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Animal Nutrition with Transgenic Plants

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1. Introduction (Global Developments)

Population ↑

Resources
per people ↓

Emissions ↑

Challenges for animal production or "Livestock's long shadow" (Steinfeld et al. 2006)

Year	Presently	2050	Percent to presently
World population (bill.)	6.5	9.0	138
Meat production (Mio. t)	229	465	203
Milk production (Mio. t)	580	1043	180

Examples for important limited global resources

Area	≈ 1,5 Bill. ha arable land; ≈ 3,3 Bill. ha Grassland
Fossil Energy	C-Sources (e.g. petrol, gas, coal)
Water	Presently about 71% used by agriculture
Phosphorus	Known resources for about 1 000 years

2. Challenges for Plant Breeding

Conditions to produce phytogenic biomass and their availability per inhabitant under consideration of the increase of population (↑ Increase, ↓ Decrease, ↔ No important influence; based on the Royal Society, 2009)

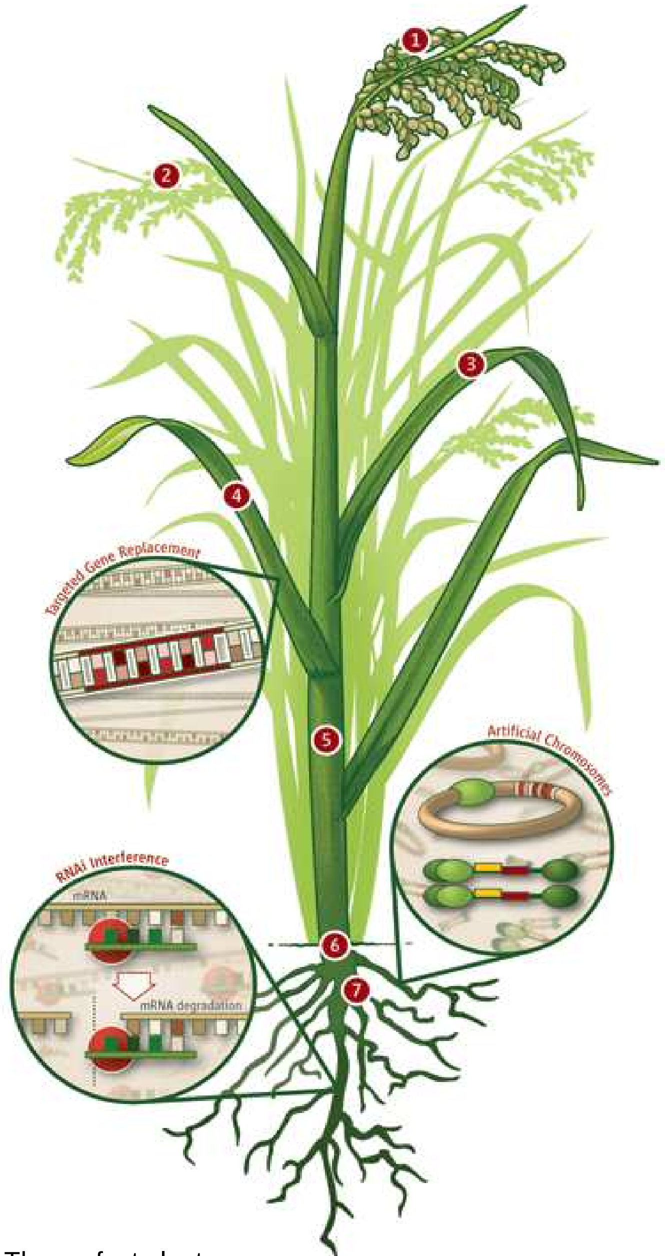
Plant nutrients in the atmosphere (N_2, CO_2)	↑↔
Sun energy	↔
Agricultural area	↓
Water	↓
Fossil Energy	↓
Mineral plant nutrients	↓
Variation of genetic pool	↑

The 'perfect plant'

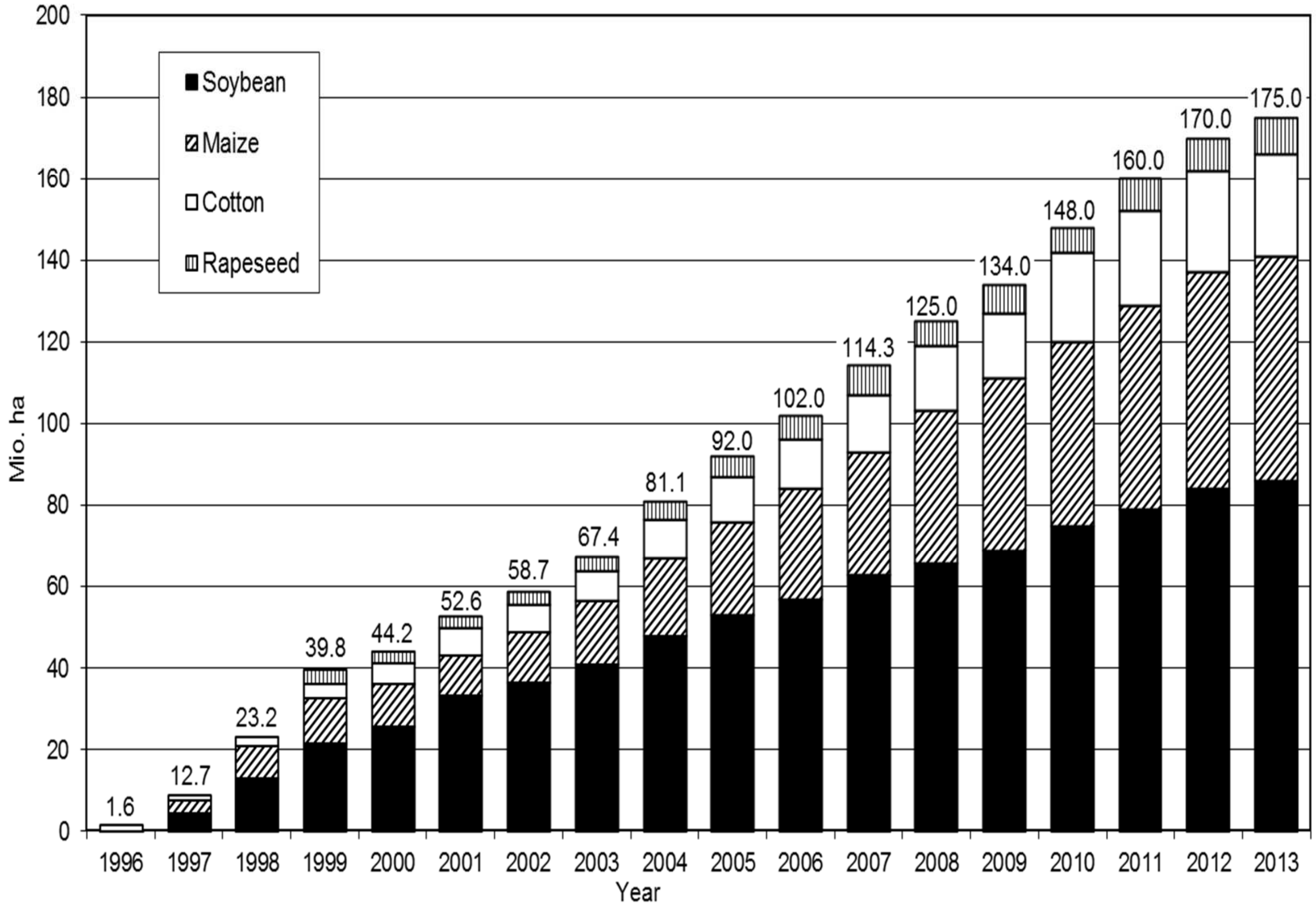
- New traits (short term)
  - Stress tolerance
  - Pharma substances

- Breeder's wish:

1. Higher nutrient content
2. Asexual reproduction
3. Optical warning signals
4. Higher water efficiency
5. Ripening & senesces control
6. Higher N efficacy
7. Better pest resistance



The perfect plant–
Science 12 February 2010



Global cultivation (Mio. ha per year) of most important GMO´s (ISAAA 2014)

Important food/feed from GM-plants and estimated portion used as food or feed

GM-Plant	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, Fullfat rapeseed	75
Cotton	Oil	15	Cotton seed (extracted oil) meal, Expeller	85

Questions to Animal/Human Nutritionists

- Nutritional and safety assessment of food/feed of the 1st generation of GMP (Plants with input traits)
- Nutritional and safety assessment of food/feed of the 2nd generation of GMP (Plants with output traits)
- Influence of GM-food/feed on human/animal health and quality of food of animal origin
- Studies on the degradation of newly expressed protein, foreign DNA, unintended effects etc.

3. Fundamentals of Animal Feeding Studies

Objectives for Animal Feeding Trials (in vivo) with GM-Products

- **Do we need additional information to compositional analysis and in silico, in vitro and/or in situ studies?**
- **Do we expect unindented effects?**
- **Do we expect long term effects (incl. effects on health, fertility etc.)?**
- **Do we need additional information from GMP with output traits (e.g. bioavailability of nutrients) or GM-animals (e.g. new energy and nutrient requirements)?**
- **Do we expect effects on product quality/composition of food of animal origin?**
- **Can we expect innovations for safety and nutritional research?**
- **Are animal studies important/necessary from the view of public concerns and/or may the studies contribute to more public acceptance?**

Present recommendation: Case by case decision

Advantages of studies for safety and nutritional assessment of GM-plants derived food/feed with laboratory or target animals

Laboratory Animals	Target Animals
Internat. agreed study protocols	Representative for animal species/categories (extrapolation??)
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 Real studies on the transfer of valueable and/or undesired substances into food of animal origin

Important types of feeding studies and animals recommended

Type of studies	Lab. 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

Influencing Factors on Type of Feeding Studies

- **Scientific question(s)**
- **Plants of 1st or 2nd generation**
- **Availability of GM-feeds (esp.in early stages of breeding) and adequate comparators**
- **Financial budget**
- **Availability of equipments, animals and qualified manpower**

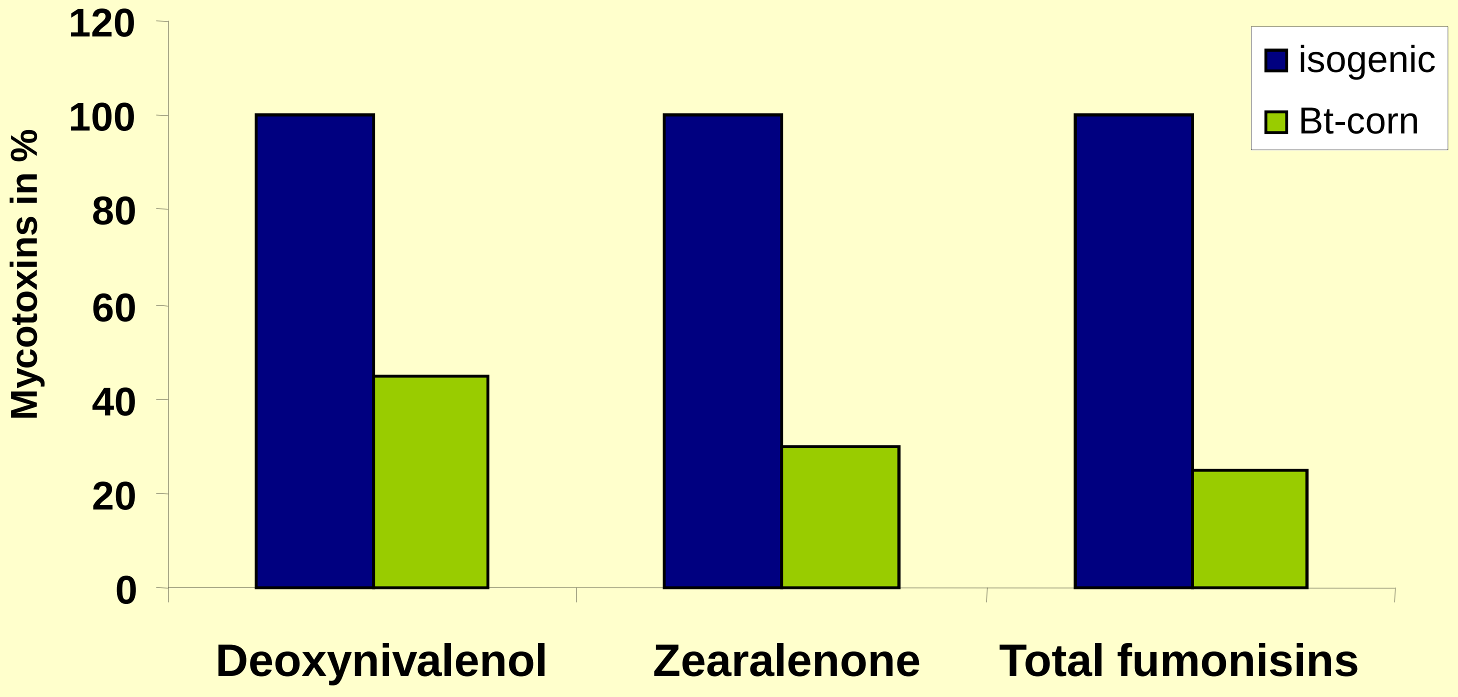
Endpoints of Feeding Studies

- **Feed intake, body weight and weight gain, yield in milk or eggs, feed conversion rate (FCR)**
- **Animal behavior, health, mortality**
- **Physiological parameters in body samples**
- **Weight of organs and tissues**
- **Chemical composition of food of animal origin (e.g. milk, eggs, tissues, organs)**

4. Assessment of feed from the 1st generation of GMP (input traits)

Crops/Grains/ Coproducts	Livestock Type	Analyte
Grain: maize, wheat, barley	Non-ruminants	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, degrable protein, NDICP, ADICP, fatty acids, fat soluble vitamins

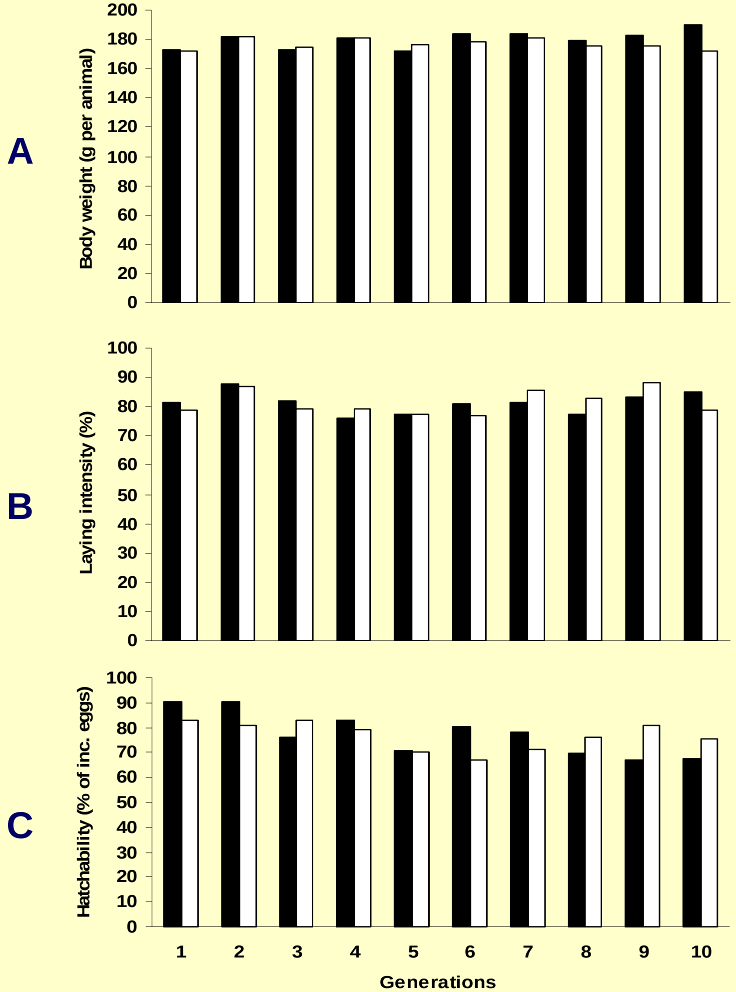
Mycotoxins in isogenic (100 %) and Bt-corn (% of isogenic corn; data from some references)



Summary of published data to compare feeds from GM plants of the first generation (with input traits) with their isogenic counterparts in food producing animals (sum. by Flachowsky 2013)

Animal (Species/categories)	Number of experiments	Nutritional assessment
Ruminants		No unintended effects in composition and contamination (except lower mycotoxins concentrations in Bt plants)
Dairy cows	23	
Beef cattle	14	
Others	10	
Pigs	21	
Poultry		No biological relevant effects on digestibility and animal health as well as no unintended effects on performances of animals and composition of food of animal origin
Laying hens	10	
Broilers	28	
Others		
(Fish, rabbits etc.)	8	

(A) Body weight of female quails (age: 6 weeks), (B) laying intensity and (C) hatchability of quails fed with isogenic (■) and transgenic (Bt, □) corn in a 10 generations experiment



Overall means (Range of generations)

	Isogenic	Transgenic
A	180.1 (172.0 – 190.1)	176.9 (171.9 – 181.7)
B	81.3 (75.9 – 87.8)	81.4 (77.1 – 88.4)
C	77.4 (66.8 – 90.5)	76.7 (67.0 – 83.2)

Conclusion: Degradation of DNA

- DNA is a permanent part of food/feed
  (daily intake: men: 0.1 – 1 g; pig: 0.5-4 g; cow: 40-60 g)
- tDNA intake amounted to ~0.005% of total DNA-intake, if 50 % of diet come from GM-crops
- DNA is mostly degraded during conservation (silage making) and industrial processing as well as in the digestive tract (pH, enzymes)
- Small fragments of DNA may pass through the mucosa and may be detected in some body tissues (esp. leucocytes, liver, spleen)
- There exist no data, that tDNA is characterized by another behaviour as native plant-DNA during feed treatment and in the animals

Conclusion: Degradation of newly expressed Proteins

- In the ruminants feed protein are mostly degraded in the rumen and microbial protein and by-pass protein is degraded by enzymes in the smaller intestine, similar to nonruminants
- The chemical and physiological properties (including microbial and enzymatic degradation) of novel proteins have been intensively tested
- Intact novel proteins were not detected outside of the digestive tract in target animals
- There is no advice, that newly expressed proteins are characterized by other chemical/physical properties as native protein

5. GM - Plants of the 2nd generation

(Substantial changes in composition; Plants with output traits)

GM - crops with output traits (GMP of the second generation; see Tillie et al. 2013)

- **Increased content of desirable/valuable substances**
  - **Nutrient precursors (e. g. β-carotene)**
  - **Nutrients (amino acids, fatty acids, vitamins, minerals etc.)**
  - **Substances which may improve nutrient digestibility (e. g. enzymes)**
  - **Substances with surplus effects (e. g. prebiotics)**
  - **Improvement of sensoric properties/ palatability (e. g. essential oils, aromas)**
- **Decreased content of undesirable substances**
  - **Inhibiting substances (e. g. lignin, phytate)**
  - **Toxic substances (e. g. alkaloids, glucosinolates, mycotoxins)**

Proposal to assess the conversion of nutrient precursors of the 2nd generation into nutrients (GM-plant with output traits; e.g. conversion of the precursor ß-carotene into vitamin A; by EFSA, 2008)

Groups[4]	Composition of diets	Measurements; endpoints[2]
1[1]	Balanced diet with typical amounts of the isogenic counterparts (unsupplemented control)	Depend on genetical modification of plants, e.g.: - Feed intake, animals growth - Concentration of specific/converted substance(s) in most suitable indicator organs (e.g. vit. A in the liver)[3] - 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 ß-carotene)	
3	Diet of Group 1 with ß-carotene supplementation adequate to Group 2	
4	Diet of Group 1 with vitamin A supplementation adequate to expected ß-carotene conversion into vitamin A	

1 Adequate feed amounts (pair feeding) for all animals; depletion phase for all animals before experimentation
2Depletion of specific nutrient in experimental animals could be necessary
3 Up to the steady state in the specific target organ
4 Some animal groups are fed with commercial/isogenic control feed to find out the biological range of the parameter(s)

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)

	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 (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 ($p < 0.05$)

6. Open Questions and Conclusions

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

Animal nutrition
(safety and nutritional assesment of feed)
between plant and animal breeding

Plant breeding

(incl. Genetical Modifications)

⇄

Animal nutrition

(Safety and Nutritional assess-ment of feeds)

⇄

Animal breeding

(incl. Clones and GM-animals)

Some open questions

- Do we need more feeding trials with feeds from GMP of the 2nd generation?
- Do we need more feeding trials with stacked events (more tDNA, more transgenic protein)?
- Do we need more feeding trials with GMP, which use resources more efficient (e.g. water, abiotic stress)?
- Do we need adequate studies with plants (feed) from traditional breeding?
- What are the consequences of a North Atlantic Free Trade Region?
- Should we harmonize the Guidance Documents for nutritional and safety assessment of GMO?

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Conclusions

- **Up to now about 1.6 Billion ha of GM-crops have been cultivated allover the world**
- **Most animal feeding studies were done with GM-crops of the 1st generation (with input traits)**
- **No unintended effects in composition (except lower mycotoxins), safety and nutritional properties were registered in more than 100 feeding studies with food producing animals**
- **tDNA and newly expressed proteins in GM-crops show similar properties during processing and in animals as plant-DNA and proteins**
- **Some other experimental designs are recommended esp. for nutritional assessment of feeds from GM-crops of the 2nd generation (with output traits, e.g. bioavailability of nutrients) and of GM-animals**
- **Furthermore case by case studies seem to be necessary to answer open questions, more groups with isogenic counterparts should be included**
- **Feeding studies with food producing animals should be also used for safety assessment**

List of some references

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