Future challenges feeding transgenic plants

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Implications

• Commercial cultivation of genetically modified (GM) plants has been conducted for more than 20 yr. Over this period of time, studies on the feeding of GM crops to animals were also conducted to evaluate the potential impact.

• The evidence revealed that animal studies feeding GM crops did not present adverse effects. Genetically modified crops have been shown to have substantially equivalent composition compared with traditional counterparts. Scientific studies have not observed biologically relevant effects on feed intake, digestibility, or animal health or unintended effects on animal performance and fertility or on the composition and quality of food of animal origin. Recombinant DNA and newly expressed proteins of GM crops do not show other chemical/physical properties as native substances in non-GM counterparts.

• Looking to the future, conventional as well as novel plant breeding methodologies that contribute to a more resource-efficient production of high and stable yields of available plant biomass should be considered. Public funding in support of plant and animal research may contribute to a better understanding of the physiological, biochemical, and molecular processes and should be considered as an important challenge to meet the future demand for animal and human feed and food.

Key words: DNA-transfer, feeding studies, future challenges, genetic modification, plant composition

Introduction

Since humans made the transition from hunter-gatherers to agriculture, crops have been subject to anthropogenic selection in an effort to improve agronomic traits and nutritional quality. For almost 80 yr, diversity in agricultural crops has been promoted through indirect genetic mutation induced by exposure to radioactivity and/or chemicals. According to the FAO/IAEA, more than 3,000 plant mutants are registered; with more than 2,000 modified plants being used for food and feed production, of which 1,400 are major staples (Ahloowalia et al., 2004; Kharkwal and Shu, 2009). In the last three decades, advances in molecular biology have made target-oriented gene transfer across the species barrier possible. The majority of first-generation commercialized genetically modified (GM) crops have been engineered for enhanced agronomic performance through transformation with genes encoding either herbicide tolerance, pest resistance, or both (Flachowsky and Aulrich, 2001). The cultivation of GM crops has become the subject of global controversy over their safety, trade, regulation, and implications for the environment throughout all sectors of society.

In 1996, the first GM crops serving as major feedstuffs for livestock entered the North American market. These included herbicide-tolerant soybeans and canola and pest-protected corn. From 1996 to 2015, the cultivated area of GM crops increased more than 100-fold to 180 million ha globally (James, 2015). Regulations concerning GM plants were established by major international organizations prior to their commercialization, including the policy of substantial equivalence, which was first introduced by the Organization for Economic Cooperation and Development (OECD, 1993) and was adopted by both the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as the most appropriate regulatory framework (FAO/WHO, 2000). Substantial equivalence was based on comparison of GM plants to an appropriate conventional counterpart from which the GM line was derived. Once defined plant traits had been deemed equivalent between the two lines, the novel transgenic trait became the focus of the safety assessment. With the 20-yr anniversary, detailed information about commercial cultivation, the feeding qualities of GM crops for livestock, and their nutritional evaluation have been reviewed previously by academia (e.g., Flachowsky, 2013; van Eenennaam and Young, 2014; Nicolia et al., 2014; Smyth et al., 2015; Flachowsky and Meyer, 2015; Harvie, 2015; Watson and Preedy, 2015; Brookes and Barfoot 2015, 2016; Panchin and Tuzhikow, 2016; Qaim 2016) and scientific bodies (e.g., JRC, 2016; NASEM, 2016; The Royal Society, 2016) analyzing socio-economic effects of cultivation, related environmental aspects, and the impact on human and animal health.

Composition of Feeds and Definitions of Feeding Studies

Feeds are vital for animal husbandry to produce food of animal origin. Feeds can be defined as edible material(s) consumed as animals diets that

“None of our “common” foods and feeds were investigated nearly as intensively, regarding human, animal and environmental safety, as were their transgenic counterpart”

Flachowsky, 2013
deliver energy and/or nutrients such as amino acids, fatty acids, minerals, and vitamins. Currently, between 40 and 70% of the total cost of animal production is related to feed. Furthermore, feed production requires substantial amounts of limited natural resources such as water, land, fuel, and fertilizers. Therefore, feed efficiency or feed conversion (amount of feed per product of animal origin such as milk, eggs, meat, or fish) is the major determinant to assess feed utilization. The objectives of feeding studies with food-producing animals were to analyze animal health and welfare, to follow the pathways of transgenic DNA and of the newly expressed proteins, and to analyze the influence of GM crops on composition and quality of food of animal origin, such as milk, eggs, meat, and fish. Possible endpoints of such feeding studies to assess any risk are shown in Figure 1.

Proposals for feeding studies are given by various scientific boards (e.g., ILSI, 2003, 2007; EFSA, 2008, 2012) and groups of researchers (e.g., Ladics et al., 2010; Flachowsky et al., 2012). Apart from livestock feeding studies, in silico, in vitro, or in sacco studies may also contribute to further characterize the impact of GM crops on animal nutrition.

Feeding Studies with First-Generation GM Crops (Input Traits)

By definition, GM crops of the so-called “first generation” are rated as substantially equivalent in composition (plants with input traits) compared with their traditional counterparts, and they are regarded as safe for human and animal nutrition (Figure 2). Consequently, dietary components of first-generation GM crops do not impact the composition or the quality of food of animal origin compared with their isogenic counterparts.

During 20 yr of commercial GM crop cultivation, most available traits were aimed at providing herbicide resistance to crops (e.g., glyphosate resistance) or the protection of crops from insect damage (e.g., Bt-corn). Numerous feeding studies with first-generation GM crops (e.g., Reuter et al., 2002a,b) or by-products, including those with more than one modification (Parisi et al., 2016), were performed (recently summarized by Flachowsky, 2013; van Eenennaam and Young, 2014; Watson and Preedy 2015). No biologically rel-
relevant differences have been shown for nutritive values, animal performance and yields, or the composition and quality of animal source foods (Figure 2).

Other researchers (e.g., Swiatkiewicz et al., 2010; McNaughton et al., 2011a,b; Czerwinski et al., 2015a,b) tested diets consisting of more than one GM source. Czerwinski et al. (2015a), for example, investigated broiler chickens fed diets containing 96.2% GM soybean meal and maize but did not find any significant evidence on animal performance, blood parameters, or the functional status of the small intestine.

In conclusion, a number of reviews summarized (e.g., Flachowsky, 2013; Van Eenennaam and Young, 2014) that the feeds and foods from first-generation GM crops are safe and can be rated equivalent to their conventional counterparts as modified feeds revealed no significant impact on the composition and the quality of food of animal origin.

On the other hand, NASEM (2016) summarized evidence that modified insect-resistant crops are beneficial to human health by reducing negative impacts related to the use of insecticide and by decreasing the potential of mycotoxin-producing fungus to colonize GM crops.

In contrast, NASEM (2016) re-examined original feeding studies on the subject of substantial equivalence and concluded that the design and/or analysis of a number of animal feeding studies are controversial. Conversely, the majority of experimental studies provided reasonable evidence that animals were not harmed by consuming GM crops. Therefore, experimental designs of animal feeding studies should be further developed to overcome such weaknesses.

Feeding Studies with Second-Generation GM Crops (Output Traits)

Similar to the objectives of traditional and mutational breeding, “green” biotechnology has demonstrated the potential to alter the composition and nutritive value of GM crops. Biofortification of plants aims to increase the content of particular nutrients, such as amino acids, minerals, vitamins and other substances like enzymes; or aims to reduce the content of undesirable plant components such as lignin, phytates, or glucosinolates. In this regard, biofortification has the potential to fill gaps in the human food supply, particularly in developing countries with enduring deficiencies of essential nutrients.

Some controversial points of biofortified plants are summarized in Figure 3. On the other hand, from the perspective of animal nutrition, it may be argued that an urgent need for biofortification does not exist because traditional feed additives are available to complete animal rations (e.g., Pape, 2006; Flachowsky and Meyer, 2015). Based on the global imbalance of nutrients available at present, biofortification of crops might be more significant for food than for feed and most needed by consumers and farmers in remote areas with less intense livestock production systems. Lower content of undesirable substances may be of greater importance for food and feed.

Feeding GM crops of the second generation (with output traits) may influence the composition and quality of food of animal nutrition, particularly in the case of fatty acids. Fatty acids, minerals, and vitamins from biofortified plants may accumulate in various animal organs and tissues and/or be excreted via milk and/or eggs. Figure 4 shows the effects of biofortification (fatty acid enriched soybean) on animal performance and parameters of food quality. Recently, Albaugh (2016) reviewed the effectiveness and safety of Golden Rice and concluded that the β-carotene-enriched rice is efficient in preventing vitamin A deficiency. In comparison, the rice can provide similar levels to the supplementation of oil capsules containing pure β-carotene.
negative effects related to human health or safety were observed in the field study.

Additional data on the influence of feeding GM crops on composition and quality of food of animal origin was recently analyzed and described in more detail by Flachowsky (2017). NASEM (2016) did not discuss crops with expected “health benefits” (e.g., higher content of valuable constituents; lower content of undesirable substances) because such crops were introduced only shortly before writing their report and a commercial cultivation had not yet occurred on a large scale.

**Long-term and Multi-generation Studies**

Numerous long-term and multi-generation feeding studies with livestock reconfirmed the substantial equivalence of feeds and co-products of first-generation GM crops. Animal health and reproduction was not negatively impacted from the feeding of diets that contained GM crops. Studies at the Federal Institute in Braunschweig with laying hens and quails (Figure 5) confirmed data and conclusions summarized by others (e.g., Snell et al., 2012; Ricroch et al., 2013).

A 10-generation study was performed with growing and laying quail and did not show any significant effect of studied parameters. Scholtz et al. (2006, 2008) used these quails (Figure 5) for further generation studies and investigated metabolic and histological traits, weight of organs, biochemical blood parameters, histomorphometrical measurements of liver, etc., from the 17th to the 20th generation. Feeding of Bt maize (40/50% during grower/layer period; over 20 generations) did not elicit consistent and analogous alterations in any of the variables recorded.

Consequently, our results (Figure 5) on GM-fed quails were confirmed by Sartowska et al. (2015) in a subsequent 10-generation study with quail diets containing both GM soya bean meal (39%) and GM maize (25%).

In summary, comparing livestock health data from long-term feeding studies, before and after the introduction of first-generation GM crops, showed no adverse effects associated with GM crops. In addition, NASEM (2016) examined epidemiological data on the incidence of cancers and other human-health problems over time but found no substantial evidence that GM crops were less safe than food from conventional crops. Furthermore, the authors could not find persuasive evidence of adverse health effects directly attributable to consumption of GM feeds. There is evidence that GM insect-resistant crops may have been beneficial for human health by reducing the amount of crop-related insecticides and subsequent poisonings as well as by decreasing the concentration and exposure to mycotoxins.

**Fate of DNA and Newly Expressed Proteins**

Functional genes of recombinant DNA (deoxyribonucleic acid) are degraded into non-coding DNA fragments during ensiling (e.g., Hupfer et al., 1999; Reuter and Aulrich, 2003; Aulrich et al., 2004), feed processing (e.g., Gawienowski et al., 1999; Berger et al., 2003), and digestion in the digestive tract of animals (e.g., Alexander et al., 2007; Einspanier, 2013). As part of the digestion, food and feed are broken down, and along with other nutrients, fragments of degraded DNA molecules enter the intestinal epithelium and become available for absorption by the host organism. Evidence of low-copy, recombinant DNA fragments passing the intestinal barrier and entering the body of livestock remains obscure while endogenous multi-copy plant gene fragments have been detected in several tissue samples (Sanden et al., 2011). In previous studies, Einspanier et al. (2001) did not find recombinant gene fragments in animal body samples and milk. Later, many authors (see Einspanier, 2013; and Furgal-Dierzuk et al., 2014, 2015; Morera et al., 2016) confirmed our previous results. Korwin-Kossakowska et al. (2016) fed quails over 10 generations 39% GM soy bean meal or 25% GM maize but did not detect transgenic DNA in any consumable quail product.

![Figure 5](source: wikipedia.commons.org)

**Figure 5.** Summary of multi-generation studies showing equivalent performance for quails and laying hens fed diets containing genetically modified or conventional crops based on data from Halle and Flachowsky (2014), Flachowsky et al. (2005), and Scholtz et al. (2006, 2008).
Similar studies attempted to detect the newly expressed proteins. Both in vitro studies and feeding experiments (see Einspanier, 2013; Bukovac, 2011; Walsh et al., 2011; Li et al., 2015) demonstrated that the novel proteins are degraded during feed processing/conservation and in the digestive tract. As proteins are broken down into small fractions, the resulting indistinguishable peptides are likely to pass the intestinal epithelium as a nutrient source.

According to Einspanier (2013), the results concerning DNA and newly expressed proteins in the animal body can be summarized as follows:

- As part of the diet, DNA/gene fragments and proteins are present in the intestinal tract. Feed DNA residues are transferred and metabolized within the animal body in a natural process.
- The presence of feed DNA residues in animal tissues does not represent a safety risk to the animal or the human consumer.
- When ingested, DNA residues are found in organs. These foreign DNA fragments do not possess/encode any biological function and do not account for apparent effects in the animal, nor have they been found to be integrated in the animal genome.
- When reviewing all available data, there is scientific evidence that milk, meat, or eggs derived from animals fed with GM crops are as safe for the human consumer as those produced with conventional feed.
- In conclusion, no scientific evidence exists to support that recombinant DNA and newly expressed proteins show other chemical and physiological properties in animals than any other conventional product.

The results shown and discussed above are summarized in Table 1. In agreement with our data, Nicolia et al. (2014) and Panchin and Tuzhikov (2016) analyzed 1,783 published articles on GM crops but did not find any significant differences related to food safety comparing genetic modified and conventional crops. Guillemaud et al. (2016) are skeptical about possible connections between researchers and the GM crop industry. They analyzed 672 papers focusing on the efficacy or durability of GM crops producing toxins and considered conflicts of interest for about 40% of the articles. According to the authors, a direct interaction between GM crop companies and the researchers cannot be excluded. Such possible interactions should be avoided in the future.

### Future Challenges

According to the FAO et al. (2014) and FAO (2016), the human population will increase globally from about 7.5 billion to more than 9 billion people in 2050; however, the production of food of animal origin is estimated to increase by approximately 70% (HLPE, 2014). The increase in human population and the need for feed and food will progressively translate into a growing demand for limited natural resources (as first nutrient and most vital, clean water) as well the elevated emissions of greenhouse gases such as carbon dioxide, methane, nitrous oxide, and trace elements (e.g., nitrogen and phosphorus). Such changes currently are and will continue to be a characteristic situation for the entire globe (e.g., Guillou and Matheron 2014; Harvie, 2015. NRC 2015). “More (food) for more people with less (limited resources and emissions)” could be a headline to describe the present situation and the challenges ahead for agricultural sciences. Malnutrition in all its forms (malnutrition, micronutrient deficiencies, e.g., concerning iron, iodine, vitamin A, etc.) and obesity—the so-called “triple burden” of malnutrition—are still recognized as a serious and intractable problems of humanity (HLPE, 2014; Thompson and Amo-rosico, 2014). The latest FAO et al. (2014) and FAO (2016) publications indicate that about 800 million people are still chronically undernourished (11.3% of global population). Many more people suffer from micronutrient deficiency. Food of animal sources has the potential to contribute to the prevention of micronutrient deficiencies. Currently, another major focus is the adaptation of crops to address climate change (e.g., Reynolds, 2010; Newman et al., 2011; Flachowsky and Meyer, 2015; Bedada et al., 2016) and to improve and/or to stabilize crop yields and the nutritive value for global food security (Fischer et al., 2014). The focus, however, on current plant breeding objectives does not contribute sustainability to global food security as summarized in Table 2.

### Table 1. Summary of published data in peer reviewed journals comparing feeds from GM plants of the first generation (with input traits) with their isogenic counterparts in food-producing animals (for details see Flachowsky 2013).

| Animals (Species/categories) | Number of experiments | Nutritional and safety assessment |
|-----------------------------|-----------------------|----------------------------------|
| Ruminants                   |                       |                                  |
| Dairy cows                  | 23                    | No unintended effects in composition of GM crops compared to conventional counterparts (except lower mycotoxin concentrations in GM crops expressing Bt-toxins) |
| Beef cattle                 | 14                    |                                  |
| Others                      | 10                    |                                  |
| Pigs                        | 21                    | No biologically relevant effects on feed intake, digestibility or animal health, and no unintended effects on the performance and fertility of animals or on the composition and quality of food of animal origin. |
| Poultry                     |                       |                                  |
| Laying hens                 | 11                    | Recombinant DNA and newly expressed proteins in GM crops show the same chemical/physical properties as native substances |
| Broilers                    | 32                    |                                  |
| Others                      | 2                     |                                  |
| Other animal species (Fish, rabbits etc.) | 9 |                                  |

### Table 2. Assessment of present modifications of plants from the view of food safety, food security and sustainability of food production.

| Objectives of plant breeding | Present significance by plant breeders | Contributions to | Food safety | Global food security | Sustainability of food production |
|------------------------------|----------------------------------------|------------------|-------------|----------------------|----------------------------------|
| More tolerance against herbicides | 0                                     |                   |             |                      |                                  |
| More resistance against insects etc. | ~                                     |                   |             |                      |                                  |
| More valuable ingredients    | 0                                     |                   |             |                      |                                  |
| Less undesirable ingredients | 0                                     |                   |             |                      |                                  |
| More efficient use of non-renewable resources (water, fuel, nutrients, area etc.) | ~ not important. |                   |             |                      |                                  |

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Challenges plant breeders may face in the future include tolerance of plants against biotic and abiotic stresses such as drought, salt content of soils, and a more efficient utilization of carbon dioxide from the atmosphere and other plant nutrients such as nitrogen. Biofortification of crops may be an important objective for plant breeders to avoid and overcome human micronutrient deficiencies in many regions of the world. Such crops should have high and stable yields, and they should grow with minimal need of globally limited resources such as arable land, water, fuel, and some minerals. Our major focus should be to make the most efficient use of the unlimited resources such as plant nutrients in the atmosphere, solar energy, and the available genetic pool (see Table 3). To accommodate future needs and demands, we likely will face the challenge of crops with staggered modifications (Parisi et al., 2016).

As mentioned before, most of the general expectations are also important for plants used for animal feeding. Considering present challenges, an ideal feed crop can be depicted by the following goals:

- High and stable yields of highly digestible biomass with low external inputs (low-input varieties) of non-renewable external resources such as water, minerals, fossil fuel, plant protection agents, etc. (see Table 3).
- Maximum and efficient use of naturally unlimited resources such as solar energy and nitrogen and carbon dioxide in the atmosphere (see Table 3).
- Higher resistance against abiotic and biotic stressors such as drought-tolerant plants (Bedada et al., 2016).
- Stable plant health and adaptation to current climate change.
- Low concentrations of anti-nutritive (toxic) substances such as secondary plant ingredients, mycotoxins from toxin-producing fungi, and toxins from anthropogenic activities or geogenic origin.

### Table 3. Potentials to produce phytoenic biomass and their availability per inhabitant under consideration of population growth.

| Plant nutrients in the atmosphere (N₂, CO₂) | ↑ ↔ |
|-----------------|------|
| Solar energy    | ↔   |
| Agricultural area | ↓  |
| Water           | ↓   |
| Fossil energy   | ↓   |
| Mineral plant nutrients | ↓ |
| Variation of genetic pool | ↑ |

(↑Increase or unlimited, ↓ Decrease or limited, ↔ no important change)

### Table 4. Objectives of inputs and outputs of future plant breeding (NASEM 2016).

| Plants with input traits | Plants with output traits |
|--------------------------|----------------------------|
| Biotic stress tolerance  | Higher nutrient content    |
| Microbiological resistance, resistance against insects | Amino acids, trace elements, vitamins, fatty acids, non-essential ingredients etc. |
| Abiotic stress tolerance | Higher food and feed safety |
| Drought resistance, increased water utilization efficiency, cold, heat and salt tolerance | Lower content in mycotoxins and further undesirable ingredients |
| Nutrient intake and utilization | Higher nutritive value |
| N, P, CO₂, trace nutrients | High feed intake and high digestibility |
| Post-harvest behavior    | Biofuel and industrial crude products |
| Microbiological resistance, improved storage properties, increased silage quality | High yield, improved properties of bio-fuel, use of by-products as valuable feeds |

Conclusions

Since humans advanced from the era of being hunter-gatherers and left their caves, plant selection and breeding can be considered as the starting point of the man-made food chain and a major foundation of our current societies. Safeguarding the physiological needs of the global population now and in the future will remain one of our greatest challenges. The real and significant challenges for future generations of plant breeders and sustainable crop production are:
1. highly digestible yields combined with low external inputs of non-renewable resources;
2. minimizing greenhouse gas emissions during cultivation;
3. increasing resistance against biotic and abiotic stressors including adaptation to climate change; and
4. reducing the concentration of undesirable substances in crops.

Twenty years of commercial cultivation of GM crops allow the following conclusions:

- Feeding studies with GM crops did not show unintended effects compared with conventional counterparts.
- There is no biologically relevant effect on feed intake, digestibility, or animal health.
- There is no unintended effects on the performance and fertility of animals or on the composition and quality of food of animal origin.
- Recombinant DNA and newly expressed proteins in GM crops do not show other chemical/physical properties than native substances.
- More education is necessary to inform human consumers about the benefits and advantages of modern plant breeding for global food security.
- The designation of financial resources and scientific expertise to educate and translate plant and animal research may contribute to a better understanding of physiological, biochemical, and molecular processes of these important traits as well as the challenges of meeting future animal and human protein demand (NRC 2015).

The question “GM or no GM” cannot be answered with a simple “Yes” or “No.” Any combination of all plant breeding methods that contribute to food/feed security and safeguarding the environment should be considered.

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