Risk assessment in the food chain: GMO‘s in food producing animals

Jürgen Zentek

Veterinary University Vienna
Free University Berlin

- What is the current situation in the EU/globally?

  - Animal products are important for the provision of high quality proteins to humans
  - > 50 % of the produced plant mass is used for animal nutrition
  - The land resources for plant production are limited

Animal nutrition – Plant production

- What is needed in animal nutrition (1)?
  - Adequate feed production (globally)
  - High-quality feed
  - Minimum use of resources, such as water, fossil energy, nutrients and land area
  - Increased resistance to pests, tolerance to drought and salt levels in soils etc.

- What is needed in animal nutrition (2)?
  - High nutritional value
    - amino acids, fatty acids, vitamins
    - greater digestibility and thus higher energy and nutrient utilisation
  - Low content of undesirable (anti-nutritional) constituents
    - Mycotoxin resistance
  - Reduced environmental pollution by animal excrements

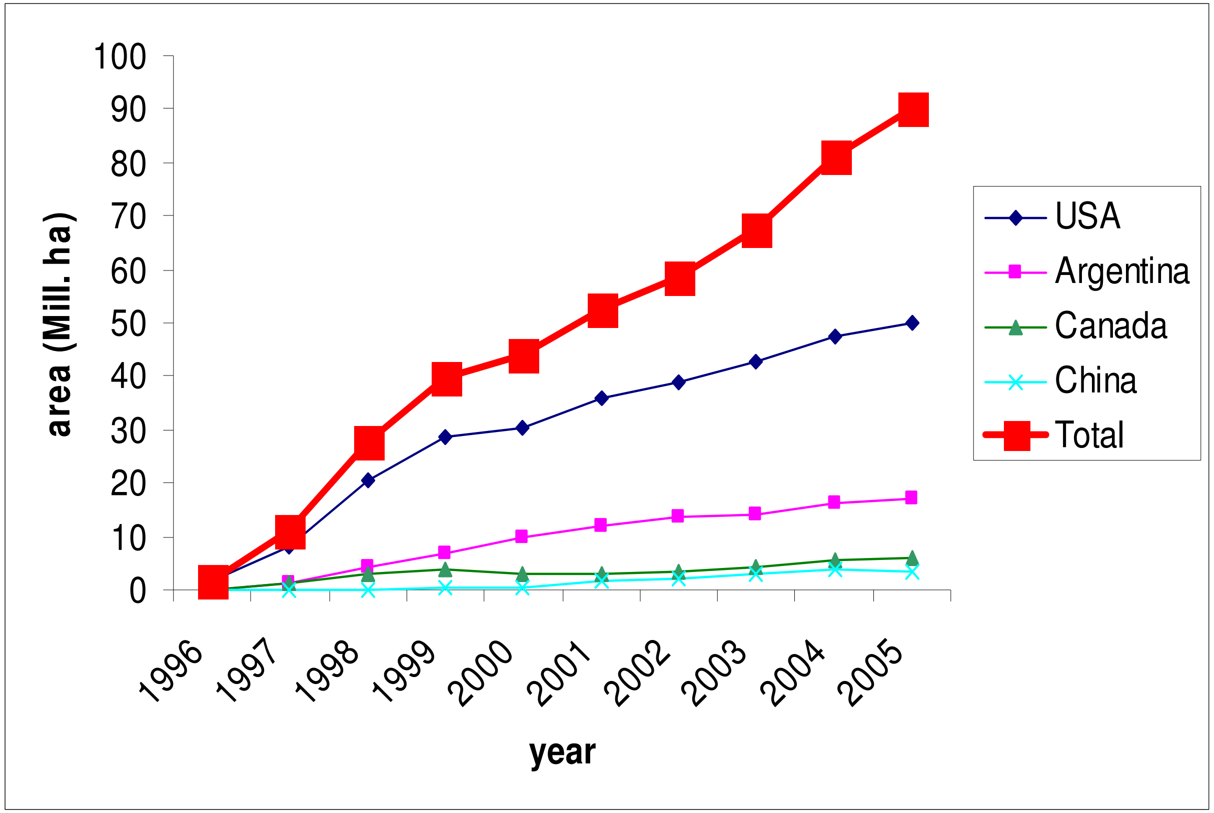
Flachowsky, 2002

- What is the challenge?
  - The global demand for food, especially for food of animal origin, will be doubled by the year 2025 and almost tripled by the year 2050
  - Different strategies heavily discussed, one are GMO's
  - GMO's are discussed controversially:

    +: less agrochemicals, higher production

    -: biodiversity, safety, unintended release,
    industrialized production -> dependency of farmers

GMO soya production

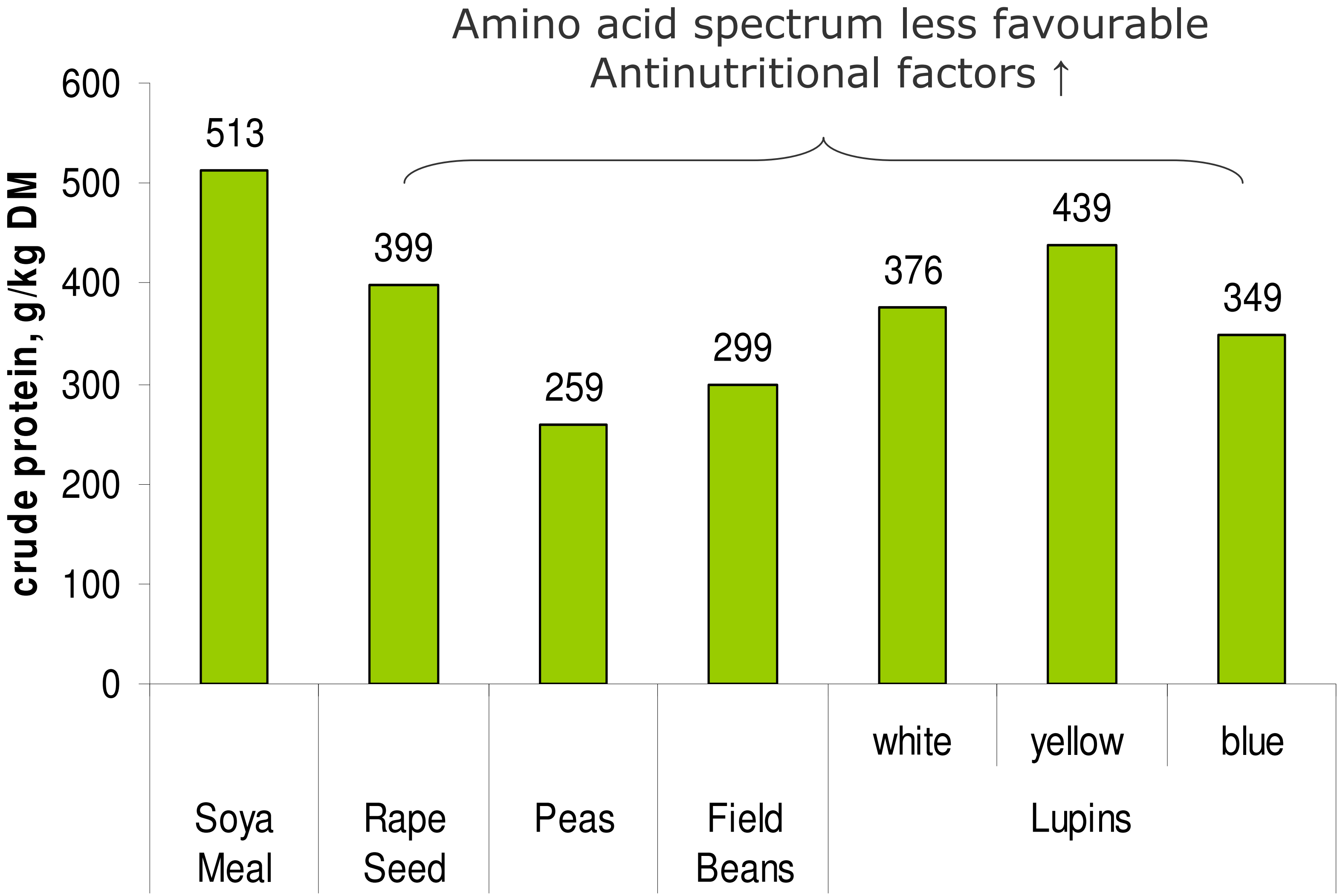


http://www.gmo-compass.org/eng/agri_biotechnology/gmo_planting/142.countries_growing_gmos.html

GMO feed import EU

- EU-25 countries account for nearly half of the world's imports of soybean meal
- Expected soybean import by the region decreased
  - Oilseed processors are seeking to convert their capacity to crush soybeans more toward rapeseed
  - Trend toward higher European feed use of wheat, rapeseed and sunflower seed
  - 2005 Ukraine made harvest records for sunflower seed, soybeans, and rapeseed

Crude protein in legumes and oil seeds



Safeguard measures

- What means precaution with regards to animal nutrition?
  - EU requires US, argentinian and brazilian soya as protein source for intensive animal production
  - Claim for safeguard measures („Precaution“) to achieve maximum consumer‘s protection
  - Political measures/restrictions in those cases when the independent, non industrial scientific evaluation is not yet finished

Cornerstones of evaluation?

1. Difference to the feed which it is intended to replace?
2. Effects of processing?
3. Effects on digestion, metabolism and health in farm animals?
4. Impairment of the distinctive features of animal products?
5. Effects on the environment?

Substantial Equivalence

- What are the starting points for the safety assessment?

  - Chemical composition
    - Nutrients
    - Transcriptom
    - Proteom

Composition

- Substantial equivalence has been demonstrated in 1st generation crops

  - Nutrients within the variation of the isogenic lines
  - “normal range of agronomic variability”

- In conventional feedstuffs considerable variation of nutrient concentration occurs

Comparison of iso- and transgenic maize

Parameter	Isogenic maize	Insect resistant maize	Isogenic maize	Herbicide tolerant maize	Isogenic maize	Herbicide/ Insect tolerant maize
Nutrients						
Crude ash	15	16	19	18	14	15
Crude protein	108	98	120	119	97	101
Crude fat	54	56	31	35	41	46
Crude fibre	23	25	34	30	36	37
N-free extracts	800	805	796	798	811	800
Starch	710	708	692	701	574	568
Sugar	n.A.	n.A.	n.A.	n.A.	18	14
Minerals						
P	3.7	3.2	4.0	3.9	3.2	3.4
Mg	1.2	1.2	1.7	1.5	1.3	1.4
Ca	0.03	0.04	0.12	0.05	0.16	0.14
Amino acids						
Lysine	2.9	3.0	3.3	3.2	n.A	n.A
Methionine	2.2	2.1	2.6	2.5	n.A.	n.A.
Cysteine	2.5	2.4	3.0	2.7	n.A.	n.A.
Fatty acids (% of total fatty acids)						
C 16:0	12.4	12.5	11.5	11.8		
C 18:1	31.1	28.6	27.7	27.4		
C 18:2	50.0	51.2	57.0	56.3		

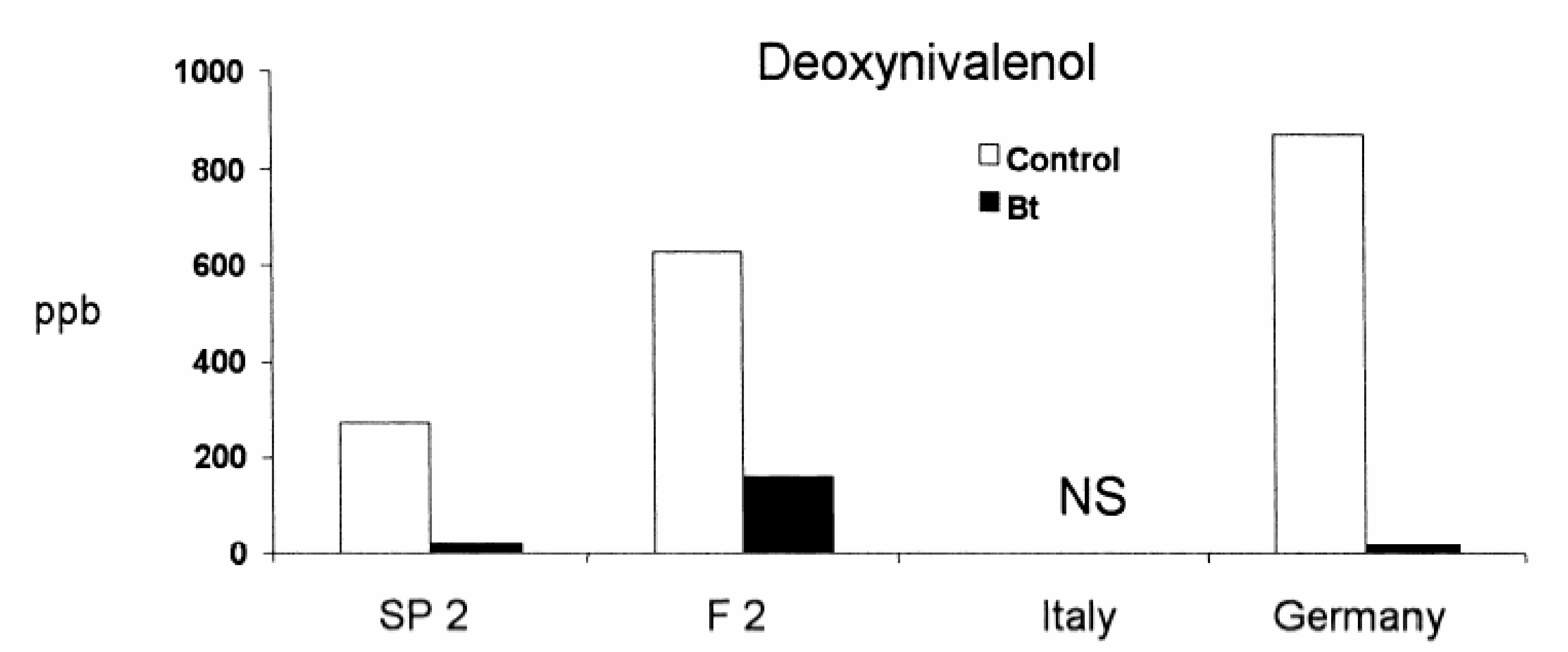
Flachowsky et al. 2005; own data

Apparent digestibility of iso- and transgenic maize

Table 4
Chemical composition and digestibility of glufosinate tolerant maize seeds for pigs as compared to the parental cultivars (Böhme & Aulrich, 1999)

Treatment	Controls (conv.)	Bt-maize seeds	
		Conv.	Basta
Dry matter (g/kg)	890	902	902
Crude nutrients (g/kg DM)			
Crudeprotein	120	119	117
Ether extract	31	35	33
Crude fibre	34	30	33
NfE	796	798	799
Digestibility, pig (%)			
Organic matter	89.6±4.1	90.0±2.1	89.3±1.8
Crude protein	81.1±12.0	80.3±9.9	79.6±6.0
NfE	93.1±2.6	94.7±1.4	95.3±1.7
Energy concentration (MJ/kg DM)			
ME	15.76±0.60	16.01±0.3	16.06±0.15

DM, dry matter; ME, metabolizable energy.



Incidence of mycotoxin contamination in control and Bt maize kernels in Spain (SP), France (F), Germany (G) and Italy in 1999; data from Cahagnier and Melcion (2000), Valenta et al. (2001) and Pietri and Piva (2000), cited from Aumaitre 2002

Further procedures

- Critical assessment of nutritional or anti-nutritional components
- Variations
  - Geographical position
  - Growth conditions
  - Soil conditions
  - Weather
- Appropriate statistical analysis

Safety evaluation process

- Genomic and postgenomic technologies

  - DNA microarrays
  - Proteomics
  - Metabolic profiling

Kuiper et al. 2000

Einspanier et al. 2004

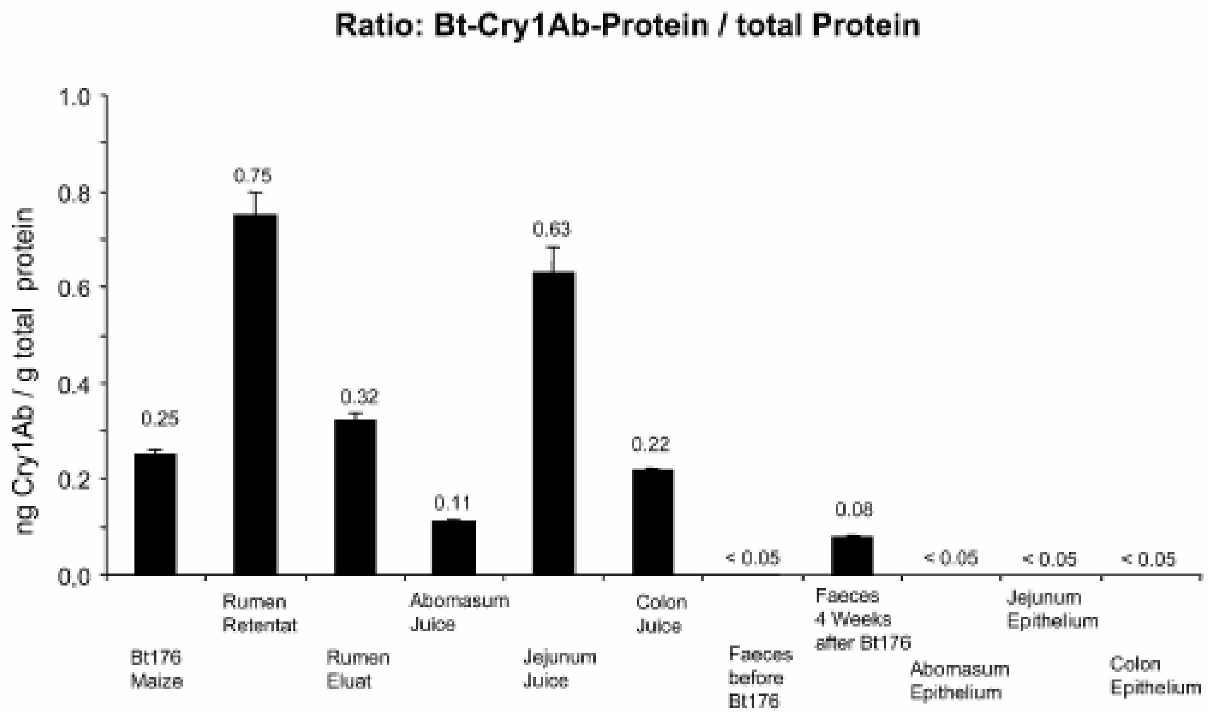


Fig. 3 Quantification of immunoreactive CryIAb protein during the intestinal passage in Bt176 maize-fed cattle. *Numbers* over the *bars* indicate the absolute CryIAb protein concentration

Effects of processing

- Few data
- Temperature x pressure x pH decrease DNA and fragment length

Bauer et al. 2003

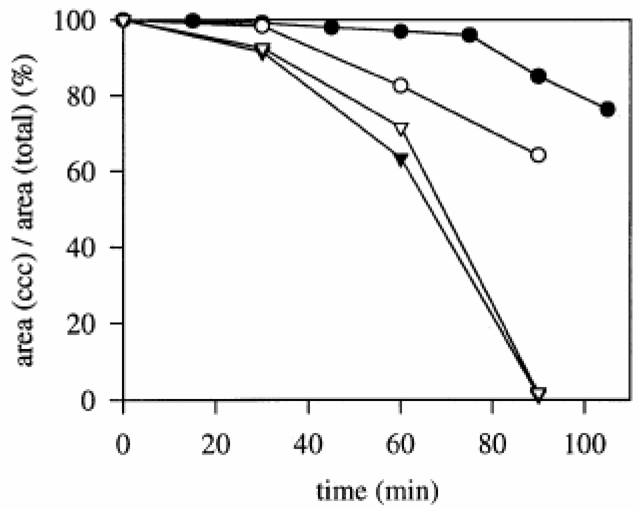


Fig. 1 Kinetics of degradation of plasmid DNA by a single strand break under different processing conditions monitored by HPLC. DNA of plasmid pSG100 was incubated in buffer at 85 °C/pH 8.4 (*solid circle*), at 37 °C/pH 4.0 (*empty circle*), at 65 °C/pH 4.0 (*solid triangle*), and in tomato serum at 65 °C/pH 4.3 (*empty triangle*)

Effects on health

- No short tem negative effects reported from admitted products
- No gene flow in the gut of chickens (Chambers et al 2002)
- Few longterm or multigeneration studies
  - No effects on reproduction and testicular development in mice or rats (Brake and Evenson 2004; Rhee et al. 2005)
  - No effects on animal health, feed intake, feed efficiency, laying performance, or hatchability,DNA-transfer and quality of meat and eggs of 10 generations of quails compared with the isogenic counterparts (Flachowsky et al. 2005)

Feeding trials – health - product safety

- Massive use in animal feeding: practically safe for animal feeds
- Problem of allergies in farm animals not significant compared to humans
- No recombinant DNA, either as whole gene or gene fragment in animal products, milk, meat and eggs
  - (except „contamination“ in milk, Agodi et al. 2006)

Composition of GM feedstuffs

- What will be the effects of the 2nd generation GM plants?
  - Substantial equivalence of isogenic and transgenic lines cannot be compared
  - Assessment will be more difficult to apply to 2nd generation GM plants due to deliberate changes in
    - Selected nutrients ↑
    - undesirable constituents ↓
    - Digestibility, bioavailability ↑

Cornerstones of evaluation?

2nd generation

- Modified nutritional properties
  - Protein enriched soya
  - Inclusion of different plant protein (eg. sunflower albumin in lupines)
  - Amino acid enriched crops, eg. methionine in lupines
  - Starch modification in cassava
  - Amylopectin starch in potatoes ↑
  - Inulin in potatoes

- Modified nutritional properties
  - Oleic acid in soya ↑
  - Lauric and myristinic acid in rape ↑
  - Phytate reduction
  - Carotene ↑
  - Enzyme expression: glucanase

Feeding trials

- 2nd generation plants may affect
  - Palatability, preference, feed consumption
  - Digestibility
  - Production (growth, meat, milk, wool)
- Concept of substantial/nutritional equivalence cannot be applied

GMO with specific functional properties

- „Pharma corn“
  - Proteins for treatment: cystic fibrosis, diabetes, hepatitis C
- Functional proteins
  - Rice: lactoferrin, lysozyme
- Antibodies against animal pathogens
  - E. coli antibodies in mice and pigs

(Chikwamba et al. 2002; Humphrey et al. 2002; Raemakers et al. 2005; Mößeler et al. 2006)

2nd generation GMO crops

- Nutritional parameters are crude measures in physiological terms
- Biological consequences of long-term exposure to certain GM crops not clear
- Much more delicate task to assess the risk as feed for food producing animals compared to the 1st generation

Flachowsky et al. 2005; Pusztai 2006

Tasks for future activities

- Composition
  - Nutrients, Genome, Proteome
- Bioavailability
  - Appropriate control diets ?
- Modification of nutritional contents in the gut
- Appearance in animal products, all aspects of product quality
- Protocols for the search for unintended effects

Future tests

- Animal health: target species, production conditions
  - Traits need to be defined, current proposals

    - Bioavailability of nutrients
    - Physiological body functions
    - Intestinal microflora
    - Immune system
    - Reproduction

- Young animals
  - Physiology
  - Pathohistology of intestinal and extraintestinal tissues
    - modifications of some nuclear features and cellular constituents in GM-fed mice (Malatesta et al. 2002a, b)
  - Bacterial flora of the gastrointestinal tract

Consequences - expectations

- What can be expected?

  - Feed quality will vary to a much greater extent in the future
  - Market transparency will be difficult to achieve
  - Traditional farming approaches will be difficult to maintain
  - Freedom of choice of farmers and consumers decreases
  - Advanced/expensive surveillance and control systems will be required: need for strictly separated distribution channels

Farm animals

- Adequate nutrition of farm animals will be more complex:

1. Characterization of raw materials
2. Purity assessment, certification?
3. Batch to batch variation?
4. Requirement for higher safety levels of nutrients in compound feeds?
5. International feed market?