

The role of animal feeding trials for the nutritional and safety assessment of feeds from genetically modified plants – Present stage and future challenges

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ABSTRACT

The world's population is still growing up and asking for more and better food. High yielding plants with low external inputs should be the main aim of plant breeding to meet the higher demand for man and animal. Apart from traditional breeding plant biotechnology seems to have a certain potential to contribute to this objective. Safety and nutritional studies with such modified plants are the most important prerequisites for public acceptance of derived feed and food.

The first step for safety and nutritional assessment of modified plants is the compositional analysis of potential food/feed and including the newly expressed proteins and other new constituents and the comparison with conventional counterparts. *In vitro* studies and experiments with laboratory animals are the next steps for assessments. Feeding studies with target animals are of special concern for nutritional assessment and are considered in more detail in the present paper.

KEY WORDS: genetically modified plants (GMP), nutritional and safety assessment, digestibility, types of feeding studies

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INTRODUCTION

Sustainability in feed and food production is a key challenge for agriculture. In the future there will be strong competition for arable land and further limited resources such as fossil carbon-sources, water, some minerals (such as phosphorus) between feed/food, fuel, fibre, areas for settlements and natural protected areas. According to the FAO (2009) human population will globally increase from currently about 6.7 to 9 billion people (about 40% more) 2050 (Steinfeld et al., 2006), but the estimated need for meat and milk will nearly double during this time (Table 1).

Table 1. Challenges for animal production or “Livestock’s long shadow” (Steinfeld et al., 2006)

Year	Presently	2050	Percent to presently
World population, billion	6.5	9.0	138
Meat production, million ton	229	465	203
Milk production, million ton	580	1043	180

Presently the global growth rate is estimated with 2.7 and 1.8% for meat and milk, respectively yearly (FAO, 2009) and varies between different regions (more than 5% in some countries in East and Southeast Asia and less than -2% in some African countries). In addition there are large differences in the per capita estimated consumption of livestock products per year (e.g., 125.6 kg meat in the USA and 367.7 kg milk in Sweden on the one hand and 3.1 kg meat in Bangladesh and 3.5 kg milk in Burundi on the other side; FAO, 2009). In consequence the per capita intake of protein from livestock products varies between 1.7 (Burundi) and 69.0 g per day (USA) averaging 21.9 g (FAO, 2009). These figures show the dramatic differentiation between the countries. There is no essential need for food of animal origin, but the consumption of meat, fish, milk and eggs contributes significantly to meet the human requirements in amino acids and some important trace nutrients (such as Ca, P, Zn, Mn, Fe, I, Se, vitamins A, D, E, some B-vitamins) esp. for children as well as for pregnant and lactating women. Human nutritionists (Waterlow, 1999; Jackson, 2007) recommend that about one third of the daily protein demand (0.75-1 g per kg body weight; Jackson 2003; Rand et al., 2003) should originate from protein of animal origin. That corresponds to about 20 g of the daily intake of 60 g should base on protein of animal origin, which corresponds to the present average consumption (see above). Another reason for foods of animal origin is the generally high availability of the nutrients and their considerable enjoyment value. Such food is also considered as an indicator for the standard of living in many regions of the world. One reason for the higher demand of food of animal origin in some countries (Table 1) is the increased income of the population (Keyzer et al., 2005).

Feed amounts needed for global animal feeding (without aquaculture) are calculated in Table 2 and compared with human food.

Increasing feed/food demands require higher plant yields (Table 2) or more area for production. Because of some limited resources, low input plants are an important prerequisite to solving future problems and to establishing a sustainable agriculture. Such plants should be very efficient in the use of mineral plant

Table 2. Estimated dry matter (DM) consumption by humans and farm animals (own calculations)

Species	Number billions; FAO Stat. 2006	Consumption DM kg/day	Billion t/year
Humans	6.5	0.45	1.1
Cattle, buffaloes, horses, camels (large animals)	1.6	8	4.6
Sheep, goats (small ruminants)	1.8	1	0.7
Pigs	0.95	1	0.35
Poultry	18.5	0.07	0.45
Total feed presently in 2050			6.1 ~ 12?
Plant yield (required; 1.5 billion ha arable land; 3.3 billion ha grassland; t DM/ha)	Presently 1.3	In 2050 2.5	

nutrients (including N), fuel, water and arable land (high yields), but they should also be able to very effectively use sun energy and unlimited plant nutrients from the air (such as N₂ and CO₂; Table 3). Furthermore the genetic pool available in plants, animals and microorganisms should contribute to optimize plants and animals for a more efficient conversion of limited resources into feed and food.

Table 3. Potentials to produce phytogetic biomass and their availability per inhabitant under consideration of the increase of population (Flachowsky, 2010)

Plant nutrients in the atmosphere, N ₂ , CO ₂	↑↔
Sin energy	↔
Agricultural area	↓
Water	↓
Fossil energy	↓
Mineral plant nutrients	↓
Mineral plant nutrients	↓

(↑ increase, ↓ decrease, ↔ no important influence)

Emissions by animals are another critical points in animal production (Steinfeld et al., 2006). These include N, P and other minerals from feed, methane (CH₄) from the anaerobic fermentation of carbohydrates in the anaerobic fermentation in the rumen and other areas (e.g., Baldwin, 1995; Flachowsky and Brade, 2007),

and carbon dioxide (CO₂) from fossil fuel. Apart from the low input of limited resources along the food chain a low output of minerals and greenhouse gases (CO₂, CH₄ and N₂O) during feed/food production are very important aims of sustainable agriculture.

As already mentioned plant breeding and cultivation are the key elements and starting points for feed and food security during the next years (Flachowsky, 2008; SCAR, 2008; The Royal Society, 2009). 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, fossil fuel, plant protection substances, etc.
- efficient use of natural unlimited resources such as sun energy, nitrogen and carbon dioxide in the air
- optimize the genetic potential of plants for a highly efficient photosynthesis
- lower concentrations of toxic substances such as secondary plant ingredients, mycotoxins from toxin-producing fungi, toxins from anthropogenic activities or 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 the nutritive value determining 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 decrease of undesirable substances is also an important objective of plant breeding. From the perspective of nutrition, an increase of essential nutrients (e.g., amino acids, fatty acids, trace elements, vitamins, etc.) could be very favourable in some regions of the world to meet the requirements of man and animal with essential nutrients. This aspect is not so important in parts of the world like Europe because of the availability of a large amount of food and feed additives on the market.

It is possible to fulfil the objectives of plant breeding mentioned above by conventional breeding. But in the future methods of biotechnology may be more flexible, more potent and faster. “New” plants, newly expressed proteins in plants and/or changed composition of plants are real challenges for animal and human nutritionists for safety aspects and the nutritional assessment of such products. Table 4 summarizes plant events which are already on the market or which could be commercialized by 2015 on the basis of genetic modification. Some traits are in the direction of low input varieties (e.g., drought tolerance, resistance against

diseases, etc.), but Table 4 shows also, that we are in the starting phase of this new technology. In 2009 about 134 million ha of GM-plants were cultivated worldwide (about 9% of total arable land; James, 2009); most of them are tolerant against herbicides and/or resistant against insects. Presently individual interests or interests of some companies are dominating and are not always in agreement with public interests as mentioned above (SCAR, 2008; The Royal Society, 2009). Fundamental research should be conducted by independent publicly sponsored research institutions and the results should be available to all those who are interested in such plants.

Table 4. Events in commercial GM - crops and pipelines worldwide, by trait (Stein and Rodriguez-Cerzo, 2009)

Trait Category	Commercial in 2008	Commercial pipeline	Regulatory pipeline	Advanced development	Total by 2015
Insect resistance	21	3	11	22	57
Herbicide tolerance	10	4	5	13	32
Crop composition	0	1	5	10	16
Virus resistance	5	0	2	3	10
Abiotic stress tolerance	0	0	0	5	5
Disease resistance	0	0	1	3	4
Nematode resistance	0	0	0	1	1
Fungus resistance	0	0	0	1	1
Other	2	0	0	11	13

note: the number of trails can be bigger than the number of GM crops; abiotic stress tolerance includes drought tolerance

Assessing the nutritive value and also the safety of foods/feeds from plant breeding and dealing with energy and nutrient requirements of GM animals including animal clones (Niemann and Kues, 2007; Robi et al., 2007) are real challenges for animals nutritionists in the future (Figure 1). Various types of studies are necessary to answer all the questions and to improve the public acceptance of such food/feed and animals, respectively. The current stage of nutritional and safety assessment of feeds from modified plants and future challenges will be analysed in the present paper. Principals of genetic modification of plants as well as socioeconomic and environmental impacts of GM plants (Brookes and Barfoot, 2008) are not covered in the present paper.

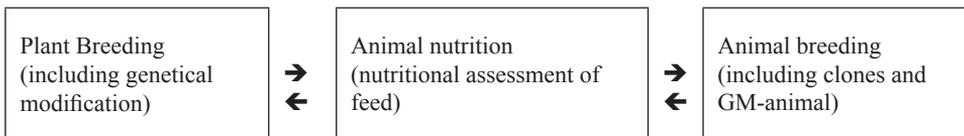


Figure 1. Animal nutrition (nutritional assessment of feeds) between plant and animal breeding

PRODUCTION OF 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) and ILSI (2003a, 2007). Genetically modified (GM) plants should be cultivated under consideration of specific isolation distances (ILSI, 2007) with their non-GM counterpart or if impossible, with GM parenteral lines as appropriate comparators. 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), preferably the studies should 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 GM-plants 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, 2002a,b,c, 2003, 2004a,b,c, 2005, or national/local feed value tables).

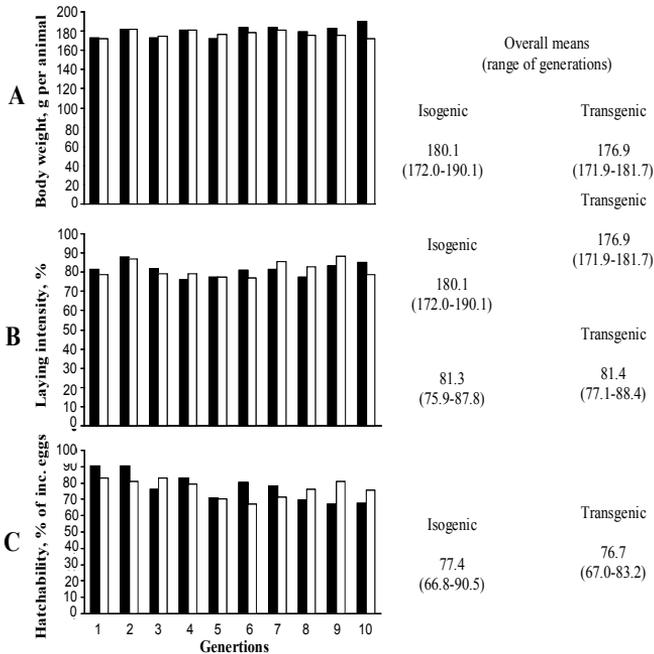


Figure 2. A. Body weight of female quails (age: 6 weeks), B. laying intensity and C. hatchability of quails fed with isogenic (■) and transgenic (□) maize in a 10 generations experiment (Flachowsky et al., 2005b)

But 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 favourable to include adequate comparators in the field studies.

Details of sampling (grain, pasture, hay, silage), handling of samples and preparation of samples for further studies are described by ILSI (2007; Figure 2).

COMPOSITIONAL ANALYSIS

Before cultivation, during growing, after harvesting, processing and manufacturing of the prepared feed 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, the most important nutrients (Table 5) and antinutritive substances such as pesticides, mycotoxins, enzyme inhibitors, glucosinolates, gossypol and further plant specific toxins 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 are 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 the cornerstone for the nutritional and safety assessment of food/feed from GMP. This comparative approach is the basis for the concept of substantial equivalence (OECD, 1993) and is suitable to assess GM-plants and derived food/feed of the first generation (GM-plants 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 GMP. The need of further studies including animal studies depends on the outcome of the compositional approach (for further details see Kok and Kuiper, 2003; EFSA, 2006; FSANZ, 2007. For GM-plants of the second generation (plants with output traits) further steps for safety and nutritional assessment seem to be necessary (see FSANZ, 2007; ILSI, 2007; EFSA, 2008).

Table 5. Recommendations for Nutrient Analysis (by ILSI, 2007)

Crops/Grains/Coproducts	Livestock type	Analyte ¹
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, tryptophan, isoleucine, arginine, phenylalanine, histidine, leucine, tyrosine, valine, fatty acids, vitamins
Oilseed meals: soyabean, 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, tryptophan, 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: soyabean, 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: soyabean, 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

¹ADF - acid detergent fibre; 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 fibre; EE - ether extract (crude fat); NDF - neutral detergent fibre; NDICP - neutral detergent insoluble protein; NDIN - neutral detergent insoluble nitrogen; NPN - non-protein nitrogen

FEEDING STUDIES

Before feeding studies compositional analysis and various *in silico* and *in vitro* methods (see DBT, 2008; EFSA, 2008) can contribute to the safety assessment of GM-plant derived food and feed. But feeding studies with laboratory and target animals are the key elements for safety and nutritional assessment of food/feed

from GM-plants.

The following types of feeding studies are established and may be carried out depending on the scientific question:

- laboratory animals models for toxicity testing of single substances (single dose toxicity testing; repeated-dose toxicity testing; reproductive and developmental toxicity testing; immunotoxicity testing, etc. (DBT, 2008; EFSA, 2008)
- 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; subchronic animal tests; for margins of safety, etc. (OECD, 1995; DBT, 2008; EFSA, 2008)
- studies to measure digestibility/bioavailability of nutrients from the GM-plants and to analyse the influence of GM-products on the metabolism in target animals/categories (Flachowsky and Böhme, 2005; ILSI, 2007; DBT, 2008; EFSA, 2008)
- tolerance studies to analyse the influence of maximal amounts of GM-feeds on animal health and welfare (ILSI, 2007; DBT, 2008; EFSA, 2008)
- studies with GM-animals to determine the energy and nutrient requirements of modified animals and to analyse quality and safety of food of animal origin
- 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 (ILSI, 2007; DBT, 2008; EFSA, 2008)
- 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; see Table 6)
- multigeneration studies to analyse the influence of GM-feed on fertility/reproduction performance of animals (BEETLE, 2009).

Table 6. Examples of life spans for growing/fattening animals, in days

Animal species/categories	Conventional/more intensive	Organic/more extensive
Chickens for fattening (broilers)	3 – 42	56 – 84
Turkeys for fattening	56 – 168	70 – 112
Growing/fattening pigs	150 – 300	200 – 400
Veal calves	80 – 200	–
Growing/fattening bulls	300 – 500	400 – 600

Laying hens and dairy cattle are usually used for longer periods:

- laying hens: about 126–140 days for growing (pullets); about 300-360 days (one year) for the laying period
- dairy cattle: about 22-36 month for growing (heifers); one to ten years for lactation (average in Europe two to five lactations)

The most important steps to prepare and carry out feeding experiments are summarized in Figure 2. In general the expense of the studies mentioned above increases from the top to the bottom. Therefore long term studies and multigeneration experiments with target animals are very seldom. Limited fed amounts in earlier breeding stadium may also restrict animal numbers and duration of studies with target animals, especially with large animals such as cattle and pigs. In summary the following factors may influence the types of animal feeding studies:

- scientific question(s)
- availability of GM-feeds (especially 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.) should be also measured in the feeding studies or after slaughtering of the animals (ILSI, 2007).

Laboratory animals

Normally, the OECD guideline tests (OECD, 1995) for chemicals are used for the safety testing of single substances including new products from the genetic modification (e.g., newly expressed proteins; EFSA, 2006, 2008). Mostly 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 the prior knowledge regarding the biology of these products, so that relevant endpoints are measured in the individual test (for more details see OECD, 1995; EFSA, 2006, 2008).

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). More details about the necessity of such studies and useful endpoints are described by OECD (1995) and EFSA (2006, 2008).

Target animals

Studies with target animals are more of nutritional concern. Less attention has presently been paid in such studies to safety aspects (EFSA, 2006, 2008). The type of study depends on the type of genetic modification in the plants or animals and the availability of GM-feed or GM-animals.

Measuring digestibility/bioavailability

In the case of substantial changes of composition of plants (GMP with output traits or GMP of the 2nd generation) studies to measure the digestibility/availability of some nutrients or nutrient precursors are necessary (Flachowsky and Böhme, 2005; ILSI, 2007; EFSA, 2008). 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. Table 7 shows results to measure the bioconversion of β -carotene into vitamin A. The retinol concentration in the liver of Mongolian gerbils as model animals was used as endpoint. The results show that the retinol concentration of 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 β -carotene from maize is almost identically converted into vitamin A as supplemented β -carotene.

Table 7. Experimental design to determine the conversion of β -carotene into vitamin A in maize (60 % of diet, Mongolian gerbils, n=10, depletion period: 4 weeks; feeding: 8 weeks; Howe and Tanumihardjo, 2006)

Item	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/d	0	106	106	106
Retinol in serum, nmol/d	1.23 \pm 0.20	1.25 \pm 0.22	1.23 \pm 0.20	1.22 \pm 0.16
Retinol in liver, nmol/d	0.10 ^a \pm 0.04	0.25 ^b \pm 0.15	0.25 ^b \pm 0.20	0.56 ^b \pm 0.15

^{a,b,c} means with different letters differ (P<0.05)

Table 8. Conventional and low-phytate maize (78.5 % of the mixture) in the feed of fattening pigs (from Spencer et al., 2000)

Item	Control		Low-phytate maize	
	0.3 g of available P per kg		1.7 g of available P per kg	
Inorganic P supplement	-	+	-	+
P content, g/kg				
29-73 kg liveweight	3.4	5.4 ¹	3.4	5.4 ¹
73-112 kg liveweight	3.2	4.7	3.2	4.7 ²
Feed intake, kg/d	2.23 ^a	2.50 ^b	2.53 ^b	2.51 ^b
Liveweight gain, g/day	730	870 ^b	900 ^b	880 ^b
Feed per gain, 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, 4 th metacarpal bone, kg	79.3 ^a	138.5 ^{bc}	132.2 ^b	153.9 ^c
Ash content, % in 4 th metacarpal bone	53.5 ^a	60.1 ^{bc}	59.3 ^b	61.2 ^c

^{a,b,c} different letters in one line indicate significant differences (P<0.05); ¹+2.0 gP/kg; ²+1.5 gP/kg

Similar studies are necessary to demonstrate the efficacy of enzymes expressed in plants or to show the higher phosphorus availability in plants with a lower phytate content. Phytate is one of the most important inhibitors of P-availability. In a pig study (Table 8) 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.

Efficacy trials

Many feeding studies were carried out during the last years to show the substantial equivalence (OECD, 1993) of feed from GM-plants of the first generation (without substantial changes in their composition or GMP +'''' with input traits). Most of the studies were done as efficacy trials and GM-feed were compared in adjusted diets with their isogenic counterparts and some

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 ¹	Measurements
Poultry for meat production	10 to 12 pens per treatment with 9 to 12 birds per pen	5 weeks or more	Balanced diets	Feed intake, gains, feed conversion
Poultry for egg production	12 to 15 replications per treatment with 3 to 5 layers per pen	18 to 40 weeks of age, at least three 28-day phases	Balanced diets	Feed intake, egg production, feed conversion, egg quality
Swine	6 to 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
	6 to 10 replications per treatment with 6 or more cattle per replication	90 - 120 days	Balanced diets	Feed intake, gain, feed conversion, carcass data
Growing and finishing ruminants	12 - 16 cows per treatment	Latin square: 28 day periods	Balanced diets	Feed intake, milk performances and composition, body weight,
Lactating dairy cows	28 cows per treatment	Randomized block design		Body Condition Score (BCS), cell counts in milk, animal health

¹ efficacy 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)

conventional commercial varieties (one to ten in some cases). The experimental designs were used according to the recommendations by ILSI (2003a, 2007; see Table 9) and EFSA (2006, 2008). Questions concerning the tolerance of some feeds in animals (tolerance studies) may be also included in efficacy trials.

During the last few years some reviews on nutritive and safety assessment of feeds from GM-plants (mostly plants from the first generation) were published (e.g., Clark and Ipharraguerre et al., 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, 2008) also summarize the present stage of knowledge in feeding of feeds from GM-plants to target animals. The animal studies did not show biologically relevant differences between animals fed with feed from GMP or their isogenic counterparts. Transgenic DNA and newly expressed proteins showed adequate properties as normal plant-DNA and proteins and are degraded during feed treatment and in the digestive tract of animals (Flachowsky et al., 2005a; Alexander et al., 2007; see Table 10).

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

Table 10. Performance and some metabolic parameters of the 1st and 2nd lactation of a long-term feeding study with dairy cows (n=18 per treatment, 25 months with Bt-maize (MON 810, 63% of roughage, 41% of concentrate from maize; Steinke et al., 2009)¹

Lactation	1 st		P-level	2 nd		P-level
	isogenic	transgenic		isogenic	transgenic	
Dry matter intake, kg/d	18.7	18.9	0.532	21.0	20.4	0.080
Milk yield, kg/d	23.9	23.7	0.566	29.2	28.8	0.419
Milk fat, %	3.95	4.03	0.015	3.75	3.86	0.055
Milk protein, %	3.62	3.71	<0.001	3.59	3.56	0.299
NEFA, µmol/l	287	281	0.991	292	290	0.988
BHBA, mmol/l	0.46	0.44	0.107	0.50	0.49	0.304
AST, U/l	92.6	89.8	0.263	94.3	88.8	0.177
GLDH, U/l	19.5	19.1	0.922	13.8	16.1	0.178
γ-GT, U/l	23.2	23.9	0.426	23.5	23.9	0.575

¹ no fragments of Cry1Ab DNA in blood, milk, faeces and urine of cows; traces of Cry1Ab protein were detected in faeces, but not in blood, milk and urine (Gürtler et al., 2009)

Long term feeding studies

Long term feeding studies cover the whole lifespan of the animals (see Table 6) or a very long period of their life, e.g., in the case of laying hens or dairy cows. Answers expected from such studies include, apart from the animal's performance their fertility and health, if 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 (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 of man, but all other possible adverse long term effects were assessed as 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 (2009) a long term feeding study with dairy cows (two lactations; Spiekers et al., 2009) was finished and partially published (see Table 10). Dry matter intake, milk yield and composition as well as physiological parameters in cows were not significantly influenced by feeding of high amounts GM-maize (MON 810). Fragments of the newly expressed protein and of the tDNA were not detected in samples of animal tissues and in the milk.

Multigeneration studies

In addition to long term feeding studies, multigeneration studies were carried out to test the reproduction and long term health effects in laboratory and target animals. In laboratory animals no negative effects were described on testicular cells or reproductive traits in mice fed Bt maize or a glyphosate tolerant soyabean compared with conventional maize or soyabean (Brake and Evenson, 2004; Brake et al., 2004). Rats and their pupils were not significantly influenced in a five generation study, if fed with 5% GM potatoes with 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 maize or conventional maize.

In a ten generation study with laying quails fed diets containing 50% Bt maize did not significantly influence production and reproduction performances of animals compared with a diet containing 50% isogenic maize.

The outcome of three studies with mice (strain OF1, Austrian mice study; Velimirov et al., 2008), including a multi-generation study, a reproductive assessment by continuous breeding and a life time study, fed with diets containing 33% NK603 x MON810 maize or its isogenic counterpart was discussed controversially because of weaknesses in experimental design, calculation errors and methodical deficiencies in the statistical analysis (Afssa, 2009; EFSA, 2009). Later (March 2010) the study was withdrawn from the Internet by the Austrian officials (Table 11).

Table 11. Comments to some studies which certain disturbances after feeding GM-crops

Authors	Study	Results	Comments
Nordlee et al., 1996	Transgen of paranut in soyabean and maize	Allergenic reactions in man	Scientific study, no practical relevance
Ewen and Pusztai 1999	Lectin potatoes to rats	Influence of intestinal tract, disturbance of reproduction	Scientific study, no practical relevance
Maletesta et al., 2002, 2003	RR soyabean to mice: comparison with wild variety	Increased cell nucleus in liver and pancreas	Methodical weaknesses, comparison with wild variety, What is normal? Relevance of results?
Hemre et al., 2005	RR soyabean to salmon	Increase of spleen, influence of spleen functions, more smaller erythrocytes	What is normal? Repetition of study
McNaughton et al., 2007	Maize event DAS-59122-7 in broilers	No differences, but liver of female rats was 3 g/kg heavier ($P < 0.05$)	Values in physiological range; overestimation of data; What is normal? Statistical significance, but biological not relevant
Poulsen et al., 2007a,b	Feeding transgenic lectin-rice to rats	Disturbances in development and fertility	Scientific study, no practical relevance
Seralini et al., 2007	New analysis of the rat feeding study by the notifier (Monsanto) with MON 863	Some differences in liver and kidney parameters	Critical analysis of the 90 days rat study, differences not directed, statistical significant, but biological not relevant (see Doull et al., 2007)
Scholtz et al., 2008	Feeding of 50% Bt-maize in longterm study in qualils	Differences in some enzymatic activities between both groups	Physiological relevance, what is normal? Other results after repetition of study
Surov, 2009	3 generations study with hamsters, added GM-soyabean to the diet, 5 pairs per treatment	Lower reproduction of hamsters fed with GM soyabean	Presently no scientific publication; only preliminary report, scientific assessment not possible
Velimirov et al., 2008	Long term reproduction studies in mice fed transgenic corn NK603xMON810	Some disturbances in reproduction of mice	For comments see above; with drawn be the Austrian officials
De Vendomois et al., 2009	Reanalysis of 90 day rat feeding studies with corn varieties MON 863, MON 810, NK 603	More significant differences than reported by the notifier (Monsanto)	Weaknesses in the statistical methods used for reanalysis (see Doull et al., 2007)

Comments to some feeding studies

There is large agreement that studies with feeds from GM-plants 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; Flachowsky et al., 2007; BEETLE, 2009). On the other hand there are some studies mainly carried out with laboratory animals, but in some cases also with target species, which show contraversial results (Table 11). They are a basis for public discussions. In some cases the authors tested GM-feed with antinutritive substances (e.g., Nordlee et al., 1996; Ewen and Pusztai, 1999; Poulsen et al., 2007a,b) in fundamental research studies. Such feeds or feed spiked with antinutritive materials do not have any practical relevance and no chance of coming onto the market because of the strong regulations for safety assessment (EFSA, 2006, 2008). In other cases GM-feeds in the diet have been compared with wild varieties (e.g., Malatesta et al., 2002, 2003). Wild varieties of legumes contain various antinutritive substances (e.g., enzyme inhibitors) and differences between both treatments must be expected (Table 11). Table 11 contains further studies or reassessments of studies (e.g., Seralini et al., 2007; De Vendomis et al., 2009) with some disturbances and comments to the results. Nevertheless, despite of weaknesses of some studies (Table 11) some authors (e.g., Dona and Arvanitoyannis, 2009) use the results uncritically and deduce health risks only from the application of genetically modified food/feed. Conflicting results in different studies indicate that there is a need for further research.

Side effects might be expected in GMP, especially in GMP with multiple modifications (multi-stacked events; Cellini et al., 2004) and must be analysed in detail on scientific basis. Furthermore the high biological range for many parameters should be considered. Presently, no other food/feed are so extensively analysed and tested in various studies as GMP-products. It can be concluded, that the safety and nutritional evaluation of GM versus conventionally bred plants is not well balanced (Kok et al., 2008).

FUTURE TRENDS

Presently, GM-plants which are tolerant against some herbicides or resistant against insects (Stein and Rodriguez-Cerezo, 2009) are dominating on the market (Table 12).

Table 12. 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	↑↑↑	↑	↑
More resistant against insects, etc. e.g., European maize borer	↑↑	↑	↑
More valuable ingredients	↑	~	(↑)
Less undesirable ingredients	(↓)	↑↑	↑
More efficient use of resources, water, etc.	(↓)	↑	↑↑↑

↑↑↑ – extremely high; ↑↑ – very high; ~ not important

But there are various trends for future objectives of plant breeding and development of GM-plants (see Table 4). The combination of some stacks to unify favourable properties in one hybrid is presently very popular in plant breeding (EFSA, 2007; Stein and Rodriguez-Cerco, 2009). In 2009 about 20% of the global area cultivated with GM-plants was stacked events (James, 2009). Plants with four stacks are already in the regulatory pipeline (Table 13), more stacked plants are in the breeders gardens.

Table 13. GM-maize stacks and their regulatory situation in the EU (Stein and Rodriguez-Cerco, 2009);

Stacking	Number stacks of world wide*	Authorised	EU situation pending	Under assessment
Double	13	4	1	8
Triple	3	4	1	2
Quadruple	1	0	0	1

* total number of the commercial GMmaize stacks and the GM maize stacks in the commercial and regulatory pipeline. Due to the differences in the regulation of stacks events on a global scale there may be more (implicity) authorised GM maize available)

Apart from stacking further objectives of plant breeding are in the pipeline of breeding companies and may be summarized as follow:

- improvement of nutritional properties (e.g., amino acids, fatty acids, minerals, vitamins, enzymes, lower content of undesirable compounds)
- higher resistance against biotic and abiotic stressors
- more efficient use of limited natural resources (water, arable land, fuel nutrients, etc.).

It is quite clear from the view of animal nutrition that studies to measure the bioavailability of changed nutrients are necessary. But:

- do we need feeding trials within target animals with stacked events (see EFSA, 2007)?

- do we need feeding trials with GM-plants, which use resources (e.g., water, abiotic stressors) more efficiently, but without changes in composition?

There exist some open questions regarding the feeding studies with GM-animals. Furthermore feeding studies with target animals should be used more intensively for safety assessment of food/feed from GM-plants.

CONCLUSIONS

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

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

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