J. Verbr. Lebensm. (2012) 7:179–194
DOI 10.1007/s00003-012-0777-9

Journal für Verbraucherschutz und Lebensmittelsicherheit
Journal of Consumer Protection and Food Safety

REVIEW ARTICLE

Animal feeding studies for nutritional and safety assessments of feeds from genetically modified plants: a review

Gerhard Flachowsky · Helmut Schafft · Ulrich Meyer

Received: 1 March 2012 / Accepted: 24 May 2012 / Published online: 24 July 2012

Abstract In the future there will be a very strong competition between arable land use for phytogenic biomass production for feed/food, fuel, fibre and other industrial materials, as well as for settlements and natural conservation areas because of the growing population and limited natural resources. Therefore plants with high and stable yields, and requiring low external inputs (low input varieties) should be the main aim of plant breeding. In addition to traditional breeding, plant biotechnology seems to have the potential to contribute to this objective. Nutritional and safety studies with feed/food made from such modified plants are one of the most important prerequisite for public acceptance, and to improve knowledge in the feed/food sciences. The first step for the nutritional and safety assessment of such modified plants is the compositional analysis of potential feed/food, including the newly expressed proteins and other new constituents, and its comparison with conventional counterparts. In vitro studies and experiments with laboratory animals comprise the next steps of the assessment. About 70–90 % of the harvested biomass from genetically modified plants (GMPs) is consumed by food producing animals. Therefore, feeding studies with target animals are of special concern for nutritional assessment, and these are considered in more detail in the present paper. Up to now most studies have been done with GMPs of the 1st generation (plants with input traits, but without substantial changes in composition). Other experimental designs for nutritional and safety assessments are recommended for GMPs with output traits or with substantial changes in composition (plants of the 2nd generation).

Keywords Genetically modified plants (GMP) · Nutritional and safety assessment · Composition · Types of feeding studies

Zusammenfassung Zukünftig ist infolge weiter ansteigender Erdbevölkerung und knapper werdender natürlicher Ressourcen ein noch intensiverer Wettbewerb um landwirtschaftliche Nutzfläche bzw. pflanzliche Biomasse für die Erzeugung von Lebens- und Futtermitteln, Energie, industriellen Rohstoffen als auch um Flächen für Siedlungen und Naturschutz zu erwarten. Deshalb sollte die Entwicklung von Pflanzen mit hohen und stabilen Erträgen bei geringem Ressourceneinsatz (low input varieties) das Hauptziel der Pflanzenzüchtung sein. Neben der traditionellen Pflanzenzüchtung scheint die Pflanzenbiotechnologie ein beachtliches Potenzial zur Realisierung dieser Zielstellung zu haben. Entsprechende Studien zur Bewertung des ernährungsphysiologischen Wertes und der Sicherheit sind eine wesentliche Voraussetzung für die öffentliche Akzeptanz der aus diesen Pflanzen hergestellten Futter- und Lebensmittel. Derartige Studien leisten auch bedeutsame Beiträge zur Verbesserung der Kenntnisse auf dem Gebiet der Futter und Lebensmittelkunde.

G. Flachowsky (✉) · U. Meyer
Institute of Animal Nutrition, Friedrich-Loeffler-Institute (FLI), Federal Research Institute for Animal Health, Bundesallee 50, 38116 Braunschweig, Germany
e-mail: gerhard.flachowsky@t-online.de

H. Schafft
Federal Institute for Risk Assessment (BfR), Unit Feed and Feed Additives, Max-Dohrn-Str. 8-10, 10589 Berlin, Germany

Der erste Schritt zur ernährungsphysiologischen und Sicherheitsbewertung von Futter-/Lebensmitteln aus gentechnisch veränderten Pflanzen (GVP) ist die Bestimmung der Inhaltsstoffe einschließlich des/der neu ausgeprägten Proteins/e sowie weiterer neu gebildeter Stoffe und der Vergleich mit herkömmlichen (isogenen) Partnern. In vitro Studien und Versuche mit Labortieren sind die nächsten Schritte der Bewertung. Da 70–90 % der von GVP geernteten Biomasse in der Tierernährung eingesetzt werden, sind Fütterungsstudien mit Lebensmittel liefernden Tiere (Zieltieren) von besonderer Bedeutung für die ernährungsphysiologische Bewertung der Futtermittel und werden im Beitrag im Detail betrachtet. Bisher wurden vor allem Fütterungsversuche mit Futtermitteln aus GVP der 1. Generation (ohne wesentliche Veränderungen von Inhaltsstoffen; Pflanzen mit Input traits) durchgeführt. Andere Versuchsansätze sind zur ernährungsphysiologischen und Sicherheitsbewertung von Futter- und Lebensmitteln aus GVP der 2. Generation (Pflanzen mit substantiellen Veränderungen von Inhaltsstoffen; Pflanzen mit Output traits) erforderlich.

1 Introduction

The world population is still growing and requires more and better food. Sustainability in feed and food production is therefore a key challenge for agriculture. In the future there will be a strong competition for arable land and other limited resources such as fossil carbon-sources, water, some minerals (such as phosphorus) between feed/food, fuel, fibre, areas for settlements and naturally protected areas. According to the FAO (2009), the human population will globally increase from currently about 7 to 9 billion people in 2050, but the estimated need for meat and milk will nearly double during this time (Steinfeld et al. 2006).

Increasing feed/food demands require higher and stable plant yields and/or more areas for production. Because of limited resources, low input plants are an important prerequisite for solving future problems and for establishing a sustainable agriculture. Such plants should be very efficient in the use of limited resources, but they should also be able to very effectively use sun energy and unlimited plant nutrients from the air (such as N_2 and CO_2; see Table 1). Furthermore the genetic pool available in plants, animals and microorganisms could also contribute to optimizing plants for a more efficient conversion of limited resources into feed and food.

Plant breeding and cultivation will be the key elements and starting points for feed and food security during the next years (Flachowsky 2008; SCAR 2008; The Royal Society 2009; Foley et al. 2011). The most important objectives for plant breeders can be summarized as follows:

- High yields with low external inputs (low input varieties) such as water, minerals, plant protection substances, fossil fuel etc.
- Efficient use of naturally unlimited resources such as sun energy, nitrogen and carbon dioxide in the air.
- Optimisation of the genetic potential of plants for highly efficient photosynthesis.
- Lower concentrations of undesirable substances such as secondary plant ingredients, mycotoxins from toxin-producing fungi, toxins from anthropogenic activities or of geogenic origin.
- Lower concentrations of substances that influence the use or bioavailability of nutrients such as lignin, phytate, enzyme inhibitors, tannins etc.
- Higher concentrations of components such as nutrient precursors, nutrients, enzymes, prebiotics, essential oils etc.

From the global view of feed and food security low input varieties have the highest priority. Furthermore, often undesirable substances cannot be removed from feedstuffs, or can only be removed with great effort (Flachowsky 2006). Therefore a decrease in such undesirable substances is also an important objective of plant breeding

It is possible to fulfil most of the objectives of plant breeding mentioned above by conventional breeding. However, in the future biotechnology methods may be more flexible, powerful and faster. "New" plants with newly expressed proteins in plants and/or changed composition of plants present real challenges for animal and human nutritionists with respect to the safety aspects and the nutritional assessment of such products. In 2011, about 160 million hectares of GMPs were cultivated worldwide

Table 1 Potentials to produce phytogenic biomass and their availability per inhabitant under consideration of the increase of population (Flachowsky 2010) (↑ increase, ↓ decrease, ↔ no important influence)

Plant nutrients in the atmosphere (N_2, CO_2)	↑ ↔
Sun energy	↔
Agricultural area	↓
Water	↓
Fossil Energy	↓
Mineral plant nutrients	↓
Variation of the genetic pool	↑

(about 11 % of total arable land; James 2012). Most of these are tolerant against herbicides and/or resistant against insects. Such plants can be considered as substantial equivalent to their isogenic counterpart (OECD 1993).

Currently, the interests of individuals or of some companies dominate, and these are not always in agreement with public interests (SCAR 2008; The Royal Society 2009; Godfray et al. 2010; Foley et al. 2011). Fundamental research should be conducted by independent publicly sponsored research institutions and the results should be made available to all those who are interested in such plants.

High portions of the yield of the most important GMPs (soybean, maize, cotton, rapeseed; Fig. 1) are fed to food producing animals (see Table 2) and only small amounts are used for human nutrition.

Therefore, in the future assessing the nutritive value, and also the safety of food/feed from plant breeding (Kleter and Kok 2010) will be real challenge for animal nutritionists (Fig. 2). Various types of animal feeding studies are required in order to answer all the scientific and public questions, and to improve the public acceptance of such food/feed. The current state of the nutritional and safety assessment of feed from modified plants and the future challenges will be analysed in the present paper.

The main objective of the paper is to consider the pros and contras of various types of animal feeding studies for the nutritional assessment of GM-feed under consideration of European guidance documents (EFSA 2006, 2008, 2011a). Sometimes it is impossible, and also not necessary to strictly separate the nutritional and safety assessment of feed and food. Animal feeds may be considered as base for food of animal origin. Furthermore, similar studies have been used for nutritional and safety assessment of feed/food. Kleter and Kok (2010) und Davis and Kuiper (2011) consider the following aspects for risk assessments which also include nutritional aspects:

- Characteristics of donor and recipient organism
- Genetical modifications and its functional consequences
- Potential environmental impact

Table 2 Important food/feed from GMPs and the estimated proportions used as food or feed (pers. estimation)

GMP	Food	%	Feed	%
Soybean	Oil, proteins	25	Soybean (extracted oil) meal, full fat soybean	75
Maize	Starch, maize meal, oil	15	Maize, oil, DDGS, gluten feed, silage, straw,	85
Rapeseed	Oil	25	Rape seed (extracted oil) meal, rapeseed expeller/cake, full fat rapeseed	75
Cotton	Oil	15	Cotton seed (extracted oil) meal, expeller	85

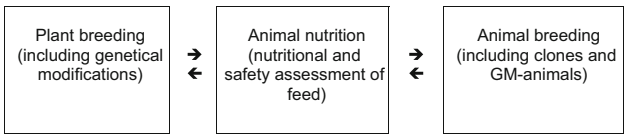

Fig. 2 Animal nutrition (nutritional assessment of feed) between plant and animal breeding

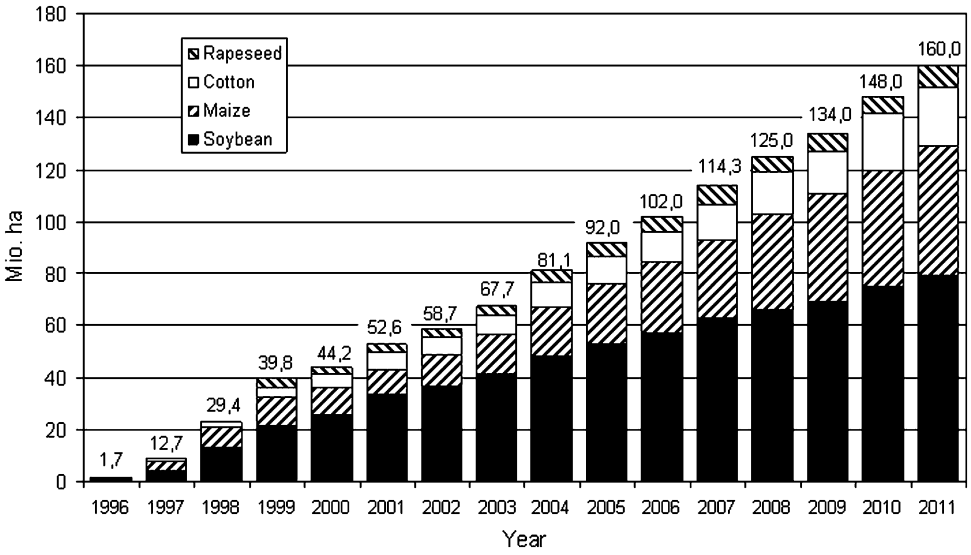

Fig. 1 Global area of transgenic crops (James 2012)

- Agronomic characteristics
- Compositional and nutritional characteristics
- Potential for toxicity and allergenicity of gene products, plant metabolites and whole GMP
- Influence of processing on the properties of food and feed
- Potential for changes in dietary intake
- Potential for long-term nutritional impact.

Principles of genetic modification of plants as well as socio-economic and environmental impacts of GMPs (Brookes and Barfoot 2008) are not covered in the present paper.

2 Definitions and guidelines for nutritional assessment

Presently, most GMPs are modified for agronomic traits such as increased tolerance against insects or higher resistance against herbicides. Most GMPs are modified only with one event, but plants with more than one event (stacked events) are in development or already in cultivation (Fig. 3). For example, such plants are resistant against insects as well as being tolerant against herbicides or other traits. There are already plants in the pipeline that contain eight and more stacked traits. Stacked events express more "new" proteins than the single events. A characterisation of the proteins is necessary and possible synergistic, additive or antagonistic effects in vitro and in vivo should be tested (Delaney et al. 2008; Ladics et al. 2010).

Plants with so-called input traits (GMPs of the 1st generation) are characterized as being without substantial changes in composition and/or nutritive value. Such plants can be considered as in the main being equivalent to their isogenic counterpart (OECD 1993). GMPs of the second generation (plants with output traits) should contain more nutrients or less undesirable substances (changes in composition, see Table 3 for some examples).

In the future we may expect plants with changed composition (plants with output traits), but also plants with increased resistance against biotic and abiotic stressors such as drought and saline soils and more efficient in using limited natural resources (low input varieties; see Table 1). Such plants (feeds) should be more or less equivalent in composition and nutritive value to their isogenic counterparts. Changes in composition and possibly nutritive effects are not intended, but still have to be considered as possible unintended effects of the genetic modification during nutritional and safety assessment.

GMPs can be used in a wide range in animal feed. Forage, silage (e.g. maize), seeds and by-products from food or biofuel industry have been fed in high amounts (see Table 2) to food producing animals. Based on the present scientific and public situation animal nutritionists should address the following aspects:

- Nutritional and safety assessment of feed from the 1st generation of GMP
- Nutritional and safety assessment of feed from the 2nd generation of GMP

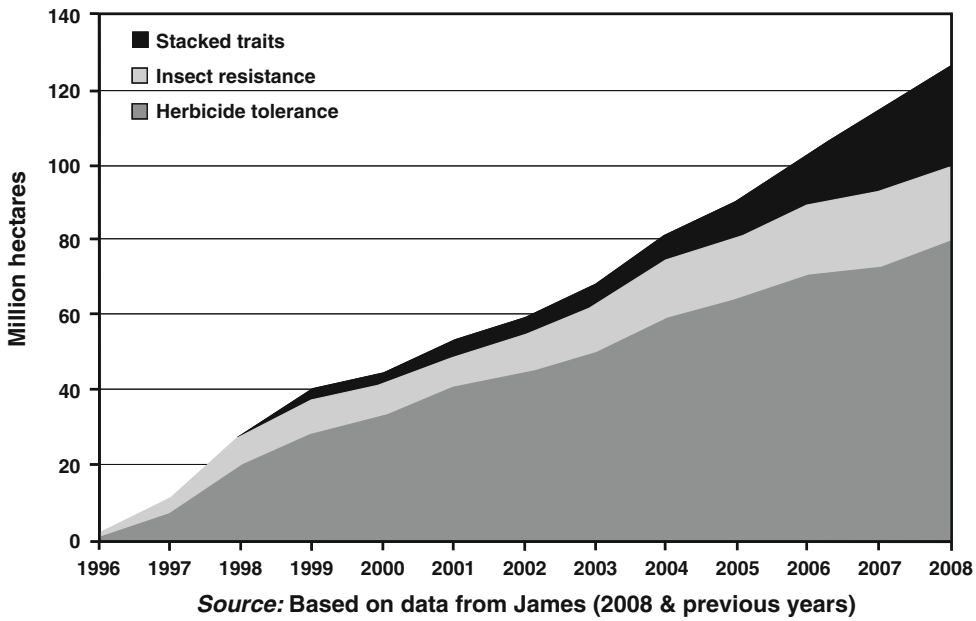

Fig. 3 Global area cultivated with the main GM traits (by Stein and Rodriguez-Cerezo 2009)

Table 3 Examples of GMP with improved characteristics intended to provide nutritional benefits (by EFSA 2008)

Plant/species	Altered characteristic	Transgene/mechanism
Maize	Improved amino acid profile ↑	Various enzymes
	Vitamin C ↑	Dehydroascorbate reductase
	Bioavailable iron ↑	Ferritin and phytase
	Fumonisin ↓	De-esterase and de-aminase
Potatoes	Starch ↑	ADP glucose pyrophosphorylase
	Solanine ↓	Antisense sterol glycotransferase
Rapeseed	Vitamin E ↑	Gamma-Tocopheryltransferase
	β-Carotene ↑	Phytoene-synthase
	Linoleic acids ↑	Various desaturases
Rice	β-Carotene ↑	Phytoene-synthetase and -desaturase, lycopene cyclase
	Iron ↑	Ferritin, metallothionein, phytase
Soybean	Oleic acid ↑	Suppression of desaturase
	Stearidonic acid ↑	Various desaturases

- Influence of GM-feed on animal health and the quality/composition of food of animal origin (e.g. milk, meat and eggs)
- Studies on the properties/degradation of newly expressed (novel) protein(s), foreign DNA, unintended effects etc.

Recommendations for production, collection and analysis of samples as well as feeding studies with GM-material are given by various international (e.g. EFSA 2006, 2008, 2011a; ILSI 2003a, 2007) and national authorities (e.g. ANSES 2011; DBT 2008; FDA 2000; FSANS 2007).

3 Materials for analysis and feeding studies

Field trials used for production of materials for analyses and feeding studies should be carried out according to the recommendations of (EFSA 2006, 2008, 2011a) and ILSI (2003a, 2007). Field trials should be designed with GMPs and the most appropriate control line as well as some conventional lines in the same field under comparable conditions to minimise environmental variability. The number of locations, growing seasons, geographical spread, replicates and statistical models are important for adequate experimental designs. Apart from the transgenic and its near isogenic counterpart (control), the studies should preferably have four or more conventional (commercial) reference varieties to help explain any unexpected differences or confirm any expected differences observed between the test and control plants (ILSI 2007). In such a case it is possible to compare the composition and nutritive value of GMPs with commercial lines. If such a cultivation is impossible, analytical data from the field studies should be compared with literature data (e.g. ILSI 2003b or updated tables; OECD 2001a, b, 2003, 2004a, b, c; or national/local feed value tables; e.g. DLG 2012). However, it should also be noted that the specific conditions (season, soil, fertilizer, weather etc.) might influence the composition of plants or processed materials. Therefore it is always advantageous to include adequate comparators in the field studies. In the case of GMPs of the second generation, it would be difficult to cultivate adequate comparators. Details of sampling (grain, pasture, hay or silage), handling of samples and preparation of samples for further studies are described by EFSA (2006) and ILSI (2007).

4 Compositional analyses

The compositional analyses of feed from GMPs are the starting point for a nutritional assessment. Before cultivation, during growing, after harvesting, processing and manufacturing of the prepared mixture for feeding, the transgenic DNA and the newly expressed protein (or proteins in the case of multi-stacked events) should be tested.

Apart from newly expressed protein(s), the most important nutrients (see Table 4) and antinutritive substances such as pesticides, mycotoxins, enzyme inhibitors, glucosinolates, gossypol and further plant specific substances should be analysed in the original material and processed substrates and compared with values of the isogenic counterpart, commercial varieties and/or values from food/feed tables. In the

present dossiers submitted to the EFSA for application on the European market, between 60 and 100 constituents have been analysed in the GM-plants or derived food/feed, its isogenic counterpart and the conventional varieties for comparison.

The compositional analysis is an important prerequisite, and indeed the cornerstone for the nutritional and safety assessment of food/feed from GMPs. This comparative approach is the basis for the concept of substantial equivalence (OECD 1993), and it is suitable for assessing GMPs and derived food/feed of the first generation (GMPs with input traits). This concept is based on the idea that an existing organism used as food/feed with a history of safe use, can serve as a comparator when assessing the safety of food/feed from GMPs. The need of further studies including animal studies depends on the outcome of the compositional approach (for further details see EFSA 2006, 2008, 2011a; FSANS 2007; Kok and Kuiper 2003). From the scientific view a case by case decision should be the basis for additional animal studies. From the present public view, animal feeding studies seem to be recommended under many circumstances.

In the case of substantial similarity between GM- and non GM-feed, from a scientific point of view animal feeding studies do not add substantially to the nutritional and safety assessment of GM feed/food (EFSA 2008, 2011a, b). These should only be performed, if scientific questions are open and new results are expected. In other cases, such animal feeding studies should be considered as wasting time, animals and money. All studies should be approved by an ethics committee under consideration of replacement, refinement and reduction of animals ("3Rs"-priciple; Russell and Burch 1959).

For GMPs of the second generation (plants with output traits such as "Golden Rice with β-carotene, soybean with modified fatty acid pattern or modified amino acid pattern; maize with lower phytate etc"; see Table 3) further steps for nutritional and safety assessment appear to be necessary (see EFSA 2008, 2011a; FSANS 2007; ILSI 2007).

5 Feeding studies

Before commencing feeding studies, compositional analysis and various in silico and in vitro methods (DBT 2008; EFSA (2008, 2011a); ILSI 2003a, 2007) can contribute to the nutritional and safety assessment of GMP derived food and feed. Nevertheless, feeding studies with target animals are key elements for

Table 4 Recommendations for nutrient analysis (by ILSI 2007)

Crops/grains/coproducts	Livestock type	Analyte
Grain: maize, wheat, barley	Nonruminants	DM, CP, EE, ADF, NDF, Ca, P, Mg, K, S, Na, Cl, Fe, Cu, Mn, Zn, ash, starch, lysine, methionine, cystine, threonine, trytophan, isoleucine, arginine, phenylalanine, histidine, leucine, tyrosine, valine, fatty acids, vitamins
Oilseed meals: soybean, linseed, cottonseed, canola meal, full-fat oilseeds	Nonruminants	DM, CP, EE, ADF, NDF, Ca, P, Mg, K, S, Na, Cl, Fe, Cu, Mn, Zn, ash, starch, lysine, methionine, cystine, threonine, trytophan, isoleucine, arginine, phenylalanine, histidine, leucine, tyrosine, valine, fatty acids, vitamins
Grain: maize, wheat, barley	Ruminants	DM, CP, EE, ADF, NDF, Ca, P, Mg, K, S, Na, Cl, Fe, Cu, Mn, Zn, Mo, ash, starch, ADIN, soluble protein, NPN, degradable protein, NDICP, ADICP, fatty acids, fat soluble vitamins
Oilseed meals: soybean, linseed, cottonseed, canola meal	Ruminants	DM, CP, EE, ADF, NDF, Ca, P, Mg, K, S, Na, Cl, Fe, Cu, Mn, Zn, ash, ADIN, soluble protein, NPN, degradable protein, NDIN, fatty acids, fat soluble vitamins
Seeds: soybean, cottonseed, sunflower	Ruminants	DM, CP, EE, ADF, NDF, Ca, P, Mg, K, S, Na, Cl, Fe, Cu, Mn, Zn, Mo, ash, ADIN, soluble protein, NPN, degradable protein, NDIN, fatty acids, fat soluble vitamins
Silage: maize, grass, legumes	Ruminants	DM, CP, EE, ADF, NDF, Ca, P, Mg, K, S, Na, Cl, Fe, Cu, Mn, Zn, Mo, ash, ADIN, soluble protein, NPN, degradable protein, NDIN, starch, sugar, pH, short chain acids such as lactic, acetic, butyric, isobutyric
Fresh/dry forages: grass, legumes	Ruminants	DM, CP, EE, ADF, NDF, Ca, P, Mg, K, S, Na, Cl, Fe, Cu, Mn, Zn, Mo, ash, ADIN, soluble protein, NPN, degradable protein, NDIN, starch, sugar, fatty acids, fat soluble vitamins

ADF acid detergent fiber, *ADIN* acid detergent insoluble nitrogen, *ADL* acid detergent lignin, *ADICP* acid detergent insoluble crude protein, *CP* crude protein, *DM* dry matter, *DNDF* digestible neutral detergent fiber, *EE* ether extract (crude fat), *NDF* neutral detergent fiber, *NDICP* neutral detergent insoluble protein, *NDIN* neutral detergent insoluble nitrogen, *NPN* non protein nitrogen

nutritional and safety assessment of food/feed from GMPs.

Depending on the scientific questions, the following types of feeding studies (see Table 5) are well-established and may be carried out:

- Laboratory animal models for the toxicity testing of single substances (single dose toxicity testing; repeated-dose toxicity testing; reproductive and developmental toxicity testing; immunotoxicity testing etc., see DBT 2008, EFSA (2008, 2011a), Ladics et al. 2010, OECD 1998a)
- Laboratory animal models for the safety and nutritional assessment of whole GM-food and feed (in general 90-day feeding studies for safety assessment; to detect unintended effects; sub-chronic animal tests; for margins of safety etc.; see DBT 2008, EFSA 2011b, OECD 1998b)
- Studies to measure digestibility/bioavailability of nutrients from the GMPs, and to analyse the influence of GM-products on the metabolism in target animals/categories (see DBT 2008; EFSA (2008, 2011a); Flachowsky and Böhme 2005; ILSI 2007)
- Tolerance studies to analyse the influence of maximal amounts of GM-feeds on animal health and welfare (DBT 2008; EFSA 2008; ILSI 2007)
- Efficacy studies to measure the influence of GM-feed on animal yield/performance, feed conversion rate (FCR), slaughtering performance as well as safety and composition of food of animal origin (DBT 2008; EFSA 2008; ILSI 2007)
- Long term studies to find out long term effects of GM-feed (e.g. whole growing period in the case of growing animals, whole laying period in the case of laying animals or one or more lactations in the case of lactating animals; whole lifespan of animals (Snell et al. 2012)
- Multigeneration studies to analyse the influence of GM-feed on fertility/reproduction performance of animals (Snell et al. 2012; BEETLE 2009).

Table 5 Important types of feeding studies with animals for nutritional and safety assessment of feed from GMP and animals recommended

Type of studies	Laboratory animals	Target animals
Testing of single substances (28 day study)	X	
90-day rodent feeding study	X	
Long-term feeding study	X	X
Multigeneration feeding study	X	X
Determination of digestibility/availability	X	X
Efficiency study		X
Tolerance study		X
Studies with GM-animals		X

In general the expense of the studies mentioned above increases from the top to the bottom of Table 5. Therefore, long term studies and multigeneration experiments with target animals are very rare (see Snell et al. 2012). Limited feed amounts in earlier plant breeding stages may also restrict animal numbers and the duration of studies with target animals, especially with large animals such as cattle and pigs. In summary the following factors (see also Table 6) may influence the types of animal feeding studies:

- Scientific question(s)
- Availability of GM-feeds (esp. in early stages of breeding) and adequate comparators
- GM-feed should be included in the diets to the highest possible amounts
- Financial budget
- Availability of equipments, animals and qualified manpower.

Feeding studies with target animals should be considered in the following paragraphs in more detail. The product quality (e.g. milk, meat, eggs etc.), and the carry over of ingredients of feed into food of animal origin (e.g. fatty acids, minerals, vitamins, undesirable substances) should be also measured in the feeding studies or after slaughtering of the animals (ILSI 2007)

5.1 Laboratory animals

Usually, the OECD guideline tests (OECD TG 407 and 408; OECD 1998a, b) for chemicals are used for the safety testing of single substances including new products resulting from genetic modification (e.g. newly expressed proteins; EFSA 2006, 2008, 2011a). In the main rodents (rats or mice) are used over a period of 28 days/one month for single dose or repeated-dose toxicity testing. The detailed testing strategy should be selected on a case-by-case basis, based on prior knowledge regarding the biology of the products, so that relevant endpoints are measured in the test (for more details see OECD 1998a, b; EFSA 2006, 2008, 2011a).

A 90-day rodent feeding study should be carried out, when indicated by molecular, compositional, phenotypic, agronomic or other analysis (e.g. changes in metabolic pathways). Such toxicity studies

Table 6 Advantages of studies for safety assessment of GMPs derived food/feed with laboratory or target animals

Laboratory animals	Target animals
International agreed study protocols	Representative for animal species/categories (extrapolation of data possible)
Small amounts of feed, higher number of repetitions	Higher amounts of GM-products are fed to animals
Lower costs for feed and equipment	All „control" animals fed with comparators (isogenic, commercial) are available for the market (no waste animals)
	Real studies on the transfer of undesired substances in food of animal origin

should only be performed on a case by case basis to provide additional information for the risk assessment (EFSA 2008). It seems to be nearly impossible from the nutritional point of view to adapt toxicity studies for testing whole feed. The OECD (1998b) guideline has been developed to assess the safety of additives and not to test whole feed/food. EFSA (2011b) mentioned as purpose for a repeated-dose 90-day oral toxicity study on whole food/feed to reassure that the GM food/feed is as safe and nutritious as its traditional comparator. In such cases high portions of the whole feed/food should be supplemented to a basal diet knowing that the energy and nutrient requirements (NRC 1995) of laboratory animals are not met and imbalances in some nutrients esp. amino acids could be expected (EFSA 2011b). This statement seems to be important from the view of many GM-feed rich in protein (e.g. soybean, cotton, rapeseed; see Fig. 1). It is nearly impossible to take scientific conclusions under imbalance conditions (NRC 1995). Adjusted diets—if possible—should be fed under those conditions.

Another point of criticism of the 90-day feeding study with rodents is the duration of the experiments for safety assessment (e.g. Seralini et al. 2011). 90 days are considered as too short for various parameters such as fertility and reproduction, histopathology of some organs (e.g. liver, kidney), hormone status etc. More details about the necessity of studies with laboratory animals and useful endpoints are described by EFSA (2006, 2008, 2011b) and OECD (1998b).

As already mentioned the scientific output of studies to contribute to nutritional and safety assessment of feed/food with such studies in rodents would be very small or negligible. Specific studies with target animals may contribute more substantially to nutritional assessment of feed and could help for safety assessment. This conclusion seems to be also very important from the view of GM-feed used in animal nutrition (Table 2).

5.2 Target animals

Studies with target animals (food producing animals such as ruminants, pigs, poultry and fish) mainly focussed on nutritional concerns. Up to now, less attention has been paid in such studies to the safety aspects (EFSA 2006, 2008, 2011a). In the future feeding studies with target animals should be also used for the safety assessment of GMPs because of the high proportion of GMPs used for animal nutrition (Table 2) and the cultivation of GMP of the 2nd generation. The type of studies depends on the type of genetic modification in the plants or animals and the availability of GM-feed or GM-animals.

5.2.1 Measuring digestibility/bioavailability

In the case of substantial changes in plant composition (GMPs with output traits or GMPs of the 2nd generation), studies measuring the digestibility/availability of some nutrients or nutrient precursors are necessary (EFSA 2008; Flachowsky and Böhme 2005; ILSI 2007). Mostly such studies are done with model animals (mice, rats, rabbits) or small target animals (chicks, quails, piglets), because of the high costs and the limited feed amounts available in some cases. The model for such studies is shown in Table 7.

Table 8 shows results to measure the bioconversion of β-carotene into vitamin A. The retinol concentration in the liver of Mongolian gerbils, as a model animal was used as endpoint. The results show that the retinol concentration by liver of gerbils fed with carotene rich maize was similar to animals fed with maize poor in carotene and supplemented with adequate amounts of β-carotene. That means in this case that β-carotene from maize is almost identically converted into vitamin A as supplemented β-carotene. In the case of "Golden Rice" the first studies to determine the vitamin A value of β-carotene were done with labels rice in humans (Tang et al. 2009).

Table 7 Examples for nutritional assessment of a GMP of the 2nd generation (GMP with output traits; e.g. higher concentration of the vitamin A precursor β-carotene; (by EFSA 2008)

Groups[a]	Composition of diets	Measurements; endpoints
1[b]	Balanced diet with typical amounts of the isogenic counterparts (unsupplemented control)	Depending on genetical modification of plants, e.g.: Concentration of specific substance(s) in target organ (e.g. vitamin A in the liver)[c] Further metabolic parameters such as depots in further organs or tissues, activities of enzymes and hormones
2	Balanced diet with adequate amounts of the transgenic counterpart (e.g. rich in β-carotene)	
3	Diet of Group 1 with β-carotene supplementation adequate to Group 2	
4	Diet of Group 1 with vitamin A supplementation adequate to expected β-carotene conversion into vitamin A	

[a] Four or more groups fed with commercial/isogenic control feed to find out the biological range of the parameter(s)

[b] Adequate feed amounts for all animals; depletion phase for all animals before experimentation

[c] Up to the steady state in the specific target organ

Table 8 Experimental design to assess the conversion of β-carotene into vitamin A in Mongolian gerbils (60 % maize in diets; n = 10, depletion phase: 4 weeks, feeding: 8 weeks; Howe and Tanumihardjo 2006)

	Unsupplemented control (maize poor in carotene)	Carotene rich maize	Control + β-carotene	Control + vitamin A
β-Carotene (nmol/g)	0	8.8	8.8	4.4
Theoretical retinol intake (nmol/day)	0	106	106	106
Retinol in serum (μmol/l)	1.23 ± 0.20	1.25 ± 0.22	1.23 ± 0.20	1.22 ± 0.16
Retinol in liver (μmol/g)	0.10[a] ± 0.04	0.25[b] ± 0.15	0.25[b] ± 0.08	0.56[c] ± 0.15

[a,b,c] Means with different letters differ ($p < 0.05$)

Similar studies are necessary to demonstrate the efficacy of enzymes expressed in plants or to show the higher phosphorus availability in plants with lower phytate content. Phytate is one of the most important inhibitors of P-availability. In a study with pigs (Spencer et al. 2000) low phytate maize showed the same results as traditional maize supplemented with 2 or 1.5 g inorganic P per kg feed, but a significantly lower P-excretion.

5.2.2 Efficiency studies including transfer of nutrients

The objective of efficiency trials is to measure the effect of feed from GMPs on the performance of food producing animals, and to compare the results with an isogenic counterpart and at least four comparable commercial products.

Many feeding studies have been carried out during the last years to show the substantial similarities (OECD 1993) of feed derived from GMPs of the first generation (without substantial changes in their composition or GMPs with input traits). Most of the studies were done as efficiency trials and GM-feed was compared in adjusted diets with their isogenic counterparts and some conventional commercial varieties (one to ten in some cases). The experimental designs were done according to the recommendations by ILSI (2003a, ILSI 2007; see Table 9) and EFSA (2006). Questions concerning the tolerance of some feeds in animals (tolerance studies) may be also included in efficiency trials.

During the last few years, some reviews on the nutrition and safety assessment of feed from GMPs (mostly plants from the first generation) have been published (e.g. Clark and Ipharraguerre 2001; Flachowsky and Aulrich 2001; Aumaitre et al. 2002; Flachowsky et al. 2005a, 2007; CAST 2006). Furthermore, the documents by ILSI (2003a, 2007) and EFSA (2006) also summarize the present state of knowledge in the feeding GMP-derived feed to target animals. About 150 studies with target animals did not show biologically relevant differences between animals fed with feed from GMPs or their isogenic counterparts (Table 10). There is a large agreement that studies with feed from GMPs of the first generation (with input traits) did not significantly influence animal health, performance, composition and quality of animals products (summaries by Alexander et al. 2007; BEETLE 2009; Flachowsky et al. 2007). Side effects might be expected in GMPs, particularly for GMPs with multiple modifications (multi-stacked events, Cellini et al. 2004), and these must be

Table 9 Some recommendations from the "Best practices for the conduct of animal studies to evaluate crops genetically modified for input traits (GMP of the first generation)"; adapted from ILSI (2003a)

Animals (species/categories)	Number of animals (coefficient of variation 4 to 5%)	Duration of experiments	Composition of diets[a]	Measurements/endpoints
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, metabolic parameters, body composition
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, metabolic parameters, 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, metabolic parameters
Lactating dairy cows	12–16 cows per treatment 28 cows per treatment	Latin square 28 day periods Randomized block design	Balanced diets	Feed intake, milk performance and composition, body weight, body condition score (BCS), cell counts in milk, animal health

[a] Efficiency studies to evaluate feed from GMP with output traits (GMP of the second generation) should be done under consideration of recommendation by EFSA (2008) and ILSI (2007)

Table 10 Summary of published data to compare feeds from GMP of the first generation (mainly maize, soybeans, cotton, and canola) of various constructs with their isogenic counterparts

Animal species/category	Number of experiments	Nutritional assessment
Ruminants		No unintended effects in composition (except lower mycotoxin concentration in Bt-plants)
Dairy cattle	23	
Beef cattle	14	
Others	10	
Pigs	21	
Poultry		No significant differences in digestibility and poultry health as well as no biological relevant unintended effects on performances of animals and composition of food of poultry origin
Broilers	48	
Laying hens	12	
Other poultry	1	
Others (fish, rabbits etc.)	8	

scientifically analysed in detail. Furthermore the high biological range for many parameters should be considered. Presently, no other food/feed are as extensively analysed and tested in various studies as is the case for GMP-products of the first generation. It can be concluded that the safety and nutritional evaluation of GM versus conventionally bred plants is not well balanced (Kok et al. 2008).

Transgenic DNA and newly expressed proteins showed comparable properties to normal plant-DNA and proteins and are mostly degraded during feed treatment and in the digestive tract of animals (Flachowsky et al. 2005a; Alexander et al. 2007). That means that small transgenic DNA-fragments and fragments of newly expressed proteins may be detected in traces in animal tissues as shown by some authors (e.g. Nemeth et al. 2004; Mazza et al. 2005; Sharma et al. 2006; Tudisco et al. 2010).

Based on the results mentioned above, the necessity of animal feeding studies with feed from GMPs of the 1st generation is often questioned concerning. According to various guidance documents (e.g. EFSA 2006, 2008, 2011a), such studies are not urgently needed. No animal feeding studies are required if the differences in compositional analyses between isogenic and transgenic plants are small or negligible (GMPs of the 1st generation) because of the costs of such studies and reduction of numbers of experimental animals.

On the other hand feeding experiments with GMPs of the 1st generation with target animals may contribute to showing the nutritional equivalence and the safety of feed to the public, and therefore improve the public acceptance of GM-feed.

Another point is the so-called wastage of animals. (the "3Rs"; Russell and Burch 1959). Under the present regulations only animals fed with not-permitted GM-feed cannot be used in the food chain. This means that if a GM-feed with its isogenic counterpart and

four commercial varieties will be tested in a feeding study, more than 80 % of the animals can be used for human nutrition. Therefore, the efficiency feeding studies with GMPs of the first generation could be useful in some cases (see Tables 6, 9).

More studies are necessary for the nutritional assessment of food/feed from the second generation of GMPs (plants with substantial changes in composition, Tables 7, 8). Experimental designs for such studies are described in detail by EFSA (2006, 2008, 2011a), Flachowsky and Böhme (2005) and ILSI (2007).

Changed composition of GMPs and derived feed may also influence the composition of food of animal origin as has been exemplarily demonstrated for soybeans with a modified fatty acid pattern. Stearidonic acid (SDA; C18:4 n-3; Fig. 4) may be transferred into the body fat of non-ruminants (Table 11) or may be used as precursor for longer fatty acid chains (e.g. C20 and C22 fatty acids) in non-ruminants (Table 11) and in ruminants.

Stearidonic soybean oil contains between 20 and 30 % SDA. Rymer et al. (2011) added soybean oil containing 240 g SDA/kg oil (45 and 50 g SDA-oil per kg grower and finisher broiler diet) and compared this with a diet comprising conventional soybean oil. The authors did not observe any significant influence of SDA-oil on feed intake, weight gain and feed conversion rate in the animals, but they found higher concentrations of SDA as well as C20 and C22 polyunsaturated fatty acids in various body fats (Table 11). Similar results are described by Bernal-Santos et al. (2010) in lactating cows after duodenal infusion of SDA-soybean oil, by Kitessa and Young (2011) after feeding of ruminal protected SDA-oil to dairy cows, by Meja et al. (2010) in laying hens and by Forster et al. (2011) in pacific white shrimp.

Gibbs et al. (2010) consider the introduction of SDA-oils in animal feed as a change in order to increase the intake of long-chain n-3 PUFA of men. However, for some polyunsaturated fatty acids there exist upper limits for human nutrition and one should be careful with supplementing of such oils in

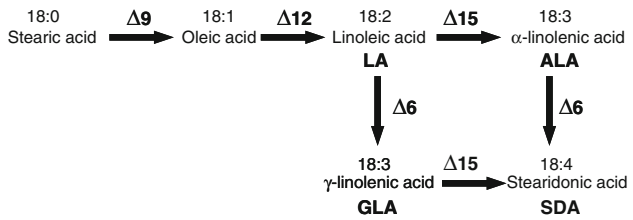

Fig. 4 Fatty acid biosynthesis in plants and the new introduced changes to produce stearidonic acid (C18:4 n-3) and the effects of various desaturases (by Ursin 2003; Whelan 2009)

Table 11 Concentrations of some n-3 fatty acids (mg/100 g fresh tissue) in body samples of broilers fed diets supplemented with 45 and 50 g soybean oil containing stearidonic acid (SDA) per kg grower and finisher broiler diet compared with a diet comprising conventional soybean oil (Rymer et al. 2011)

Sample	Control diet	+ 45 or 50 g SDA oil per kg grower and finisher diet
Breast meat		
C18:4n-3	3	231
C20:5n-3	12	28
C22:6n-3	7	14
Leg meat		
C18:4n-3	10	442
C20:5n-3	5	53
C22:6n-3	8	21
Skin		
C18:4n-3	111	3,673
C20:5n-5	31	317
C22:6n-6	21	78

animal nutrition. Therefore, animal body composition may be also an endpoint of animal feeding studies to measure the transfer of some ingredients of GMP of the second generation into animal tissues, milk or eggs. Animal body samples or products from animals such as milk, eggs etc. should be considered and analysed adequately (Table 12).

5.2.3 Long term feeding studies

Long-term feeding studies cover the whole lifespan, or a very long period of this, of the animals e.g., in the case of laying hens or dairy cows. Apart from the animal's performance, the answers expected from such studies include their fertility and health, when fed with high amounts of GM-feed. Can animal feeding trials contribute to the assessment of long term effects? This was the main question of the BEETLE-study (BEETLE 2009). The assessment of the data and the results from the Online Survey of BEETLE (2009) on animal health did not show any new aspects. Some participants of the Online Survey expect only potential long term effects in relation to allergenicity in humans, but all other possible adverse long term effects were assessed as being negligible. In general, a methodical improvement of the risk assessment procedure has been recommended, including a higher number of replications and additional control groups to demonstrate the biological range of measured parameters.

After publishing the BEETLE-study (BEETLE 2009), a long term feeding study with dairy cows (two

Table 12 Proposal of endpoints for comparative analysis of food of animal origin from animals fed with feed from GMP of the second generation and of GM animals (pers. proposal)

Group of GM-animals	Mammals	Birds	Aquaculture (e.g. fishes, mollusks)	Insects (honey bees)
Samples from the animal body	Tissues Meat, muscle (*M.long.dorsi*; *M.bic.femoris*) Body fat Blood Some organs (liver, kidney, spleen, brain? etc.) Residue body (meat and bone meal as feed)	Tissues Meat, muscle (breast, thigh) Abdominal fat Blood Some organs (liver, kidney, spleen etc.) Residue body (animal body meal as feed)	Edible fraction (e.g. fillet) Residue body (e.g. fish meal as feed)	
Food/feed produced by animals	Milk	Eggs	Caviar	Honey

lactations; Spiekers et al. 2009) was finished and has been partially published. Dry matter intake, milk yield and composition as well as physiological parameters in cows were not significantly influenced by feeding high amounts of GM-maize (MON 810). Fragments of the newly expressed protein and of the tDNA were not detected in samples of animal tissues or in the milk. Recently Snell et al. (2012) examined 12 long-term feeding studies (of more than 90 days) and came to the conclusion that the studies do not suggest any health hazards. Furthermore, they did not observe statistically significant differences within the parameters observed. Some small differences were observed, though these fell within the normal variation range of the considered parameters and thus had no biological or toxicological significance in the case of GMPs with input traits (1st generation). The authors conclude that a 90-day rodent feeding study is generally considered sufficient in order to evaluate the health effects of GM feed on animals.

5.2.4 Multigeneration studies

In addition to long term feeding studies, multigeneration studies (mostly five generations) were carried out to test the influence of GM-feed on reproduction, long term health and metabolic effects in laboratory and target animals. In laboratory animals, no negative effects were described for growth, testicular cells or reproductive traits in mice fed Bt corn, a glyphosate tolerant soybean or a transgenic triticale grain resistant to the "Basta"-herbicide when compared with conventional corn, soybean or triticale (Baranowski et al. 2006; Brake and Evenson 2004; Brake et al. 2004). Rats and their offspring were not significantly influenced in a five generation study, if fed with 5 % GM potatoes containing the bar gene or conventional potatoes (Rhee et al. 2005). Kilic and Akay (2008) did not find any differences in the organ weights of the offspring and no differences in reproduction rate of rats fed up to 20 % Bt corn or conventional corn. Krzyzowska et al. (2010) fed pellets containing 20 % control triticale or 20 % Basta-herbicide resistant GM-triticale to mice for five consecutive generations and found some changes in lymph nodes and in immune response (increased IL-2 levels and decreased IL-6 levels) in the fifth generation. Snell et al. (2012) examined 12 multigenerational studies (from 2 to 5 generations), mainly carried out with rodents, but also with sheep (44 months; Trabalza-Marinucci et al. 2008) and laying hens (4 generations; Halle et al. 2006). Results of all 12 studies did not show any health hazards.

In a ten generation study, feeding laying quails with a diet containing 50 % Bt corn did not significantly influence production and reproduction performances of animals compared with a diet containing 50 % isogenic corn (Fig. 5). Unfortunately, further multigeneration studies with food producing animals are missing.

6 Conclusions

Feeding studies with feed from genetically modified plants contribute substantially to the nutritional and safety assessment of such feed. Presently feeding studies with laboratory animals have been mainly used for safety assessment, and those with target animals for nutritional assessment. In the future, studies with target animals should also be used more intensively for safety assessment. For this purpose,

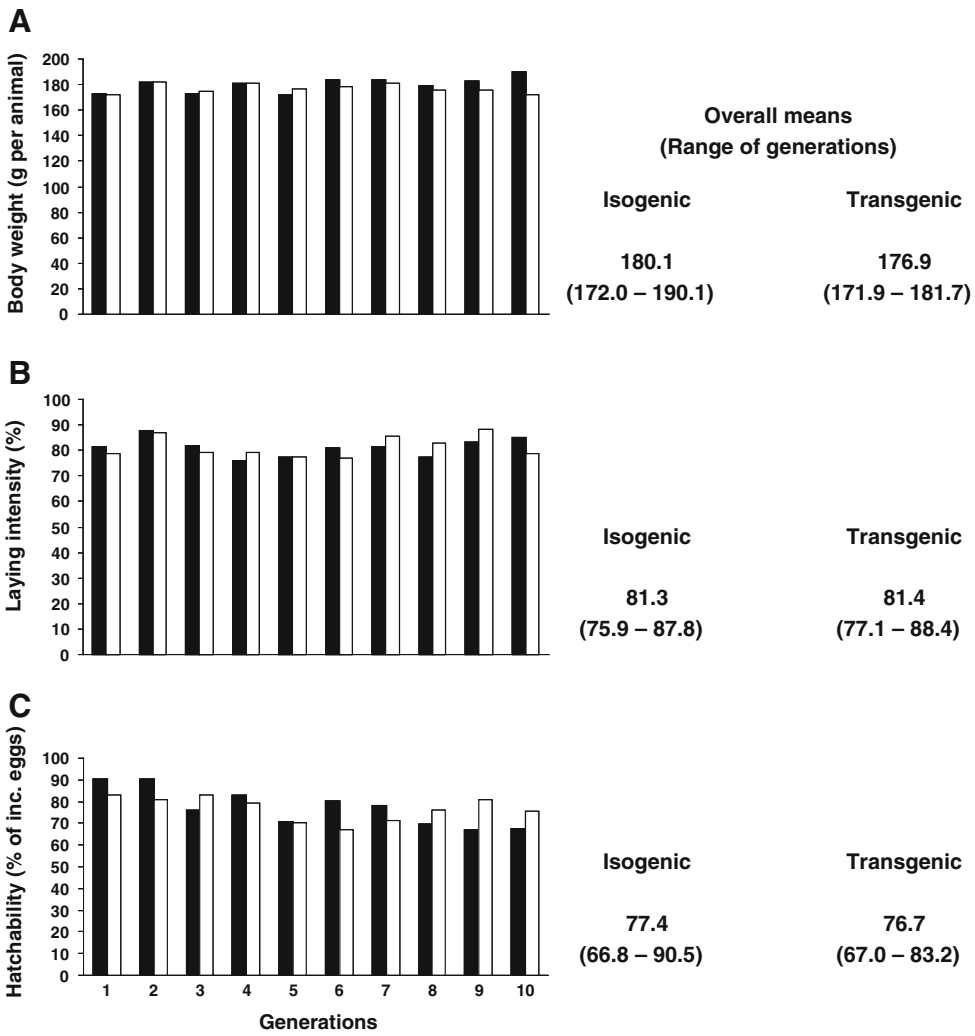

Fig. 5 **a** Body weight of female quails (age 6 weeks). **b** laying intensity and **c** hatchability of quails fed with isogenic (*filled bars*) and transgenic (Bt, *open bars*) corn in a ten generations experiment (Flachowsky et al. 2005b)

more endpoints such as weight of inner organs, parameters relating to the gastrointestinal tract, metabolic parameters, histopathology, urine, faeces etc. should be included in the studies.

Table 13 Assessment of present modifications of plants from the view of food safety and food security

Objectives	Present significance	Contributions to	
		Food safety	Global food security
More resistant against herbicides	↑↑↑	↑↑	↑
Mores resistant against insects etc.	↑↑	↑	↑
More valuable ingredients	↑	~	(↑)
Less undesirable ingredients	(↑)	↑↑	↑
More efficient use of resources (water etc.)	(↑)	↑	↑↑↑

↑↑↑ extremely high, ↑↑ very high, ↑ high, ~ not important

New developments in plant (and animal) breeding will pose real challenges for animal nutritionists for the nutritional and safety assessment of such products.

In summary, the genetic modification of plants is a big chance and a large challenge for improving the food security all over the world. Unfortunately, the contributions of present GMPs to this aspect are relatively low and need to be further improved (Table 13). Further efforts on a more efficient use of restricted resources (development of low input varieties) should therefore be one of the main objectives of plant breeding.

References

Alexander TW, Reuter T, Aulrich K, Sharma R, Okine EK, Dixon WT, McAllister TA (2007) A review of the detection and fate of novel plant molecules derived from biotechnology in livestock production. Anim Feed Sci Technol 133:31–62

ANSES (Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail) (2011) Opinion of the French Agency for Food, Environmental and Occupational Health and Safety—recommendation of carrying our statistical analyses of data from 90-day rat feeding studies in the context of marketing authorisation applications for GM organisms. Request no 2009-SA-0285

Aumaitre A, Aulrich K, Chesson A, Flachowsky G, Piva G (2002) New feeds from genetically modified plants: Substantial equivalence, nutritional equivalence, digestibility, and safety for animals and the food chain. Livest Prod Sci 74:223–238

Baranowski A, Rosochacki S, Parada R, Jaszczak K, Zimny J, Poloszynowicz J (2006) The effect of diet containing modified triticale on growth and transgenic DNA fate in selected tissues of mice. Anim Sci Pap Rep 24:129–142

BEETLE (2009) Long-term effects of genetically modified (GM) crops on health and the environment (including biodiversity), Executive Summary and Main Report. Federal Office of Consumer Protection and Food Safety (BVL), BLaU-Umweltstudien and Genius GmbH, Berlin

Bernal-Santos B, O'Donell SM, Vicine JL, Hartnell GF, Bauman DE (2010) Hot topic: enhancing omega-3 fatty acids in milk fat of dairy cows by using stearidonic acid-enriched soybean oil from genetically modified soybeans. J Dairy Sci 93:32–37

Brake DE, Evenson DP (2004) A generational study of glyphosate-tolerant soybeans on mouse fetal, postnatal and adult testicular development. Food Chem Toxicol 42:29–36

Brake DG, Thaler R, Evenson DP (2004) Evaluation of Bt (*Bacillus thuringiensis*) corn on mouse testicular development by dual parameter flow cytometry. J Agric Food Chem 52:2097–2102

Brookes G, Barfoot P (2008) GMcrop: global socio-economic and environmental impacts 1996–2006. PG Economics Ltd, Dorchester

CAST (2006) Safety of meat, milk, and eggs from animals fed crops derived from modern biotechnology. No 34

Cellini F, Chesson A, Coquhonn I, Constable A, Davies HV, Engel K-H, Gatehouse AMR, Kärenlampi S, Kok EJ, Legnay JJ, Lehesranta S, Noteborn HPJM, Pedersen J, Smith M (2004) Unintended effects and their detection in genetically modified crops. Food Chem Toxicol 42:1089–1123

Clark JH, Ipharraguerre IR (2001) Livestock performance: feeding biotech crops. J Dairy Sci 84(E. Suppl. E9–E18):237–249

Davis HV, Kuiper HA (2011) GM-risk assessment in the EU: Current perspective. Aspects Appl Biol 110:37–45

DBT (Department Biotechnology) (2008) Protocols for food and feed safety assessment of GE crops. Dept. of Biotechn., Min. Science and Technol., India

Delaney B, Astwood JD, Cunny H (2008) Evaluation of protein safety in the context of agricultural biotechnology. Food Chem Toxicol 46:S71–S97

DLG (2012) DLG-Datenbank Futtermittel (database feedstuffs). http://www.dlg.org/futtermitteldatenbank.html

EFSA (European Food Safety Authority) (2006) Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed. EFSA J 99:1–100

EFSA (European Food Safety Authority) (2008) Safety and nutritional assessment of GM plant derived food and feed. The role of animal feeding trials. Food Chem Toxicol 46:2–70

EFSA (European Food Safety Authority) (2011b) Scientific opinion on EFSA guidance on repeated-dose 90-day oral toxicity study in rodents on whole food/feed. EFSA J 9(12):2438

EFSA Panel on Genetically Modified Organisms (GMO) (2011a) Scientific opinion on guidance for risk assessment of food and feed from genetically modified plants. EFSA J 9(5):2150

FAO (Food and Agricultural Organisation of the UN) (2009) The state of food and agriculture. Livestock in the balance. Rome

FDA (Food and Drug Administration; Redbook) (2000) Guidance for industry and other stakeholders, toxicological principles for the safety assessment of food ingredients. U.S. Department of Health and Human Services, Food and Drug Administration, Centre for Food Safety and Applied Nutrition. http://www.cfsan.fda.gov/guidance.html

Flachowsky G (ed) (2006) Possibilities of decontamination of "Undesirable substances of Annex 5 of the Feed Law (2006)". Landbauforschung Völkenrode – FAL Agric Res, Spec. Issue 294 (in German)

Flachowsky G (2008) What do animal nutritionists expect from plant breeding? Outlook Agric 37:95–103

Flachowsky G (2010) Global food security: is there any solution? NovoArgumente 105(3–4):64–68 (in German)

Flachowsky G, Aulrich K (2001) Nutritional assessment of GMO in animal nutrition. J Anim Feed Sci 10(Suppl. 1):181–194

Flachowsky G, Böhme H (2005) Proposals for nutritional assessments of feed from genetically modified plants. J Anim Feed Sci 14:49–70

Flachowsky G, Chesson A, Aulrich K (2005a) Animal nutrition with feeds from genetically modified plants. Arch Anim Nutr 59:1–40

Flachowsky G, Halle I, Aulrich K (2005b) Long term feeding of Bt-corn—a ten generation study with quails. Arch Anim Nutr 59:449–451

Flachowsky G, Aulrich K, Böhme H, Halle I (2007) Studies on feeds from genetically modified plants (GMP) contributions to nutritional and safety assessment. Anim Feed Sci Technol 133:2–30

Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS (2011) Solutions for a cultivated planet. Nature 478:337–342

Forster IP, Dominy WG, Obaldo LC, Hartnell GF, Sanders EF, Hickman TC, Ruebelt MC (2011) The effect of soybean oil containing stearidonic acid on growth performance, n-3 fatty acid deposition and sensory characteristics of pacific white shrimp (*Litopenaeus vannamei*). Aquacult Nutr 17:200–213

FSANS (2007) The role of animal feeding studies in the safety assessment of genetically modified foods. Rep. Workshop of Food Standards Australia New Zealand, Canberra

Gibbs RA, Rymer C, Givens DI (2010) Long-chain n-3 PUFA intakes in the UK and the potential of a chicken meat prototype to increase them. Proc Nutr Soc 69:144–155

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Wawrence D, Muir J, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: the challenge of feeding 9 billion people. Science 327:812–818

Halle I, Aulrich K, Flachowsky G (2006) Four generations feeding GMO-corn to laying hens. Proc Soc Nutr Physiol 15:114 (Abstr.)

Howe JA, Tanumihardjo SA (2006) Cartenoid-biofortified maize maintains adequate vitamin A status in Mongolian gerbils. J Nutr 136:2562–2567

ILSI (2003a) Best practices for the conduct of animal studies to evaluate crops genetically modified for input traits. International Life Sciences Institute, Washington, D.C. http//www.ilsi.org/file/bestpracticescas.pdf

ILSI (2003b) Crop compositional database. Accessed 2003 July 14; Update 2009

ILSI (2007) Best practices for the conduct of animal studies to evaluate crops genetically modified for output traits. Int Life Sci Inst, Washington DC

James C (2012) Global status of commercialized biotech/GM crops: 2011. ISAAA Brief No. 43. ISAAA, Ithaca

Kilic A, Akay MT (2008) A three generation study with genetically modified Bt corn in rats: biochemical and histopathological investigation. Food Chem Toxicol 46:1164–1170

Kitessa SM, Young P (2011) Enriched milk fat with n-3 polyunsaturated fatty acids by supplementing grazing dairy cows with ruminally protected echium oil. Anim Feed Sci Technol 170:35–44

Kleter GA, Kok EJ (2010) Safety assessment of biotechnology used in animal production, including genetically modified (GM) feed and GM animals—a review. Anim Sci Pap Rep 28:105–114

Kok E, Kuiper H (2003) Comparative safety assessment of biotech crops. Trends Biotechnol 21:439–444

Kok EJ, Keijer J, Kleter GA, Kuiper HA (2008) Comparative safety assessment of plant-derived feeds. Reg Toxicol Pharmacol 50:98–113

Krzyzowska M, Wincenciak M, Winnicka A, Baranowski A, Jaszczak K, Zimny J, Niemialtowski M (2010) The effect of multigenerational diet containing genetically modified triticale on immune system in mice. Polish J Vet Sci 13:423–430

Ladics GS, Knippels LM, Penninks AH (2010) Review of animal models to predict the potential allergenicity of novel proteins in genetically modified crops. Regul Toxicol Pharmacol 56:212–224

Mazza R, Soave M, Morlacchini M, Piva G, Marocco A (2005) Assessing the transfer of genetically modified DNA from feed to animal tissues. Transgenic Res 14:775–785

Meja L, Jacobs CM, Utterback PL, Parsons CM, Rice D, Sanders C, Smith B, Iiams C, Sauber T (2010) Evaluation of the nutritional equivalency of soybean meal with the genetically modified trait DP-3O5423-1 when fed to laying hens. Poultry Sci 89:2634–2639

Nemeth A, Wurz A, Artim L, Charlton S, Dana G, Glenn K, Hunst P, Jennings I, Shilto R, Song P (2004) Sensitive PCR analysis of animal tissue samples for fragments of endogenous and transgenic plant DNA. J Agric Food Chem 52:6129–6135

NRC (National Research Council) (1995) Nutrient requirements for laboratory animals. Forth revised ed., National Academy Press, Washington, DC

OECD (1993) Safety evaluation of foods derived by modern biotechnology. Concepts and principles. OECD, Paris

OECD (1998a) OECD guidelines for the testing of chemicals—Repeated dose 28-day oral toxicity study in rodents, 407. http://browse.oecbookshop.org/oecd/pdfs/free/9740701e.pdf

OECD (1998b) Guideline for the testing of chemicals—repeated dose 90-day oral toxicity study in rodents, 408. http://browse.oecdbookshop.org/oecd/pdfs/free/9740801e.pdf

OECD (2001a) Series on the safety of novel foods and feeds no. 1. Consensus document on key nutrients and key toxicants in low erucic acid rapeseed (canola). OECD, Paris

OECD (2001b) Series on the safety of novel foods and feeds no. 2: Consensus document on compositional considerations for new varieties of soybean: key food and feed nutrients and anti-nutrients. OECD, Paris

OECD (2003) Series on the safety of novel foods and feeds no. 7: consensus document on compositional considerations for new varieties of bread wheat (*Triticum aestivum*): key food and feed nutrients, anti-nutrients and toxicants. OECD, Paris

OECD (2004a) Series on the afety of novel foods and feeds no. 10: consensus document on compositional considerations for new varieties of rice (*Oryza sativa*): key food and feed nutrients and anti-nutrients. OECD, Paris

OECD (2004b) Series on the safety of novel foods and feeds no. 11: consensus document on compositional considerations for new varieties of cotton (*Gossypium hirsutum* and *Gossypium barbadense*): key food and feed nutrients and anti-nutrients. OECD, Paris

OECD (2004c) Series on the safety of novel foods and feeds no. 12: consensus document on compositional considerations for new varieties of barley (*Hordeum vulgare* L.): key food and feed nutrients and anti-nutrients. OECD, Paris

Rhee GS, Cho DH, Won YH, Soek JH, Kim SS, Kwack SJ, Lee RD, Chae SY, Kim JW, Lee BM, Park KL, Choi KS (2005) Multigeneration reproductive and developmental toxicity study of bar gene inserted intogenetically modified potato on rats. J Toxicol Environ Health 68:2263–2276

Russell WMS, Burch RL (1959) The principles of humane experimental technique, Special Ed., Universities Fed. for Animal Welfare, Potters Bar

Rymer C, Hartnell GF, Givens DI (2011) The effect of feeding modified soyabean oil enriched with C18:4 n-3 to broilers on the deposition of n-3 fatty acids in chicken meat. Br J Nutr 105:866–878

SCAR (EU Commission—Standing Committee on Agricultural Research (2008) New challenges for agricultural research. Climate change, rural development, agricultural knowledge systems. The 2nd SCAR Foresight Exercise, Brussels

Seralini GE, Mesnage R, Clair E, Gress S, de Vendomois JS, Cellier D (2011) Genetically modified crops safety assessments: present limits and possible improvements. Environ Sci Eur 23:10

Sharma R, Damgaard D, Alexander TW, Dugan MER, Aalhus JL, Stanford K, McAllister TA (2006) Detection of transgenic and endogenous plant DNA in digesta and tissues of sheep and pigs fed Roundup Ready canola meal. J Agric Food Chem 54:1699–1709

Snell C, Bernheim A, Bergé JB, Kuntz M, Gerard P, Paris A, Ricroch AE (2012) Assessment of the health impact of GM plant diets in long-term and multigenerational animal feeding trials. A literature review. Food Chem 50:1134–1148

Spencer JD, Allee GL, Sauber TE (2000) Phosphorus bioavailability and digestibility of normal and genetically modified low-phytate com for pigs. J Anim Sci 78:675–681

Spiekers H, Meyer HHD, Schwarz FJ (2009) Effects of long term utilization of genetically modified maize (MON 810) in dairy cattle feeding on performance and metabolism parameters. Schriftenreihe, Bayerische Landesanstalt für Landwirtschaft (LfL), vol 18 (in German)

Stein AJ, Rodriguez-Cerezo E (2009) The global pipeline of new GM-crops. JRC/ipts, EUR 23496 EN

Steinfeld H, Gerber P, Wassenaar T, Castel V, Rosales M, de Haan C (2006) Livestock's long shadow: environmental issues and options. Food and Agric Organization of the United Nations (FAO), Rome

Tang G, Qin J, Dolnikowski GG, Russell RM, Grusak MA (2009) Golden rice in an effective source of vitamin A. Am J Clin Nutr 89:1776–1783

The Royal Society (2009) Reaping the benefits: Science and the sustainable intensification of global agriculture. RS policy document 11/09, issued Oct. 2009 RS 1608. ISBN: 978-0-85403-784-1

Trabalza-Marinucci M, Brandi G, Rondine C, Avelini L, Giammarini C, Costarelli S, Acuti G, Orlandi C, Filippini G, Chiradia E, Malatesta M, Crotti S, Antonini C, Amagliani G,

Manuali E, Mastrogiacomo AR, Moscati L, Haounet MN, Gaiti A, Magnani M (2008) A three-year longitudinal study on the effects of a diet containing genetically modified Bt176 maize on the health status and performance of sheep. Livest Sci 113:178–190

Tudisco R, Mastellone V, Cutrignelli MI, Lombardi P, Bovera F, Mirabella N, Piccolo G, Calabro S, Avallone L, Infascelli F (2010) Fate of transgenic DNA and metabolic effects in goats fed genetically modified soybean and in their offsprings. Animal 4:1662–1671

Ursin VM (2003) Modification of plant lipids for human health: development of functional land-based omega-3 fatty acids. J Nutr 133:4271–4274

Whelan J (2009) Dietary stearidonic acid is a long chain (n-3) polyunsaturated fatty acid with potential health benefits. J Nutr 139:5–10