a} \pm 2.6$
Metabolizable energy [MJ/kg DM]			
Maize	15.8 ± 0.6	16.0 ± 0.3	16.1 ± 0.3
Sugar beets	13.7 ± 0.4	14.2 ± 0.3	14.0 ± 0.4

*Determined with the difference method; 30% maize or sugar beets in the diet (DM base); five pigs per treatment.
$^{a,b}P < 0.05$.

(see Table XII). The bulls consumed a daily average of 18.8 kg and 18.7 kg fresh weight of the conventional and GM silages respectively in the 246-day lasting experimental period, and the daily weight gain was 1487 and 1482 g respectively. This can be described as very high for Black Pied cattle. No significant differences emerged between the bulls in the two groups for any of the criteria investigated (see Table XIII). This finding also applied to the health of the animals and to the quality of meat and fat they produced.

Erickson et al. (2003) compared in three experiments high portions of Gt maize in concentrate of finishing diets of feedlot steers (175, 196 and 200 steers per experiment) with nontransgenic hybrids. No differences were observed between Gt maize and nontransgenic lines in dry matter intake, daily weight gain, carcass weight, *Musculus longissimus dorsi* area and marbling scores in any of the experiments. The fat depth of steers fed with Gt maize varied from controls, however, the variation was not consistent between the experiments. Similar results were reported by Berger et al. (2003) comparing high portion of Bt maize with control lines in feedlot steers (see Table XIV).

Donkin et al. (2003) compared in three experiments the effect of feeding silages and grain from conventional and Bt maize and conventional Gt maize. They, too, found no effect on the composition of grains and silages (see Table XV) or on the milk yield and milk composition (see Table XVI). Similar results are also reported from feeding of insect-protected cottonseed in lactating buffaloes (Singh et al., 2003).

In addition to the studies described above, about 40 papers have now been published describing feeding experiments with ruminants in which a feed containing GMP was compared with a corresponding feed made with conventional varieties. These are summarized in Table XVII.

3.1.6. Other food producing animals. A few studies have been reported in which GMP were incorporated in feeds intended for other food-producing animals (rabbits, fish, see Table XVIII). As previously, the presence of the GM ingredient had no significant effect on performance or health.

Table X. Influence of isogenic and transgenic (Bt) maize on the performance of piglets (from Piva et al., 2001a).

Parameter	Isogenic maize	Bt maize (in % of isogenic maize)
Mycotoxins (isogenic = 100%)		
Fumonisin B_1	100	31
Deoxynivalenol	100	86
Final body weight [kg]	22.0[a]	22.6[b]
Body weight gain [g/d]	375[a]	396[b]

[a, b] $P < 0.05$.

Table XI. Comparison of chemical composition and nutritional value to pigs of feeds from GMP and conventional parenteral or near isogenic lines.

Authors	Transgenic feed ingredients⋆	Results of compositional analysis[#]	Pig categories	Results of nutritional assessment[#]
Böhme et al. (2001)	Pat maize	≈	Growing	≈
	Pat sugar-beets	≈	Growing	≈
Gaines et al. (2001b)	Bt maize	≈	Growing	≈
	Gt maize		Growing	
Piva et al (2001a)	Bt maize	≈	Piglets	≈ (↑)[‡]
Stanisiewski et al. (2001)	Bt maize	≈	Growing	≈
Weber and Richert (2001)	Bt maize	≈	Growing/finishing	≈(↓)[§]
Bressner et al. (2002)	Gt maize	≈	Growing/finishing	≈
Cromwell et al. (2002)	Gt soybean meal	≈	Growing/finishing	≈
Fischer et al. (2002)	Gt maize	≈	Growing/finishing	≈
Reuter et al. (2002a,b)	Bt maize	≈	Growing/finishing	≈
Aalhus et al. (2003)	Gt canola meal	≈	Growing	≈
Bressner et al. (2003)	Corn root worm protected corn	≈	Growing/finishing	≈
Fischer et al. (2003)	Corn root worm protected corn	≈	Growing/finishing	≈
Peterson et al. (2003)	Gt wheat	≈	Growing/finishing	≈
Hyun et al. (2004)	Gt maize	≈	Growing/finishing[†]	≈
Cromwell et al. (2004)	Pat rice	≈	Growing/finishing	≈
Custodio et al. (2004)	Bt maize[¶]	No data	Growing/finishing	≈(↓)[‖]
Stein et al. (2004)	Bt maize⋆⋆	No data	Growing/finishing	≈
Aulrich et al. (2005)	Gt soybeans	≈	Growing/finishing	≈

⋆Bt: *Bacillus thuringiensis*; Gt: Glyphosate-tolerant. [#]Meaning of symbols: ≈ no significant changes ($P > 0.05$); ↑ significant increase, improvement ($P < 0.05$); ↓ significant decrease, reduction ($P < 0.05$). [‡]Higher BWG as a consequence of lower mycotoxin content in Bt maize. [§]Lower 10th rib fat depth in Bt maize fed pigs. [†]Two studies at two locations. [¶]Bt maize in comparison with pooled non-biotechnologically derived inbred lines. [‖]Feed efficiency was lower for pigs fed the Bt diet. ⋆⋆Bt maize contains the Cry1F gene from *Bacillus thuringensis var. azawai* and protects the plant from several insects including the European corn borer and the black cutworm.

3.1.7. Summary of digestion and feeding experiments with the 1st generation GMP. The results of the many animal experiments already completed have mostly revealed no significant differences between isogenic and transgenic hybrids (see Tables I, II, IV–XVIII). The occasional finding of a significant difference are plausibly explained by other external factors such as differences in the level of mycotoxin contamination (see Table III) or a weakness of experimental design (see Flachowsky & Aulrich, 1999). All of these studies were made with relatively few constructs and all would be considered as 1st generation crops in which no significant change in composition is to be expected. However, a minor cause for concern is that many of the experimental results compiled in Tables VII, XI, XVII and XVIII derived from reports made at scientific congresses and are available as abstracts. Relatively few of these studies have gone on to be published as full papers in peer-reviewed journals. Journals, however, are reluctant to publish experiments which are repetitive, produce only negative findings and which do not obviously contribute to the developments of the science.

Despite this minor concern, the weight of evidence shows that for these 1st generation plants, the concept of 'substantial equivalence' between transgenic feedstuffs and their isogenic foundation lines, as set out in the Novel Food Guideline (OECD, 1993), can be confirmed.

The published literature also contains no indications of any disturbance to the health of animals fed long-term with feeds from GMP and therefore these feedstuffs can be assessed as safe for animals. There are also no indications that incorporation of GM material had any effect on the quality of the animal products. Clark and Ipharraguerre (2001) came to a similar conclusion, after they had analysed the results of 23 published studies in which isogenic plants were compared with transgenic plants (maize, soybeans). The insect resistance or herbicide tolerance introduced into existing commercial GM varieties are agronomic traits and have little or no measurable effect on feed composition or the bioavailability of nutrients. As Tables I and II and some other tables show, the gross composition of such GM varieties falls within the range normally associated with conventional varieties of same feedstuff and the evidence to date is that they behave as any other varieties. This suggest that for those GMP with modified input traits, provided that the gross composition and presence of any anti-nutritional factors falls within the expected range, routine feeding studies made with food producing

Table XII. Constituents of silage from isogenic (Cesar) and from transgenic (Bt) maize hybrids, and digestibility and energy content of maize silages fed to wethers* (from Daenicke et al., 1999).

Parameter	Cesar maize	Bt maize
Dry matter [g/kg]	337	321
Crude nutrients [g/kg DM]		
Crude ash	45	42
Crude protein	84	87
Ether extract	29	28
Crude fibre	186	191
NFE	656	652
Digestibility [%]		
Organic matter	75.0 ± 2.5	74.5 ± 2.0
Ether extract	76.3 ± 3.2	79.8 ± 5.1
Crude fibre	66.7 ± 4.4	68.1 ± 3.6
NFE	81.2 ± 2.3	80.8 ± 1.3
Metabolizable energy [MJ/kg DM]	10.95 ± 0.03	10.91 ± 0.04

*Four wethers per treatment.

Table XIII. Fattening and slaughter performance of Schwarzbunt [Black Pied] bulls fed with silage made from isogenic (Cesar) and transgenic (Bt) maize (from Daenicke et al., 1999)*.

Parameter	Cesar maize	Bt maize
Dry matter intake		
Concentrates [kg/d]	1.78 ± 0.05	1.80 ± 0.03
Maize silage [kg/d]	6.33 ± 0.35	6.00 ± 0.29
Crude protein [g/d]	1102 ± 35	1110 ± 29
Metabolizable energy [MJ/d]	91.2 ± 4.2	88.6 ± 3.2
Weight gain [g/d]	1487 ± 97	1482 ± 121
Energy efficiency [MJ/kg BWG]	61.5 ± 3.3	60.1 ± 4.1
Carcass yield [%]	52.4 ± 1.5	52.8 ± 1.1
Leaf fat# [kg]	49.6 ± 5.5	48.7 ± 8.1

*Duration of the experiment: 246 d; 20 bulls per treatment; mean initial BW: 188 kg. #Total of stomach, intestinal, pelvic and kidney fat.

Table XIV. Influence of corn rootworm protected maize (CRW: event MON863) on fattening and slaughtering data of feedlot steers (Berger et al., 2003)*.

	Commercial control hybrids		Isogenic control	Transgenic maize	
Parameter	RX740	DK647	RX670	CRW	SEM
Initial BW [kg]	456	458	458	457	3
Final BW [kg]	598	609	614	609	7
DM intake [kg/d]	7.57	7.46	7.94	7.76	0.16
BWG [kg/d]	1.39	1.49	1.53	1.49	0.06
Weight gain per kg DM[kg/kg]	0.184	0.198	0.193	0.193	0.008
Carcass characteristics					
Carcass weight [kg]	367	374	377	374	4
Marbling score	484	470	489	493	9
M. longissimus dorsi area at 12th rib [cm^2]	97.3	99.5	95.6	97.2	1.5
Fat [cm]	0.85	0.89	0.99	0.92	0.05
Yield grade	1.9	1.9	2.3	2.1	0.1

*Duration of the experiment: 102 d; 49 steers per treatment.

Table XV. Constituents of silage and grain from isogenic and transgenic (Bt) maize hybrids (from Donkin et al., 2003) (Means ± SD, $n = 3$).

	Silage		Grain	
Parameter	Control maize	Bt maize	Control maize	Bt maize
Dry matter [%]	38.7 ± 2.9	38.6 ± 1.6	87.5 ± 1.9	87.7 ± 1.8
Crude protein [% of DM]	8.2 ± 0.4	7.9 ± 0.6	9.4 ± 0.1	9.4 ± 0.1
ADF [% of DM]	21.2 ± 1.3	21.9 ± 1.1	3.4 ± 0.7	3.3 ± 1.0
NDF [% of DM]	35.9 ± 2.5	36.9 ± 3.9	10.9 ± 2.4	9.7 ± 2.5
NEL [MJ/kg]	7.16 ± 0.38	6.99 ± 0.30	8.67 ± 0.08	8.67 ± 0.08

Table XVI. Influence of feeding maize silage and maize grain from isogenic and transgenic maize (Bt) on feed intake, milk production, and composition of dairy cows (from Donkin et al., 2003)*.

Parameter	Control maize	Bt maize
Dry matter intake [kg/d]	23.2	24.1
Milk yield [kg/d]	32.2	32.2
Milk Fat [%]	3.66	3.75
Milk Fat [kg/d]	1.02	1.03
Milk protein [%]	3.19	3.24
Milk protein [kg/d]	1.17	1.21
Milk lactose [%]	4.56	4.59
Milk lactose [kg/d]	1.48	1.49

*Duration of the experiment: three 21-day periods; 6 cows per treatment.

animals would appear to add little to a real safety assessment. But such experiments seem to be necessary for the public, especially in Europe.

3.2. Second generation of GMP

The concept of substantial equivalence is not readily applicable to the 2nd generation of genetically modified plants, in which the content of certain constituents is intentionally altered. Experiments with GM maize in which the phytate content is lowered (Spencer et al., 2000a, b) confirm this view. In experiments with fattening pigs, phosphorus from the 'low-phytate' maize was utilized distinctly better, and therefore no supplementation with mineral phosphorus was necessary (see Table XIX). Edwards et al. (2000) compared constituents and energy content of soybean meal from conventional (47.5% CP) and soybean lines with increased protein (52.5; 53.4 and 62.7% CP in the original matter, respectively) and content of limiting amino acids and came to a better assessment of the protein- or amino acid enriched soybeans (see Table XX).

Examples for undesirable secondary plant constituents from a nutritional viewpoint are alkaloids, glucosides, glucosinolates, lectins and phenol derivatives, such as tannins and gossypol, and protease inhibitors (see ILSI, 2003a; Jeroch et al., 1993; Kling & Wöhlbier, 1983; OECD, 2001a, b; 2002a, b, c). Experimental constructs exist in which the concentration of these undesirable substances have been substantially increased or reduced. Simple determinations of composition are not sufficient to make a nutritional/safety assessment of changes of this type. Animal experiments are needed to assess the digestibility of the changed nutrient or its influence on the digestibility/availability (e.g., bioavailability of β-carotene from Golden Rice) of other constituents and other effects on the physiological processes in the animal (see Table XXIII). For example, Molvig et al., (1997) investigated the digestibility of methionine-rich lupines (3.9 *vs.* 2.0 g methionine/kg) in rats and found protein digestibility increased from 89.4 to 95.7% ($p < 0.05$). Similar data are reported by Ravindran et al. (2002) after nutritional evaluation of transgenic high methionine lupins with broilers. White et al. (2001) increased the efficiency of wool growth and live weight gain in Merino sheep feed transgenic lupine seed containing sunflower albumin. Humphrey et al. (2001) tested rice in broiler rations containing a higher level of lactoferrin and lysozyme. These genetically modified changes had a marked effect on the microbial colonization of the digestive tract of chicks and on the mucosa of the small intestine, and caused a reduction in feed consumption.

In the future, 2nd generation GMP are likely to be more widely available, particularly those in which the proportion desirable constituents is increased and undesirable or anti-nutritional constituents reduced.

Expressing of enzymes by GMP may be a further way of genetic modification. Armstrong et al. (2002) developed a potato cultivar capable of expressing 1,3 – 1,4-β-D-glucan 4-glucano hydrolase from *Fibrobacter succinogenes*. The enzyme concentration can be found in concentrations as high as 0.05% of the fresh tuber weight with a specific activity of 3013 units mg^{-1} glucanase. Baah et al. (2002) included the transgenic potato cultivar in barley-based diets for broiler chickens at 0.6 kg t^{-1} and found an improved feed conversion by 8.8%. Digesta viscosity was reduced. Such potatoes may have a similar potential as an enzyme additive.

The following can be regarded as desirable changes or are currently one of the objectives of genetic engineering (see also Beever & Mueller-Harvey, 2000; Harlander, 2002; ILSI 2004; USDA, 2002; Table XXI):

- Increased content of protein or certain amino acids (e.g., lysine, methionine);
- Increased content of fat or certain fatty acids.
- Increased quantity and modified forms of starch (high amylopectin starch with different breakdown characteristics);
- Increased content or better availability of certain minerals, trace elements and vitamins;
- Increased content of certain substances which contribute to the well-being of animals (e.g., essential oils) or can assist the digestive process (e.g., enzymes, prebiotical substances);
- Better digestibility/availability of certain constituents or energy.

Recently Zimmermann et al. (2004) made a health economics approach within an *ex ante* study of Golden Rice in the Philippines. Depending on the underlying assumptions, internal rates of return on research investments range between 66 and 138%. This confirms that micronutrient-dense staple crops can be an efficient way to reduce deficiency problems among the poor.

By reducing the content of undesirable constituents it is also possible to improve the utilization of constituents, which determine nutritional value, as the results from the studies by Spencer et al. (2000b) and Mendoza (2002) demonstrate. However other effects are more difficult to predict. While low lignin content can contribute to better digestibility of the plant cell wall fractions, it can also be highly detrimental to the resistance of plants to lodging and to infestation by pests (e.g., bm_3 hybrids of maize).

The consequences for the nutritional assessment of 2nd generation GMP with changed constituents are discussed later (see Figure 1, Table XXIII and ILSI 2004).

4. Persistence of 'foreign' deoxyribonucleic acids (DNA)

From the moment a plant is harvested, autolytic process and microbial attack results in a decline in total DNA and RNA and a reduction in polymer size. The rate at which this decline occurs and consequently the period during which genetic information is retained intact is determined by many factors including ambient conditions, the extent and nature of any processing and finally, for those products destined for use as food or feed, the digestive process of the gastrointestinal tract.

4.1. DNA degradation during feed treatment

With the obvious exception of forages grazed directly, feedstuffs are usually treated by methods designed to preserve the feed, improve palatability or to increase nutritive value

Table XVII. Comparison of chemical composition and nutritional value to ruminants of feeds from GMP and conventional parenteral or near isogenic lines.

Authors	Transgenic feed ingredients*	Results of composition analysis[#]	Species/category of ruminants (investigations)	Results of nutritional assessment[1]
Hammond et al. (1996)	Gt soybeans	≈	Dairy cows (Performance, composition, digestibility)	≈ (↑)[§]
Faust and Miller (1997)	Bt maize -green plant	≈	Dairy cows (Performance, composition)	≈
Daenicke et al. (1999)	Bt maize -silage	≈	Sheep (Digestibility)	≈
			Growing/fattening bulls (Performance)	≈
Rutzmoser et al. (1999)	Bt maize -silage	≈	Dairy cows (Performance, composition)	≈
Donkin et al. (2000)	Gt maize -silage and grain	No data	Dairy cows (Performance)	≈
Faust (2000)	Bt maize-silage	≈	Dairy cows (Performance)	≈
Folmer et al. (2000a)	Bt maize -plant residue	No data	Growing steers (Performance)	≈
	-silage		Growing steers (Performance)	≈ (↑)[¶]
Folmer al. (2000b)	Bt maize -silage	No data	Dairy cows (Performance)	≈
Hendrix et al. (2000)	Bt maize -plant silage	No data	Steer calves (Performance)	≈ (↑)[¶]
	-crop residues		Beef cows (Performance)	≈
Russel et al. (2000)	Bt maize -crop residues	≈	Beef cows	≈
Russel et al. (2001)	Bt maize -crop residues	≈	Beef cows	≈
Barriere et al. (2001)	Bt maize -silage	≈	Sheep (Digestibility)	≈
			Dairy cows (Performance, composition)	≈
Böhme et al. (2001)	Pat sugar beets,	≈	Sheep (Digestibility)	≈ (↓) (↑)[†]
	Pat sugar beet top silage	≈		
Castillo et al. (2001a,b)	Bt cottonseed and Gt cottonseed	No data	Dairy cows (Performance, composition)	≈
				≈
Hvelplund and Weisbjerg (2001)	Gt sugar beets	No data	Sheep (Digestibility)	≈
	Gt fodder beets			≈
	Beet pulp from sugar beets			≈
Kerley et al. (2001)	Bt maize	≈	Beef cattle (Performance)	≈
Petty et al. (2001a)	Bt maize -grain	No data	Beef cattle (Performance, carcass characteristics)	≈
Petty et al. (2001b)	Gt maize -grain and silage	No data	Beef Cattle (Performance, carcass characteristics)	≈
Weisbjerg et al. (2001)	Gt fodder -beets	≈	Dairy cows (Performance, composition)	≈
Berger et al. (2002)	Gt maize -grain	No data	Feedlot steers (Performance, carcass characteristics)	≈
Folmer et al. (2002)	Bt maize -plant residues	≈	Beef steers (Performance)	≈

(*continued*).

Table XVII. (*continued*).

Authors	Transgenic feed ingredients*	Results of composition analysis#	Species/category of ruminants (investigations)	Results of nutritional assessment[1]
	Bt maize -silage and grain	≈	Growing beef cattle and dairy cows (Performance, composition)	≈ (↑)‡
Ipharraguerre et al. (2002)	Gt maize -grain and -silage	≈	Dairy cows (Performance, composition)	≈
Grant et al. (2002)	Gt maize-silage and grain	≈	Dairy cows (Performance, composition)	
Stanford et al. (2002)	Gt canola meal	No data	Lamb (Digestibility, performance, carcass characteristics)	≈
Van der Pol et al. (2002)	Corn rootworm protected corn -grain	No data	Beef cattle (Performance)	≈
Simon et al. (2002)	Gt maize -grain	≈	Feedlot steers (Performance, carcass characteristics)	≈
Berger et al. (2003)	Corn root worm protected corn	≈	Feedlot steers (Performance, carcass characteristics)	≈
Erickson et al. (2003)	Gt maize -grain	≈	Feedlot steers (Performance, carcass characteristics)	≈
Donkin et al. (2003)	Bt maize -silage and grain	≈	Dairy cows (Performance, composition)	≈
	Gt maize -silage and grain	≈	Dairy cows (Performance, composition)	≈
Calsamiglia et al. (2003)	Gt and Bt maize -silage	≈	Dairy cows (Performance, composition)	≈ (↑)‖
Faust et al. (2003)	Bt maize -grain and silage	No data	Dairy cows (Performance, health, metabolism)	≈
Grant et al. (2003)	Gt maize -silage and grain	≈	Dairy cows (Performance, composition)	≈ (↓)**
	Corn rootworm protected corn -silage and grain	≈	Dairy cows (Performance, composition)	≈
Ipharraguerre et al. (2003)	Gt maize -silage and grain		Dairy cows (Performance, composition)	≈
Yonemochi et al. (2003)	Bt maize -grain	≈	Dairy cows (Performance, health)	≈
Singh et al. (2003)	Bt cottonseed	≈	Murrah buffaloes (Lactation, haematobiochemistry)	≈
Stanford et al. (2003)	Gt canola meal	≈	Sheep (Digestibility)	≈
			Lambs (Performance, carcass characteristics)	≈
Wilson et al. (2003)	Corn rootworm protected corn -plant residues	No data	Grazing steers (Performance)	≈
	Gt maize -plant residues	No data	Grazing steers (Performance)	≈
Castillo et al. (2004)	Bt cottonseed	≈	Dairy cows	≈
	Gt cottonseed	≈	(Performance, composition)	≈

*Bt: *Bacillus thuringiensis*; Gt: Glyphosate-tolerant. #Meaning of symbols: ≈ no significant changes ($P>0.05$); ↑ significant increase, improvement ($P<0.05$); ↓ significant decrease, reduction ($P<0.05$). §Increase of FCM-performance in consequence of weaknesses in experimental design (see Flachowsky and Aulrich, 1999). ¶Increase of weight gain of steer calves fed Bt silage. †Lower digestibility of CF and higher digestibility of NFE of sheep fed diets containing Pat sugar beet top silage. ‡Higher DMI and higher daily gain in Bt fed growing beef cattle. ‖Slightly higher milk protein and lactose in milk from cows fed Bt maize silage. **Lower DMI of cows fed Gt diet, resulting in a lower milk production.

Table XVIII. Chemical composition and nutritional value to other food producing animals of feeds incorporating GMP compared to equivalent feeds produced with conventional parental or near isogenic lines.

Authors	Transgenic feed ingredients*	Results of composition analysis#	Species/category of animals	Results of nutritional assessment
Hammond et al. (1996)	Gt soybeans	≈	Cat fish	≈
Maertens et al. (1996)	Gt rapeseed	≈	Rabbits	≈
Chrastinova et al. (2002)	Bt maize	≈	Rabbits	≈
Brown et al. (2003)	Gt rapeseed	≈	Rainbow trout	≈

*Bt: *Bacillus thuringiensis*; Gt: Glyphosate-tolerant. #Meaning of symbol: ≈ no significant changes ($P > 0.05$).

before being presented to an animal. Virtually all such methods have an effect on DNA, either by increasing availability to micro-organisms or leading directly to the breakdown (fragmentation) of the polymer.

DNA is unstable under the acid conditions (pH 3.5 – 5.0) found in silage and has been shown to be degraded to smaller fragments (Aulrich et al., 2004; Einspanier et al., 2004; Hupfer et al., 1999). Aulrich et al. (2004) detected 1016 base pairs (bp) fragments of DNA in maize cob silage and whole plant maize silage made from Pat maize up to 5 d and 28 d after ensiling respectively. Fragments of 680 bp could be detected up to 28 d in the maize cob silage and up to 35 d in whole plant silage. Thereafter only small fragments of 194 bp could be detected. In studies by Einspanier et al. (2004) the concentrations of specific plant DNA similarly decreased during the ensiling of Bt and conventional maize, finally representing only 1.3 – 3% of that initially present in the starting material. No quantitative differences were observed between the rate and extent of breakdown in the isogenic and transgenic ensiled maize. DNA fragments from silages are less stable in the rumen than fragments from seeds. A 1914 bp DNA fragment was still amplificable from rumen fluid sampled 5 h after feeding maize grains (Duggan et al., 2003). The same target sequence, however, could not be amplified from rumen fluid sampled from sheep fed silage. PCR amplification of a shorter (211 bp) target sequence was possible with rumen fluid sampled up to 3 and 24 h after feeding silage or maize grains, respectively. These findings indicate that intact transgenes from silage are unlikely to survive significantly in the rumen. DNA in untreated maize persists for a significant time in the rumen.

Intact DNA and protein in crops conserved by air-drying or in pulps obtained by low-temperature aqueous extraction (e.g., sugar beet) can be detected throughout the normal duration of storage (Chiter et al., 2000). Unless subject to some other form of processing, which is unlikely in the case of hays which are not normal ingredients of manufactured feeds, protein and DNA from such sources are consumed by the animal largely in an entire form.

Chemical or physical extraction of oils, sugar or starch from plants or processing to produce beer or other foods of plant origin invariably causes significant and, in some cases, complete DNA degradation (Alexander et al., 2002; Berger et al., 2003; Chiter et al., 2000; Gawienowski et al., 1999; Gryson et al., 2002 and 2004). As a rule, grinding and dry milling have little direct effect on DNA structure, but if shear forces are accompanied by localized heating then some degradation can occur (Berger et al., 2003). In contrast only highly fragmented DNA could be detected in oilseed meals following chemical extraction of the oil (Chiter et al., 2000).

Table XIX. Conventional and low-phytate maize (78.5% of the mixture) in the feed of fattening pigs (from Spencer et al., 2000b).

	Control maize (0.3 g of available P per kg) *		Low-phytate maize (1.7 g of available P per kg) *	
Inorganic P supplement	–	+	–	+
P content [g/kg]				
29 – 73 kg BW	3.4	5.4 #	3.4	5.4 #
73 – 112 kg BW	3.2	4.7 §	3.2	4.7 §
Feed intake [kg/d]	2.23 [a]	2.50 [b]	2.53 [b]	2.51 [b]
BWG [g/d]	730 [a]	870 [b]	900 [b]	880 [b]
FCR [kg/kg]	3.05 [a]	2.87 [b]	2.81 [b]	2.85 [b]
P excreted [g/kg]	4.6 [a]	8.9 [c]	3.8 [b]	8.8 [c]
Strength (4th metacarpal bone) [kg]	79.4 [a]	138.5 [bc]	132.2 [b]	153.9 [d]
Ash content (4th metacarpal bone) (%)	53.5 [a]	60.1 [bc]	59.3 [b]	61.2 [c]

[a, b, c, d] different letters in one line indicate significant differences ($P < 0.05$). *35 pigs per treatment. # +2.0 g P/kg. §+1.5 g P/kg.

Table XX. Constituents and key feed values (~90% DM) of soybean meal from protein-enriched GM soybeans (from Edwards et al., 2000).

	Control soybean meal		GM soybean meal	
Parameter	1	2	3	4
Crude protein [%]	47.5	52.5	53.4	62.7
Lysine [%]	3.02	3.23	3.27	3.40
Methionine [%]	0.66	0.70	0.75	0.72
Threonine [%]	1.90	1.94	2.12	2.03
NDF [%]	7.1	12.8	9.8	5.2
True ME_N [MJ/kg]	9.3	9.1	8.7	10.3

4.2. Fate of DNA in animals

Humans and animals have always been confronted with 'foreign' DNA as part of the diet. In humans the dietary intake of DNA ranges between 0.1 and 1 g per day (Doerfler, 2000), and includes more or less degraded fragments of various genes of plant and animal origin, as well as bacterial DNA. In the case of a fattening pig (DM intake 2 kg/d) and the dairy cow (DM intake 20 – 25 kg/d), much higher intakes of DNA are to be expected. Phipps and Beever (2000) calculated a DNA intake of a cow of 54 – 57 g/d, given a DM intake of 24 kg/d. When cows were fed rations containing 40% silage and 20% grain from transgenic (Bt) maize, the same authors calculated the DNA originating from the transgenic DNA as 54 μg/d or 0.000094% of the total DNA intake. The genes newly introduced into a feedstuff by gene transfer therefore change the quantity of ingested DNA only to a negligible extent.

DNA and DNA fragments are partially degraded after ingestion in the digestive tract by gastric acid or microbial activities incl. various endonucleases (Alexander et al., 2002; Duggan et al., 2000; Ruiz et al., 2000; Sharma et al., 2004; Zhu et al., 2004). Alexander et al. (2004) investigated the stability of the cp4epsps transgene from Roundup Ready canola in the intestinal, ruminal, and faecal contents of sheep and found a rapid degradation of free DNA at neutral pH in duodenal fluid. Free transgenic DNA was least stable in duodenal fluid at pH 7 where fragments less than 527 bp were detected for up to 2 min and fragments as large as

Table XXI. Examples of crops genetically modified with nutritionally improved traits intented to provide benefits to consumers and domestic animals (ILSI, 2004).

Crop	Trait	Transgene
Alfalfa	+Phytase	Phytase (*Aspergillus)*
	+Resveratrol	Resveratrol glucoside
	Lignin↑	Downregulation of caffeic acid 3-O-methyltransferase and caffeoyl CoA 3-O-metyhltransferase
Canola	Vitamin E↑	γ-tocopherol methyl transferase (*Arabidopsis*)
	Lauric acid↑	Lauroyl ACP thioesterase (California bay tree)
	γ-linolenic acid↑	δ-6- and δ-12 desaturases
	+ ω-3 fatty acid	δ-6 Desaturase gene (*Mortierella*)
	+ β-carotene	Phytoene synthase (daffodil)
	8:0 and 10:0 fatty acids	Phytoene desaturase (*Erwinia*)
	Medium chain fatty acids↑	Lycopene cyclase (daffodil)
		Ch FatB2, a thioesterase cDNA *(Cuphea hookeriana)*
Cassava	Cynaogenic glycosides↑	Hydroxynitril lyase
Lupin	Methionine↑	Seed albumin (sunflower)
Maize	Methionine↑	mRNA stability by intron switiching Dzr1 target
	Fumonisin↓	de-esterase-de-aminase (mbial)
	Insect resistance	Avidin (chicken)
	Protein with favorable amino acid profile↑	α-lactabumin (porcine)
	Sulphur amino acids↑	Maize 15kDa-zein
	Vitamin C↑	Wheat dehydroascorbate reductase (DHAR)
Potato	Starch↑	ADP glucose pyrophosphorylase (*Escherichia coli)*
	Very-high-amylose starch↑	inhibition of SBE A and B
	Inulin molecules↑	1-SST (sucrose:sucrose 1-fructosyltransferase) and the 1-FFT (fructan:fructan 1-fructosyltrans-ferase)genes of globe artichoke (*Cynara scolymus*)
	+sulphur-rich protein	Nonallergenic seed albumin gene (*Amaranthus hypochondriacus)*
	Solanine ↓	Antisense sterol glyco transferase (Sgt) gene
Rice	+ β-carotene	Phytoene synthase (daffodil)
	Iron ↑	Phytoene desaturase (*Erwinia)*
		Lycopene cyclase (daffodil)
		Ferritin (*Phaseolus*)
		Metallothionein (rice)
		Phytase (mutant, *Aspergillus*)
	Allergenic protein↓	Antisense 16kDa allergen (rice)
	+ Puroindolinone compounds: softer rice kernels, flour yields more finer particles, less damage to starch	Wheat puroindoline genes
Sorghum	Improved digestibility of livestock feed	Mutated Brown midrib (Bmr) encodes cafeic acid O-methyltransferase (COMT), a lignin-producing enzyme
Soybeans	Improved amino acid composition	Synthetic proteins
	Increased sulfur amino acids	Overexpressing the maize 15 kDa zein protein
	Oleic acid↑	Δ-12 desaturase (soybean, sense suppression)
	Oleic acid↑	Ribozyme termination of RNA transcripts down-regulate seed fatty acid
	Immunodominant Allergen↓	Gene silencing of cysteine protease P34 (34kDa)
Sweet potato	Protein content↑	Artificial storage protein (ASP-1)gene
Wheat	Glutenins↑	High molecular weight subunit genes
	Caffeic and ferulic acids↑	Wheat gene

1363 bp were detected for 0.5 min. Degradation reduces the likelihood that intact transgenic DNA would be available for absorption through the Peyers's Patches (Schubbert et al., 1997) in the distal ileum. However, the possibility that gene fragments are endocytosed and enter the intestinal epithelium and are absorbed by the host organism cannot be excluded. In model experiments, in which mice consumed large quantities of phage DNA over varying lengths of time, between 0.01 and 0.1% of the DNA fed was detected in the blood as DNA fragments (of up to 1000 base pairs) 2 to 8 h after feeding (Schubbert et al. 1994, 1997). The 'foreign' DNA was then found mainly in the cells and tissues belonging to the body's immune system, into which they were randomly integrated. After a single dose, fragments were found up to 8 h after a meal in the leucocytes and up to 24 h after a meal in the spleen and liver – these being the body's principle disposal routes.

The physiological importance of such findings cannot be assessed at present (Schauzu, 1997). Hohlweg and Doerfler (2001) speculated a possible role of DNA ingested with food in the mutagenesis and oncogenesis of cells in an animal's body. In a more recent study, Schubbert and his colleagues (1998) fed marker DNA to pregnant mice daily over one to two weeks. The DNA fragments were found in cells of some of the foetuses ($\approx$ 8%) and also in the newborn mice. As a route of transfer, the transfer of maternal leucocytes across the placenta was considered the most likely route of transfer. There are, as yet, no indications of the appearance of transgenes in milk. In one study in dairy cows, Klotz and Einspanier (1998) studied leucocytes when concentrates with small quantities of transgenic soybean meal were fed. Although plant DNA fragments were found in the leucocytes, none were found in milk and no fragments of the transgenic DNA were found, in any tissue examined. However these results are not conclusive and further work to trace the route taken by 'foreign' DNA in the animal's body and in foodstuffs of animal origin would seem desirable (Fenton et al., 1999; Glenn, 2001; Harlander, 2001; Kuiper et al., 1999). If, as is the case, fragments of conventional plant DNA can be detected in the tissues of mammals including cows' milk (Beever & Kemp, 2000), it would seem only a question of time until fragments of transgenes are also detected. There is no reason to suppose that transgenic DNA behaves any differently to other sources of DNA.

The few studies available on the persistence of DNA or on the transfer of DNA fragments into warm-blooded animals are summarized in Table XXII. These findings, that the transfer of plant DNA fragments into the body is to be expected, are also confirmed by the results of a complex study following the feeding of Bt maize to broilers, layers, growing cattle and dairy cows (Einspanier et al., 2001). In summary, the gastrointestinal tract and the blood and lymph transport system of ruminants eliminate free DNA very effectively but are not a complete barrier to 'foreign' DNA. However, transfer events seem to be too rare to be used for examinations to detect recombinant DNA from feeds in milk (Poms et al., 2003).

Although, at present, it is not possible to make a full assessment of the physiological consequences of the absorption of DNA fragments, Beever and Kemp (2000) believe that no risk is likely to arise from the consumption of milk, meat and eggs from animals which have received feed containing GMP. In studies made by Phipps et al. (2003) concentrates containing herbicide-tolerant soybean meal and Bt maize were fed to dairy cows in comparison with concentrate containing the isogenic counterparts. DNA-fragments of single-copy genes were only detected in the solid phase of rumen and duodenal digesta. In contrast, fragments of *rubisco* (ribulosobiphospatecarboxylase gene, a multicopy plant plastid gene) were detected in the majority of samples analysed in both the liquid and solid phase of ruminal and duodenal digesta, milk and faeces, but rarely in blood. The size of the detected *rubisco* gene fragments decreased from 1176 bp in ruminal and duodenal digesta to 351 bp in faecal samples. Reuter and Aulrich (2003) investigated, beside fragments of the *rubisco* gene in

tissue samples, also the passage of fragments of the Bt gene in the gastrointestinal tract. 72 h after last feeding, a Bt maize containing diet fragments of the Bt gene were not detectable in any sample from the gastrointestinal tract. The detection of plant DNA fragments in body samples such as meat, milk or eggs can only occur as a result of either DNA passing from the gastrointestinal tract into blood or as a result of contamination during sample collection or preparation (Klaften et al., 2004) but this seems to be very unlikely because many efforts were made in the last published studies with respect to this possible effect (Phipps et al., 2003). Furthermore, if plant DNA is absorbed, it could indicate that transgenic DNA may also be absorbed (Phipps et al. 2003), but if this occurs, the frequency is likely to be exceedingly low.

5. The fate of genetically modified (novel-) protein

In non-ruminants, feed proteins are degraded into di- and tripeptides as well as free amino acids, which are absorbed in the upper gut (Webb, 1990). In ruminants, most of the proteins are degraded in the rumen by micro-organisms and incorporated into microbial protein or fully degraded to ammonia. Relatively small amounts of proteins pass through the rumen and are degraded in the small intestine in a manner akin to non-ruminants. Traces of proteins also may pass through the digestive tract and be detected in the faeces (Einspanier et al., 2004). *In vitro* tests intended to mimic conditions in the stomach or the upper gastrointestinal tract are routinely used to determine the likely extent of protein degradation for non-ruminants. These have shown that, with a single exception, protein products of the transgenes introduced into current commercial crops are as rapidly degraded as other dietary proteins (Noteborn et al., 1994; Harrison et al., 1996; Wehrman et al., 1997; Okunuki et al., 2002). The exception is the product of *cry9C*, a bacterial lectin, which, in common with a sub-group of other plant and microbial lectins and protease inhibitors, is highly resistant to proteolysis (EPA, 1998). However, comparable *in vitro* methods using rumen fluid and of direct relevance to ruminants are rarely reported. Recently the value of *in vitro* digestibility studies was critically analysed (Anonymous, 2004). The authors underline that they are not providing an accurate representation of the *in vivo* situation as:

- Protein can be 'protected' by food/feed;
- Nutrients included in the food/feed may effect digestion;
- Peptides as breakdown products may have effects;
- Widely used protein-pump inhibitors might reduce gastric acidity;
- Proteins used in vitro studies are usually produced from micro-organisms and might therefore have an altered stability.

Even more unusual are studies in which the fate of protein from expressed transgenes is studied *in vivo*, although a few have been done.

Chowdhury et al. (2003a, b) investigated the degradation of Cry1Ab protein from genetically modified Bt 11 maize in the digestive tract of calves and pigs. They observed no pathological lesions in the digestive tract. Trace amounts of Cry1Ab protein were detected in the gastrointestinal tract, but not in the liver, spleen, kidney, muscle and other tissues. Einspanier et al. (2004) found about 0.08 ng of Bt-protein per g total protein in faeces of cattle. Other authors have shown that during the degradation processes, newly expressed proteins from herbicide-resistant soybeans were destroyed along the digestive tract at the same rate as other soybean proteins (Ash et al., 2003, Faust et al., 2000, Harrison et al., 1996, Jennings et al., 2003b). In addition, Ash et al. (2003) failed to detect any modified protein

Table XXII. Studies of the transfer of 'foreign' DNA fragments into experimental animals.

Authors	DNA source	Animal species	Results	
			Detection of transgenic DNA	Detection of 'foreign' nontransgenic DNA
Schubbert et al. (1994)	Phage DNA	Mouse	Not investigated	DNA fragments in the blood
Schubbert et al. (1997)	Phage DNA	Mouse, pregnant	Not investigated	DNA fragments up to 8 hrs in leucocytes, up to 24 hrs in kidney and liver
Schubbert et al. (1998)	Phage DNA	Mouse, pregnant	Not investigated	Transfer of DNA fragments across placenta into the foetus
Klotz and Einspanier (1998)	Soybean meal	Dairy cows	No transgenic DNA in blood and milk	Plant DNA fragments in blood leucocytes, not in milk
Einspanier et al. (2001)	Bt maize -grain and silage	Broiler Layer Feeder 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 fattening bulls, in eggs and feces of broilers and layers and in feces of dairy cows
Hohlweg and Doerfler (2001)	Soy leaves	Mouse	Not investigated	Plant DNA fragments up to 121 hrs in feces, in liver and spleen
Khumnirdpetch et al. (2001)	Gt soybean meal	Broiler	No transgenic DNA in muscle, skin, liver and duodenum	Not investigated
Phipps et al. (2001)	Bt maize	Dairy cows	No transgenic DNA in milk	Not investigated
Weber and Richert (2001)	Bt maize -grain	Grower-finisher pigs	No transgenic DNA in loin tissue	No plant DNA in loin tissue
Aeschbacher et al. (2002)	Bt maize	Broiler	Transgenic DNA fragments up to the crop	Plant DNA fragments in liver and spleen and muscle and up to the small intestine
Klotz et al. (2002)	Bt maize -grain	Pig	No transgenic DNA in tissues and gastrointestinal tract	No plant DNA fragments in blood, muscle, liver, spleen, plant DNA up to 12 hrs in the ileum
		Broiler from supermarket		Plant DNA fragments in leg, breast and wing muscle and in stomach
Phipps et al. (2002)	Gt soybean meal	Dairy cows	No transgenic DNA in milk	Not investigated
Yonemochi et al. (2002)	Bt maize-grain	Broiler	No transgenic DNA in blood, liver and muscle	Not investigated
Calsamigglia et al. (2003)	Gt and Bt maize-silage	Dairy cows	No transgenic DNA in milk	Not investigated

(*continued*).

Table XXII. (*continued*).

Authors	DNA source	Animal species	Results	
			Detection of transgenic DNA	Detection of 'foreign' nontransgenic DNA
Chowdhury et al. (2003a)	Bt maize	Pig	Transgenic DNA-fragments in gastrointestinal tract, not in blood	Plant DNA fragments in gastrointestinal tract, no plant DNA in blood
Jennings et al. (2003a)	Bt maize-grain	Broiler	No transgenic DNA in breast muscle	No plant DNA in breast muscle
Jennings et al. (2003b)	Gt soybean meal	Pig	No transgenic DNA in longissimus muscle	No plant DNA in longissimus muscle
Jennings et al. (2003c)	Bt cotton seed	Dairy cows	No transgenic DNA in milk, liver, kidney and spleen	No plant DNA in milk, liver, kidney and spleen
Phipps et al. (2003)	Gt soybean meal and Bt maize	Dairy cows	Transgenic DNA in ruminal and duodenal digesta, not in feces, blood and milk	Plant DNA fragments in ruminal and duodenal digesta, milk, feces, rarely in blood
Poms et al. (2003)	Maize, Soybeans	Dairy cows	Not investigated	No plant DNA in milk
Reuter and Aulrich (2003)	Bt maize-grain	Pig	Transgenic DNA fragments up to 48 hrs up to the rectum, not in blood, organs and tissues	Plant DNA fragments in the gastrointestinal tract, in blood, organs and tissues
Tony et al. (2003)	Bt maize-grain	Broiler	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
Yonemochi et al. (2003)	Bt maize-grain	Dairy cows	No transgenic DNA in milk, blood, liver and muscles	Not investigated
Castillo et al. (2004)	Bt cottonseed Gt cottonseed	Dairy cows	No transgenic DNA in milk	No plant DNA in milk
Einspanier et al. (2004)	Bt maize-silage	Cattle	No transgenic DNA in the gastrointestinal tract	Plant DNA fragments in the gastrointestinal tract, No plant DNA in feces
Beagle et al. (2004)	Maize contained glutamate dehydrogenase gene	Pig	Transgenic DNA in stomach, No transgenic DNA in small and large intestine, in blood, liver and muscle	Not investigated
Qiu et al. (2004)	Maize contained glutamate dehydrogenase gene	Pig	No transgenic DNA in ileal digesta	Not investigated
El Sanhoty (2004)	Bt potato	Broiler	No transgenic DNA in muscle, liver, kidney and spleen	Plant DNA fragments in muscle, liver, kidney and spleen til 8 h after feeding

from engineered soybean meal in the liver, eggs or faeces of laying hens although the protein could be detected in the soybean prior to feeding using an ELISA technique. Calsamiglia et al. (2003) did not find the Cry1Ab protein encoded in Bt-maize in cow milk (detection limit 0.1 ng/ml). Weber and Richert (2001) could not detect the Cry1Ab protein in extracts of loin samples from pigs fed Bt corn. These findings were confirmed by results from Yonemochi et al. (2002; 2003) who could not detect the Cry9C protein in blood, liver and muscles of broiler and dairy cows and also not in milk after feeding transgenic maize.

In conclusion, the metabolic processes involved in the digestion, absorption and utilization of amino acids and peptides by livestock species do not wholly preclude the incorporation of intact (transgenic) proteins into animal products. However, the vast majority of proteins are synthesized *de novo* from an amino acid pool. Thus it would be very unlikely for an expressed protein of any plant gene to be found intact in food of animal origin and none have been detected to date. More *in vivo* studies to investigate degradation of novel protein seem to be necessary.

6. Future approaches to the nutritional and safety assessment of GMP

The following conclusions can be drawn from the existing studies:

- The transgenic plants used in animal feeds so far investigated (input-traits, sometimes so-called first generation plants) do not differ to any significant extent from their isogenic foundation lines in their composition and delivery of nutrients;
- The uptake of DNA fragments into the body is constantly taking place and therefore is not an issue specific to genetic modification. Nevertheless, this aspect should be further studied because of the unknown nature of future constructs (e.g., use of viral genes).

From the standpoint of animal nutrition further studies are needed:

- On ways to assess the impact of modifications intended to significantly alter composition and/or bioavailability (the so-called 2nd generation genetically modified plants, see Table XXIII);
- To establish the extent of any influence of (2nd generation) genetically modified plants on animal health and product quality;
- To determine the value of nutritional experiments as a contribution to safety assessment and the search for unintended effects (Chesson & Flachowsky, 2003; Cockburn, 2002; EFSA, 2004; EU, 2000b, 2004; ILSI, 2003b, 2004; OECD, 2003);
- Effects of genetically modified micro-organisms (probiotics) when used in animal (and human) nutrition.

Table XXIII presents a proposal for the nutritional assessment of genetically modified plants intended for feed use.

Given the almost complete lack of public acceptance of 'green genetic engineering' today it is essential that the approach taken to any safety assessment is clearly laid out and is transparent to all stakeholders. The proposal of a 'decision tree' shown in Figure 1 offers a clear and stepwise series of actions (Flachowsky & Aulrich, 2001b). Depending on the results of each step, further studies follow in order to identify any unforeseeable effects. The questions on the left and right sides of Figure 1 are a reflection of the present situation and the need for a large measure of standardization of studies into the nutritional assessment of GMP. In case of crops modified for agronomic input traits with stacked genes, the need for longer-

term feeding studies should be assessed on a case-by-case basis (see EFSA, 2004; ILSI, 2003b, 2004).

In the case of GM crop plants with improved nutritional characteristics (2nd generation plants), livestock feeding studies with target species should be conducted on a case-by-case basis to confirm the expected nutritional benefits (e.g., lower content of phytate, see Table XXI, bioavailability of higher ß-carotene etc.). These studies ideally should span either the growing/finishing period for chickens, pigs, and beef cattle or a major part of a lactating cycle for dairy cattle and should be conducted according to internationally agreed standard protocols (e.g., EFSA, 2004; ILSI, 2003b, 2004) on a scientific basis (Kuiper & Kleter, 2003). Some recommendations from the ''Best practices for the conduct of animal studies to evaluate feeds from GMP for input traits'' are summarized in Table XXIV.

EFSA (2004) proposed the following guidelines to test feed from GMP, especially from the 2nd generation:

- In the case of GM crops modified for improved bioavailability of nutrients, livestock studies with target species should be conducted to determine the bioavailability of individual nutrients in the GM crop and a range of conventional varieties;
- In the case of GM crops specifically modified with traits to enhance animal performance through increased nutrient density (e.g., increased oil concentration) or an enhanced level of a specific nutrient (e.g,. lysine), an appropriate control diet using its nearest genetic counterpart should be formulated by supplementing it with the specific nutrient to the extent of the change effected in the GM crop;
- In the case of co-products (e.g., oilseeds meals) from which the modified ingredient has been extracted, these can be compared with those derived from an appropriate counterpart and other commercial varieties on the basis that they are essentially free from the modified component;
- In the case where the nutritional content of animal-based foods may be modified following the feeding of animals with nutritionally modified GM feed, then the content of these nutrients should be assessed in the animal products.

Table XXIII. Proposal for the nutritional assessment of GMPs (from Flachowsky and Aulrich, 2001a)*.

	Generation of GMPs	
Parameter	1st	2nd
Determinations of important constituents		
• Crude nutrients	+	++
• Genetically modified nutrients (e.g., amino acids, fatty acids, vitamins, enzymes etc.)	–	++§
• Genetically modified undesirable substances (e.g., plant constituents such as lignin, inhibitors, glucosides, etc., or secondary substances, such as mycotoxins, pesticides etc.)	(+)	++§
Digestibility, conversion studies, availability of modified nutrients in the target animal species	(+)	++
In vitro studies of nutritional assessment	(+)	(+)
Feeding experiments with species/categories of target animal		
• Performance of animals and quality of foods of animal origin	(+)	++
• Animal health	(+)	(+)
• Route taken by modified protein and/or DNA#	+	+

*Meaning of symbols: - not necessary; (+) may be advantageous; + recommended; ++ necessary. §For modified components. #For scientific purposes.

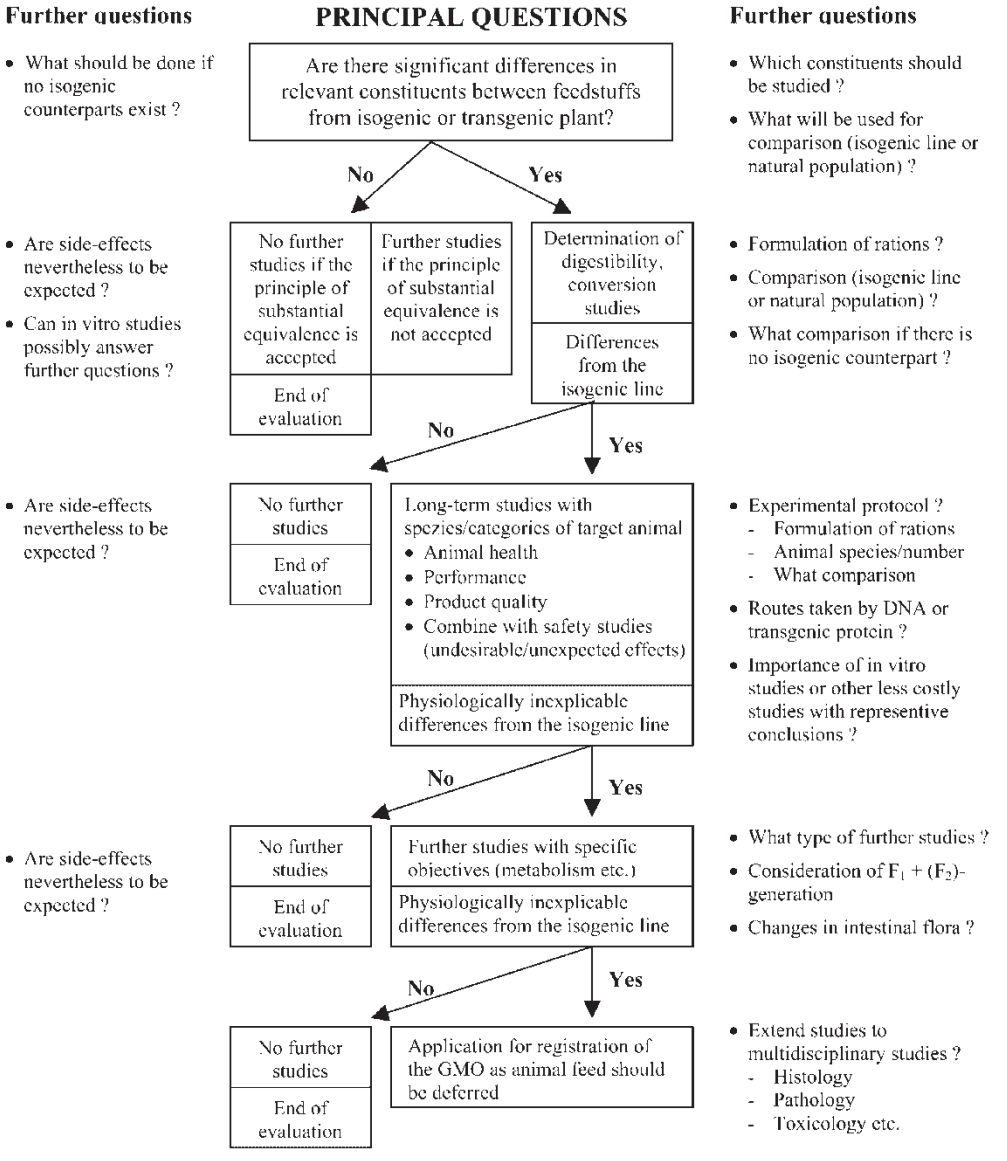

Figure 1. Proposal for a decision tree for the nutritional assessment of feedstuffs from GMPs (Flachowsky and Aulrich, 2001b).

The genetically modified material to be tested should be included in the diet to a maximum amount consistent with a good diet design and should be compared to diets containing their isogenic counterpart. More details for animal experimentation are given by ILSI (2003b). Apart from the recommendations given in Table XXIII, slaughtering results should be measured in meat producing animals. Animal behaviour and quality of foods of animal origin are further parameter, which could be considered.

The data in Table XXIV give some guidance for good practice in studies of animal nutrition. However, ultimately, there are only two essentials in the conduct of nutritional

experiments made as part of a safety assessment of GM feedstuffs. Firstly, it is essential that with all studies made with livestock the purpose of the study should be clearly understood and stated. Adding nutritional studies without a clear purpose simply because they are possible does not add to a safety assessment and may simply give a spurious illusion of thoroughness. The second element is an analysis of power (Berndston, 1991) to demonstrate that the experimental design is capable of meeting the stated purpose of the study. An *a priori* power analysis should be made and then the limitations imposed because of practical considerations (e.g., number of experimental units it is possible to house) considered rather than relying on a post hoc analysis of power. If this is done, then nutritional studies can and will play an important role in ensuring the safety of future genetically modified plants, particularly those intended to deliver improved nutrition to livestock.

The Council for Agricultural and Technology (CAST, 2001), and the Food and Drug Administration (FDA, 1991) have similar requirements to the food safety evaluation of crops produced through biotechnology. The principal food safety issues for new varieties crops are (Chassy, 2002):

- The newly added DNA,
- The safety of the newly introduced gene product,
- Potential toxicity of the nearly introduced protein,
- Potential changes in allergenicity,
- Changes in nutrient composition,

Table XXIV. Some recommendations from the best practices of animal studies to evaluate genetically modified crops for input traits (GMP of the 1st generation; adapted from ILSI, 2003b).

Animals (Species/ categories)	Number of animals (Coefficient of variation 4 to 5%)	Duration of experiments	Composition of diets*	Measurements
Poultry for meat production	10 – 12 pens per treatment with 9 – 12 birds per pen	5 weeks or more	Balanced diets	Feed intake, gain, feed conversion
Poultry for egg production	12 – 15 replications per treatment with 3 – 5 layers per pen	18 – 40 weeks of age, at least three 28-day phases	Balanced diets	Feed intake, egg production, feed conversion, egg quality
Swine	6 – 9 replications per treatment with 4 or more pigs per replication	Piglets (7 – 12 kg), 4 – 6 weeks; growers (15 – 25 kg), 6 – 8 weeks	Balanced diets	Feed intake, gain, feed conversion, carcass quality
Growing and finishing ruminants	6 – 10 replications per treatment with 6 or more cattle per replication	90 – 120 days	Balanced diets	Feed intake, gain, feed conversion, carcass data
Lactating dairy cows	12 – 16 cows per treatment	Latin/square: 28-day periods	Balanced diets	Feed intake, milk performance and composition, body weight;
	28 cows per treatment	Randomized block design		Body Condition Score (BCS), cell counts in milk, animal health

*Feed from GMP should be included in high portion in diets and compared with isogenic counterparts

- Unintended effects giving rise to allergenicity or toxicity,
- The safety of antibiotic resistance marker – encoded proteins included with the transgene,
- The overall safety of the balance of the food.

Such an evaluation seeks to establish that there is a reasonable likelihood of safety and that new varieties are as safe as or safer than crops produced by traditional methods. Indeed, after extensive safety testing and some five years of experience with such crops on the market, there is not a single report that would lead an expert food scientist to question the safety of such transgenic crops now in use (Chassy, 2002). Similar conclusion can be given for GM-feeds.

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