

Feed nutrition evaluation

Working Group "Nutritional aspects of genetically modified feeds": Eddy Decuypere, Remi De Schrijver, Leonel Fiems, Robert Renaville, Marcel Vanbelle; Secretariat: Ellen Van Haver

1 Introduction

There has been a permanent selection for thousands of years to create new varieties of plants to supply better feeds for animal nutrition. Selection purposes may result in an increased dry matter yield per hectare, an altered dry matter composition, an improved organic matter digestibility and consequently an increased energy value, a reduction of the presence of anti-nutritional factors or a better resistance against diseases or unfavourable environmental conditions.

During the last decades, crops for livestock feeding were increasingly developed based on genetic engineering, where foreign DNA fragments with specific characteristics were inserted into the genome. The safety of these genetically modified organisms should be assessed for both livestock feeding and human nutrition. Additionally, it has to be evaluated if the DNA of inserted or modified genes (such as antibiotic resistance genes), or their products, can cause detrimental effects, if transferred into animals, or if the proteins can accumulate in the end products (milk, meat, eggs...) of animals which are fed with these novel feeds.

This chapter describes in first instance the data and information needed for the compositional analysis of the feed derived from the genetically modified crop. Next, the possible implications of genetically modified crops in the animal diet as outlined in this chapter should be considered to determine which studies are necessary for the further feed safety assessment.

2 Compositional analysis

2.1 Compositional data and methods

This section has to present the proximate analysis of the matter, to describe the sampling procedure, to refer to the analysis methods and to precise the statistical distribution of the results.

2.1.1 Major and minor constituents

A non-exhaustive checklist as presented hereafter provides information on critical parameters of feed safety and nutrition. Depending of the crop and/or derived feed product to be considered, several components may be not relevant.

Checklist for proximate composition analysis

- Moisture % of wet weight
- Protein % of dry matter (DM)
- Total fat % of DM
- Crude fibre % of DM
- Total ash % of DM
 - soluble ash
 - insoluble ash
- Other carbohydrates (nitrogen-free extractives) % of DM

In recent years the proximate analysis procedure has been replaced by other analytical procedures. Alternative procedures for fibre have been developed (Van Soest):

- Neutral Detergent Fibre (NDF), eNDF, peNDF
- Hemicellulose
- Acid Detergent Fibre (ADF)
- Lignin (ADL)
- Cellulose

Also the carbohydrate methodology has been revised:

- Non-structural carbohydrates (NSC): sugars, starches, fructans, galactans, pectins, -glucans, etc.
- Non-starch polysaccharides (NSP): NSC minus starch and sugars

Protein can also be specified:

- NPN (non-protein nitrogen) % of DM
- Amino acids % of DM and % of total amino acids
 - Essential and semi-essential amino acids: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and others according to the species of monogastric animals
 - Non-essential amino acids: alanine, asparagine, aspartic acid, cysteine, cystine, glutamic acid, glutamine, glycine, proline, serine, tyrosine

Triacylglycerols (triglycerides), which make up the major fraction of usual dietary fats, are characterised by the identity, the position and the combination of the fatty acids they contain.

Conjugated linoleic acids are synthesized from linoleic and linolenic acid in the fore-stomachs of ruminants, and from vaccenic acid in adipose tissue in growing ruminants and mainly in the mammary gland in lactating ruminants, or in the liver of other species; they are secreted in the milk and deposited in the meat. Anti-carcinogenic and other properties of these compounds have been reported.

Table 1: Classification of fatty acids (% of total fat)

Saturated fatty acids	Unsaturated fatty acids		
	n-9 family	n-6 family	n-3 family
Butyric acid	Oleic acid	Linoleic acid	-linolenic acid
Caproic acid	Erucic acid	-linolenic acid	Eicosapentaenoic acid (EPA)
Caprylic acid		Arachidonic acid	Docosapentaenoic acid
Capric acid			Docosahexaenoic acid (DHA)
Lauric acid			
Myristic acid			
Palmitic acid			
Stearic acid			
Aracidic acid			

- Mineral composition and trace-elements
 - Minerals (g/kg): Ca, Cl, K, Mg, Na, P, S
 - Trace-elements (mg/kg): Co, Cu, Fe, I, Mn, Mo, Se, Zn
- Vitamins
 - Fat soluble vitamins: Vitamin A (retinol) ($\mu\text{g}/100\text{g}$), Vitamin D₃ (cholecalciferol) ($\mu\text{g}/100\text{g}$), Vitamin D₂ (ergocalciferol) ($\mu\text{g}/100\text{g}$), Vitamin E (-tocopherol) (mg/100g), Vitamin K (phylloquinone) (mg/kg), -carotene (mg/kg)
 - Water soluble vitamins: Vitamin B₁ (thiamine) (mg/kg), Vitamin B₂ (riboflavin) (mg/kg), Vitamin B₆ (pyridoxine) (mg/kg), Niacin (mg/kg), Pantothenic acid (mg/kg), Folic acid (mg/kg), Biotin (mg/kg), Vitamin B₁₂ (cobalamin) (mg/kg), Vitamin C (ascorbic acid) (mg/kg)

2.1.2 Analytical methods

Reference methods must be used and mentioned. European standardized validated methods will be preferred but other official methods will be considered. Depending on the feed involved, appropriate and currently available methods are used. See Chapter VI for an extended description of analytical methods.

2.1.3 Statistical and sampling aspects

The sampling method must be explained and must take into account the requirements linked to the statistical analysis as well as the distribution of the components in the raw material.

A very important point to consider is the variability of the raw material for example by taking into account the impact of the geographical origin, the climate, the agronomic practices, the annual variations... Enough samples are to be analysed with the help of a sampling plan and the results are to be evaluated on a statistical basis.

Plants used to obtain samples for compositional analysis should be grown under conditions that represent normal practice for the crop plant. For example, studies on herbicide tolerant crops should be done on herbicide treated crops (with a waiting period afterwards). If the transgenic plant inactivates the herbicide, (metabolised) degradation products might be present in the plant.

2.2 Nutritional aspects

Whenever changes are made to the way in which a feed is produced or processed, the implications on the nutritional value require consideration. Information will be needed on any issue relating to this aspect. Feeds are usually complex mixtures of macro- and micronutrients, which provide energy and nutrients and contribute to animal welfare.

2.2.1 Identification of key nutrients

If a genetically modified crop is expected to have an important role in the diet, then appropriate information on nutritional composition is needed. Both macro- and micronutrients of nutritional value are already given. It is clear that not all these nutrients are relevant for every specific genetically modified crop. For every such crop, the place (value) within the animal diet should be determined. It is well known that different feed groups contribute in different ways to animal feeding. Depending on the composition and the (estimated) consumption of the genetically modified crop, it appears justified to limit the testing to the most relevant nutrients, which are specified in Table 2. This table should be considered as an example and not as an exhaustive list.

Table 2: Identification of relevant nutrients for different feed groups

Feed group	Key nutrients
Grass and forage crops	Energy, protein, fibre, vitamins, minerals and trace elements
Dried forages and straw	Fibre, minerals and trace
Silages	Energy, protein, fibre, minerals and trace elements
Roots tubers and related by-products	Energy, minerals and trace elements
Cereals and related by-products	Energy, minerals and trace elements
Protein concentrates	Protein, energy, minerals and trace elements
Vitamin and trace mineral premixes	Vitamins, minerals and trace elements

2.2.2 Intake

High-performing animals require a high feed intake. Feed intake capacity may not be deteriorated by the use of genetically modified feeds in the diet. As it may not be possible to predict such events, a surveillance programme should accompany the marketing of a genetically modified feed. Such a programme should encompass information on changes in the conditions for processing and preparation as well as effects of possible replacement of other feeds or feed component of dietary importance. If surveillance reveals changes in those factors, which raise concerns regarding wholesomeness, a reappraisal of the acceptability of the genetically modified feed would be required.

2.2.3 Digestion

Genetically modified feeds in animal diets come into close contact with the host within the digestive tract. Different digestive processes occur between the categories of livestock husbandry, due to different anatomical and enzymatic aspects of the digestive tract.

Mechanical, chemical and microbial activities are involved in the digestion process. Large differences exist between monogastric and ruminant animals, mainly with regard to the microbial digestion. Extensive microbial digestion occurs in the rumen, while this is restricted to the large intestine in monogastric animals, but in the latter this process is of less nutritional importance.

In monogastric animals carbohydrates are broken down by enzymes to monosaccharides and actively transported to the liver. Protein digestion results in the formation of free amino acids and peptides. Amino acids pass into the portal blood and then to the liver. Ingested DNA and RNA are rapidly cleaved into small fragments by pancreatic and gastro-intestinal enzymatic digestion and acid hydrolysis in ruminants (McAllan, 1980; Flint and Thomson, 1990). D'Mello (1982) concluded that there is an extensive catabolism of exogenous bases from nucleic acids, but the salvage of preformed purines and pyrimidines occurs widely in non-ruminant animals. The enzymes involved in DNA hydrolysis include high concentrations of DNase I. This endonuclease, with an optimal activity at neutral pH, disrupts the double stranded DNA and is produced and secreted by salivary glands, as well as the pancreas, the liver and the Paneth cells of the small intestine. DNase II, with a pH optimum of between 5.2 to 6.4, is also secreted but its primary function is in lysosomes within phagocytes, involved in the catabolism of DNA as well as the fragmentation of genomic DNA during apoptosis (Yamanaka et al., 1974). However, with more accurate analytical techniques, the degradation of DNA may be re-evaluated. Tests made with simulated gastric and intestinal conditions have confirmed that protein products of the genes introduced into current commercial crops are as rapidly degraded as other dietary proteins (Harrison et al., 1996; Wehrman et al., 1997). Nevertheless, protein fragments of Cry9C, a bacterial lectin, which, in common with a sub-group of other plant and microbial lectins and protease inhibitors, are highly resistant to proteolysis (Peferoen, 1998). With regard to transformation of DNA from genetically modified feeds, it seems likely that the sites preceding the acidic stomach, i.e. the mouth, the oesophagus, the rumen and the avian crop, might see the highest concentration of intact DNA entering with the feed. McAllan (1982) estimated that more than 85% of the plant DNA consumed by ruminants is reduced to nucleotides or smaller constituents before entering the duodenum, with most of the larger nucleic acid fragments in the small intestine arising from rumen microbes. In addition to enzymatic digestion, low pH conditions in the stomach or the abomasum should remove most adenosine and guanine from naked DNA.

The digestion of fibre takes place in the large intestine, due to microbial activity. However, this digestion is small compared with the microbial activity taking place in the rumen. Triacylglycerols are broken down into partial glycerides and free fatty acids. These are incorporated into micelles and absorbed from the jejunum, involving bile salts. Following absorption, there is a resynthesis of triacylglycerols. They are formed into chylomicrons, which then enter the thoracic duct. It is possible to vary fatty acid composition of body tissues by altering the composition of dietary fat.

In ruminants, carbohydrates are broken down to simple sugars by the rumen flora. These simple sugars are immediately fermented to carbon dioxide, hydrogen, methane and various volatile fatty acids (VFA), depending on the composition of the diet. In the case of starch, part of it may be stable and by-pass rumen fermentation. This undegraded starch is then digested in a similar way as in non-ruminant animals. Proteins are also degraded in the rumen with peptides and ammonia as end products. When fermentable organic matter (FOS) is sufficiently available in the

rumen, ammonia is used for microbial protein synthesis. Otherwise, part of the ammonia is lost for the animal through excretion via the urine. Part of the feed protein may escape microbial degradation, depending on feed origin, processing and feeding level. This protein is then similarly digested as in non-ruminant animals. The rumen flora also enables protein synthesis from NPN.

Lipids are to a large extent hydrolysed in the rumen by bacterial lipases and unsaturated fatty acids are hydrogenated afterwards. Short-chain fatty acids are absorbed directly from the rumen, while long-chain fatty acids reach the small intestine. The formation of micelles and the absorption of long-chain fatty acids are dependent on the conjugated bile salts and the phospholipids present in bile. In contradiction with monogastrics, the predominant fatty acid of adipose tissue is stearic acid, resulting from rumen hydrogenation. However, fat can be protected, so that it bypasses the rumen and modifies body and milk fat.

In horses most microbial digestion takes place in an enlarged colon. Some microbial activity occurs in the avian crop. Rabbits take advantage of the hind-gut microbial fermentation by practising coprophagy.

2.2.4 Nutritive value

The nutritive value is obtained as a result of chemical composition and digestibility. It is mainly determined by the energy and protein value.

- o Energy value
 - Net energy lactation (VEM/kg DM for dairy cattle)
 - Net energy fattening (VEVI/kg DM for beef cattle)
 - Net energy (kcal/kg DM for pigs)
 - Metabolizable energy (kcal/kg DM for poultry)
- o Protein value
 - Protein digestible in the small intestine (DVE; g/kg DM for ruminants)
 - Rumen degradable protein balance (OEB; g/kg for ruminants)
 - Digestible crude protein (g/kg DM for pigs and poultry)
- o Structural value (only for ruminants)

2.3 Anti-nutrients and toxicants

Information is requested with respect to the presence of anti-nutrients. This applies particularly to the key anti-nutrients for the product. The examples given below are to be considered as examples and not as an exhaustive list.

Naturally occurring toxins that are inherently present in the plant should be determined. Data on the sensitivity of the crop towards the formation of mycotoxins, pathogenic microorganisms, biogenic amines and other toxic substances or organisms formed in the product have to be given, if relevant.

2.3.1 Examples

- o **Protease inhibitors** inhibit the activity of trypsin, chymotrypsin and other proteases. They are found in legumes such as beans and peas, but also in

cereals, potatoes and other products. Their presence results in impaired growth and poor feed utilisation.

- **Amylase inhibitors** have a similar activity against amylases.
- **Lectins or hemagglutinins** are glycoproteins mainly found in legumes: beans, peas, and lentils. They bind to intestinal epithelial cells. They cause agglutination of erythrocytes in vitro. Their presence results in poor food utilization and impaired growth.
- **Cyanogens** are cyanogenic glucosides found in cassava, linseed, peas, beans and other products. They may cause HCN poisoning.
- **Glucosinolates** are thioglycosides found in rapeseed meal and related species. Effects upon the thyroid function have been demonstrated.
- **Saponins** are also glycosides found in soybeans, lucerne, sugar beet and others. Haemolytic effects in vitro have been shown. Saponins may result in bloat in ruminants due to the formation of stable foam in the rumen.
- **Alkaloids** occur in certain plants. They are of particular interest since many of them have poisonous properties.
- **Gossypol** is particularly important in cottonseed. Several toxic effects have been demonstrated.
- **Phytic acid** occurs in several vegetable products. This compound has a strong chelating activity. Its presence may affect bioavailability of minerals.
- **Mycotoxins** are very diverse in chemical structure and in the characteristics of the mycotoxicoses they produce. These toxins include the aflatoxins, the tricothecenes, the fumonisins, zearalenone, moniliformin and fusaric acid.
- **Phytotoxins** (solanine, etc.).

2.3.2 *Potential effects of cultivation conditions and processes*

If relevant for the particular feed, information is requested about the presence of the anti-nutrient and particularly about the quantity. Moreover this information should include data about the potential effect of different cultivation conditions. In addition it is very well established that processing may have profound effects upon the level of anti-nutrients present. Two approaches at least have to be followed:

- the effect of inactivation processes
- the effect of separation processes.

In order to have a real picture of these effects, the commonly applied processing steps have to be followed. The use of flow sheets is highly recommended.

Inactivation studies under conditions equivalent to normal processing may give information about the stability of the anti-nutrient. Inactivation may be due to heat treatments, enzymatic activity, leaching or others.

Separation processes like dry milling, wet milling, extraction, centrifugation or equivalent may affect the level of anti-nutrients. In this case it is easily understood that information on the localisation of the anti-nutrient is of great help, such as the distribution in endosperm, aleurone layer, bran or germ for cereals. Data about the presence of the particular anti-nutrient in fractions for feed use are necessary.

2.4 **Secondary plant and bacterial metabolites**

Secondary plant metabolites are neither nutrients nor anti-nutrients. They are part of the characteristic composition of a plant. They are important for the compositional

analysis and for the comparative approach. Even more than in the previous paragraphs, an exhaustive list cannot be given.

Some of these substances may have undesirable effects, others may have beneficial effects.

2.4.1 Examples

- **Phenolic compounds** are considered to be of great importance. Detailed information about the qualitative and quantitative composition of the phenolic fractions is necessary.
- **Phyto-estrogens** naturally occur in soybeans and clover species.
- **Key enzymes** may affect the utilisation of the plant material. Information about the relevant enzymes is necessary.
- **Organic acids** are another group. This includes:
 - aliphatic plant acids like citric and malic acid and others,
 - aromatic acids like benzoic acid and analogues,
 - phenolic acids like caffeic, coumaric, ferulic acids and others.
- Additional information on **N substances**, if not covered in a previous section, is requested. This includes low molecular N substances, unusual amino acids and others.
- **Biogenic amines** are produced as a result of proteolysis during silage preservation and may induce physiological effects when toxic amounts are consumed.
- **Carbohydrates** are mainly covered in section 2.1.1 (major and minor constituents). In addition to simple sugars and polysaccharides, complex sugars such as raffinose, stachyose and verbascose have to be covered in this section.
- With respect to **lipids**, information not covered in Table 1 is requested. This includes complex lipids and others.

2.5 Derived products

Processing may have a pronounced effect upon the content and distribution of nutrients and anti-nutrients.

This aspect is, by preference, covered by means of flow sheets indicating the major steps in the processing scheme. As the global and detailed composition is already dealt with in the previous sections, further information on particular nutrients and anti-nutrients is necessary if this information is essential for the assessment of the product.

As an example it is felt that when discussing soybeans some aspects related to inactivation of anti-trypsin factors during toasting have to be included.

3 Implications of genetically modified crops in animal diets

3.1 Effects on animal performance and animal health

As regards genetically modified crops subdivision into two classes can be made between first and next-generation plants (see Introduction, Chapter I). Agronomic characteristics are mainly improved in first-generation plants, while changes in the content of major or minor constituents are intended by next-generation plants. Results from several experiments indicate that the current genetically modified feeds are substantially equivalent to their conventionally counterpart in composition, are

similar in digestibility, and have a similar feeding value for livestock (Clark and Ipharraguerre, 2001). Genetic engineering may however lead to enhanced levels of some essential amino acids and increased nutritive values (Molvig et al., 1997). Other studies showed that the nutritional value can be improved through recombinant techniques, resulting in improved daily gain and feed efficiency as reported by von Wettstein et al. (2000) for broiler feed. Furthermore, the number of chickens with adhering sticky droppings was drastically reduced. Panaccione et al. (2001) reported that alkaloid toxicoses in livestock could be reduced by genetic modification of the endophyte responsible for ergovaline production in perennial ryegrass. Piva et al. (2001) reported that pigs fed Bt maize had 2.8% higher final weights compared to isogenic maize, which may be attributed to lower levels of fumosin (69%) and deoxynivalenol (14.4%). On the other hand Leeson (1998) reported an increased mortality of male broilers fed with genetically modified maize. Ewen and Pusztai (1999) reported that snowdrop lectins in genetically modified potatoes altered the mucosa of the gastrointestinal tract of rats.

However, if a genetically modified feed is expected to have an important role in the animal diet, then appropriate assessment data are needed. Attention should be paid to the particular physiologic characteristics and metabolic requirements. Information will be needed on long term as well as on short-term effects of the consumption of the genetically modified feed.

Nucleotides are generally abundant in feed. Assuming that 85% of plant DNA is degraded before entering the small intestine (McAllan, 1982), a small proportion of the plant or microbial DNA fragments could potentially be absorbed from the digesta through the intestinal mucosa, either directly by epithelial cells or by antigen presenting cells of the immune system. Beever and Kemp (2000) suggested that most of this DNA is phagocytised by tissue macrophages. Nevertheless, it is conceivable that micro-environments exist where DNA is not degraded. Klotz and Einspanier (1998) reported the detection of a plant DNA fragment in white blood cells of a cow fed a diet containing genetically modified soybean meal. In other studies (Doerfler et al., 1997; Schubbert et al., 1994; 1997; 1998) viral DNA not only survived the passage through the gastrointestinal tract of mice, but was detected in host tissues (see also annex 1 to this chapter).

Gene transfer between bacteria is very extensive in natural ecosystems, so that it is thought that any transfer of transgenes may be negligible. Rare transfer events can have an enormous significance and can be amplified very rapidly under favourable circumstances. With regard to transgenes, pertinent questions are whether the release of a modified organism is likely to create a new route of acquisition of novel genes by organisms that are unlikely to have been able to acquire them naturally, and whether such acquisition could have detrimental consequences.

There are several reasons to assume that the rumen environment is more favourable for inter and intraspecies gene transfer (Forano and Flint, 2000). The microbial population in the rumen is very dense. The bacteria live in adhesion with feed particles, so that it is likely to have a permanent contact with exogenous free DNA. Furthermore, plasmids and bacteriophages have been found in rumen bacterial species.

Fragmentation of DNA is affected by feed processing. Chiter et al. (2000) reported that temperatures of 95°C or above for more than a few minutes are sufficient for degradation of DNA to take place to the extent that it should be incapable of transmitting genetic information. Furthermore, they mentioned that chemical

expulsion and extrusion of oilseeds resulted in residues with completely degraded genomic DNA. For feeds that are not subjected to high temperatures, such as wet sugarbeet pulp silage and cereal grain, is intact DNA still present and potentially taken up by microbes in the digestive tract (Chiter et al., 2000). This is in agreement with earlier findings of Hupfer et al. (1999), where amplification of a 211 bp sequence of the cry1A(b) gene was detected in maize seven months after ensiling. Duggan et al. (2001) concluded that free DNA from transgenic maize could survive in rumen fluid and saliva under in vitro condition. Extended exposure of maize grain to steepwater could result in some DNA degradation (Gawienowski et al., 1999).

There is no evidence to date that feeding genetically modified crops has detrimental effects on animal welfare. However, it can not be completely excluded, if foreign DNA is partly absorbed.

On the other hand, genetically modified plants may exert a positive effect on health: reduced fumosin concentrations may be expected in transgenic Bt-maize due to a decreased incidence of Fusarium ear rot (Munkvold et al., 1997 and 1999). Gregg et al. (1998) reported markedly reduced toxicological symptoms after fluoroacetate poisoning in sheep inoculated with ruminal bacteria, transformed with a gene encoding fluoroacetate dehalogenase.

The agro-industrial processing of genetically modified plants may increase transgenic protein in the involved end-products. For instance, the protein content in full fat soybeans used in ruminant diets may increase from ± 400 g crude protein per kg DM to about 900 g in soy isolates used in milk replacers.

Many proteins are degraded by heat. However, Peferoen (1998) found no effect of a heat treatment at 90°C for 10 minutes on Cry9C activity. Van Wert and Noteborn (1999b) have shown that the Cry9C protein is resistant to the digestive enzymes from the stomach (pepsin) and the pancreas (trypsin), besides its heat resistance. Faust (2000) reported the detection of transgenic protein in soybean meal and maize silage.

3.2 Effects on the quality of end products

Data dealing with the effect of genetically modified crops in animal nutrition on the quality of the end product are rather scarce. Effects on milk composition are reported in the literature (Vilotte et al., 1997), but this is a consequence of the use of transgenic animals rather than feeding of genetically modified crops. Faust (2000) reviewed the literature on the composition and detection of transgenic protein and DNA in a range of livestock products. It was concluded that transgenic protein and transgenic DNA had not been found in milk, meat and eggs. Ash et al. (2000) neither found genetically modified protein in whole egg, egg white, liver and faeces of laying hens. Einspanier et al. (2001) reported that only short DNA fragments (<200 base pairs) derived from plant chloroplasts could be detected in the blood lymphocytes of cows. In all other cattle organs investigated (muscle, liver, spleen, kidney) plant DNAs were not found, except for faint signals in milk. However, in all chicken tissues (muscle, liver, spleen, and kidney) the short maize chloroplast gene fragment was amplified. In contrast to this, no foreign plant DNA fragments were found in eggs. Bt-gene specific constructs originating from recombinant Bt-maize were not detectable in any of these poultry samples either (see also annex 1 to this chapter).

4 Feed safety assessment

As described in Chapter 1, three outcomes of the comparative approach can be considered (FAO,1996). The first category is this, which have the same composition as the parent crop (substantially equivalent). The second class has the same composition as the parent crop with the exception of a well-defined trait (substantially equivalent apart from defined differences). Finally, there are genetically modified crops, which are different from the parent crop (not substantially equivalent).

The concept of substantial equivalence has been accepted as a useful framework for the hazard assessment of genetically modified feed. Data for comparison should take into account agronomic, molecular and compositional aspects and the need for further studies depend on the nature of the differences and whether or not they are well characterised. Anti-nutritive factors should be determined when an altered composition is envisaged by the genetic modification. Nutritive value can be screened with in vitro tests. However, in vivo digestibility trials and balance studies in target animal should confirm results of in vitro studies. Long term feeding experiments, with ad lib. feeding of diets containing a high content of the genetically modified feed, are necessary to verify if there is no harmful effect on feed intake capacity, growth rate, feed efficiency, or yield of milk or eggs, animal health, reproduction, quality of end products and fate of modified protein and /or DNA. Special attention is necessary in case of young animals, which are more susceptible to deleterious effects because of an immature immune system.

Unintended effects are considered to be consistent differences between the genetically modified crop and its conventional counterpart, which go beyond the primary expected effect(s) of the introduced target gene(s). There remains a remote possibility that unintended effects in the plant are not detected by the approach of substantial equivalence. Novel methods and concepts are needed for the safety evaluation of next generation genetically modified crops to probe into compositional, nutritional, toxicological and metabolic differences between genetically modified and conventional crops.

Integrity of recombinant DNA should be studied with regard to processing (heating, flaking, crushing, ensiling,...). Industrial by-products of genetically modified crops involved in animal feeding should be subjected to a similar investigation as genetically modified crops.

A decision tree for the nutritional assessment can be recommended (see Annex 2 to this chapter), as proposed by Flachowsky et al. (2002).

4.1 Nutritional assessment of genetically modified feeds from a physiological point of view

The many manufactured feeds for animals make use of the same crops (or by-products of the same crops) used for human food. The safety assessment of animal feeds must take into account any risk to the animals consuming the feed and any indirect risk to the consumer of animal products (meat, milk and eggs).

An important question in this discussion concerns the definition of “safety of genetically modified feeds for animals”. A much wider range of factors than merely the aspect of “safety for animals” has to be taken into consideration but environmental aspects (biodiversity, soil, water), antibiotic resistance and human beings as consumers of these foodstuffs of animal origin should be included. As it is unlikely that any introduced protein will become directly incorporated into animal products, it is not considered necessary to test routinely for the presence of introduced genes or their products unless their characteristics suggest cause of

concern. The evaluation of the environmental aspects is beyond the scope of this report.

Studies on the composition of feeds, digestibility, feeding experiments, animal health and performance, quality of livestock products and fate of DNA demonstrate no significant differences between feeds from isogenic and transgenic plants where the genetic modification has introduced an agronomic characteristic (so called “first-generation plants”).

Although there is a growing body of scientifically valid information available that indicates no significant risk associated with the consumption of DNA or the resulting proteins from any genetically modified crops that are registered yet, a more complex nutritional assessment will remain necessary for genetically modified plants where the genetic modification has changed the feed composition (so called “next-generation plants”). They cannot be considered substantially equivalent to isogenic plants, as far as substantial changes in composition and nutritive value of those products are incorporated. A combination of nutritional and safety assessment in animal experiments is necessary and next-generation of genetically modified plants should be subject to the full range of physiological-nutritional studies with representative groups of animals. This requirement should apply for any new genetically modified plant when first introduced and it should include besides chosen animal test species and/or age categories, also feeding trials with the species concerned or for those categories of animals for which the modification is significant.

Indeed, farm animal species are not necessarily equally sensitive to possible toxic effects, their lifespan may be very different from one species to another or according to their production aim and moreover the quantity of a certain product consumed on daily basis and per kg BW may also be very different.

Nutritional efficacy is a legitimate indicator of product (genetically modified plant) quality, and therefore, wholesomeness. Animal performance studies are sensitive methods to measure feed quality and safety since one of the first, if not the first, indicator of a health problem of the animal is reduced performance.

However in the context of far reaching selection for performance in farm animals, subacute toxicity may be masked or overruled by their genetic predisposition for specific production goals (growth, milk, egg production) and additional safety studies have to be performed in addition to performance or wholesomeness studies.

Besides the determination of important ingredients in genetically modified feeds (see section 2, Compositional Analysis), the following parameters should be studied:

- digestibility of total novel feed
- balance studies
- availability of modified nutrients in target animals
- animal performance, health, welfare
- quality of foods of animal origin

Further in vitro studies to assess nutritional value or to study the fate of modified protein and/or DNA could be useful and of scientific value but is not necessary for risk assessment in farm animals.

As for the further physiological studies in case of new feeds or feed products, where there is expression of new proteins or other substances this can be regarded as foreign substances, hence possible toxic products.

Therefore the physiological tests should be equivalent as for chemicals or products with sub-acute oral toxicity in the worst case. The following test proposal is in first instance designed for the next-generation of genetically modified crops, taking the limitations of extrapolating results to other animal species into account. Special attention must be paid to the avoidance of problems of nutritional imbalance.

4.2 Proposal of test method

The genetically modified feed is administered orally in daily graduated doses to several groups of experimental animals for an appropriate period (28 or 90 days); the genetically modified feed should be performance tested in target species that would normally consume the plant products, besides rats as the preferred species as an experimental animal.

As a general rule, younger animals are more sensitive to nutritional imbalances or performance enhancement compared with older animals. On this basis it can be argued from a scientific point of view that young animals could serve as a bioassay model for their species.

However, from an industry acceptance point of view, producers are most interested in phases of performance in which the greatest quantities of feed are utilized. These are typically grower/finisher types of studies. As more data become available which support that such residues are not in marketed products, use of younger animal models may become more appropriate. Therefore, commonly used laboratory strains of young healthy rats, less than 6 weeks old and certainly not older than 8 weeks should be used. Perhaps the ideal animal model, when appropriate, is the broiler chicken, which allows testing over the full life of an animal in a rapidly growing and sensitive species. At the commencement of the study, weight variation in the animals used, whatever the species, should not exceed $\pm 20\%$ of the mean value and preferably be less. The number of animals to be tested should be depended on the variation in the animals at the start of the trial for the variable to be tested in order to be able to detect significant effects.

The subacute oral toxicity test should be carried out in the target species according to the appropriate OECD protocol (see also section 3.4.7 of Chapter III).

5 Conclusion

In order to subject genetically modified feeds to a thorough safety assessment, notifiers should submit data as presented in this chapter. This includes investigation of the nutritional and physiological aspects of the genetically modified feed on a case-by-case basis, taking into account following considerations:

- the category of the genetically modified crop (first or next-generation) and a thorough description of the genetic modification; the potential effects of cultivation conditions and processes on the quality of the end product and the influence of the fate of modified DNA/protein on the composition;
- the concept of substantial equivalence as a useful framework for the safety assessment of first-generation products; next-generation of genetically modified plants should be subjected to physiological-nutritional studies;
- the nutritional and the food safety aspects of either feed or food derived from genetically modified crops, as well as health and welfare aspects of the animal. Investigation of the physiological aspects is important for the detection of possible unintended effects. The integrity of the animal should be

unaffected and the animal should function in the same manner as the animal that has been fed with the same non-genetically modified counterpart. Additionally the ecological safety of the genetically modified crop should be considered, which implies the evaluation of the effects of the genetically modified feed on the gut flora;

- the full range of physiological-nutritional studies should be carried out with representative groups of animals and special attention should be given to the target species itself.

6 References

- Aeschbacher, K., Messikommer, R., Wenk, C. (2001). Physiological characteristics of Bt 176-corn in poultry and destiny of recombinant plant DNA in poultry products. *Ann. Nutr. Metab.* 45 (Suppl. 1) 376 (Abstr.).
- Ash, J.A., Scheideler, S.E., Novak, C.L. (2000). The fate of genetically modified protein from Roundup Ready soybeans in the laying hen. *Poultry Sci. Assoc. Ann. Abstr., Suppl.* 1: 26.
- Beever, D.E., Kemp, C.F. (2000). Safety issue associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. *Nutr. Abstr. Rev. B* 70: 173-182.
- Chiter, A., Forbes, J.M., Blair, G.E. (2000). DNA stability in plant tissues: implications for the possible transfer of genes from genetically modified food. *FEBS Letters* 481: 164-168.
- Clark, J.H., Ipharraguerre, I.R. (2001). Livestock performance: feeding biotech crops. *J. Dairy Sci.* 84 (E. Suppl.): E9-E18.
- D'Mello, J.P.F. (1982). Utilization of dietary purines and pyrimidines by non-ruminant animals. *Proc. Nutr. Soc.* 41: 301-308.
- Doerfler, W., Schubbert, R., Heller, H., Kammer, C., Hilger-Eversheim, K., Knoblauch, M., Remus, R. (1997). Integration of foreign DNA and its consequences in mammalian systems. *Trends Biotech.* 15: 297-301.
- Duggan, P.S., Chambers, P.A., Heritage, J., Forbes, J.M. (2001). Survival of free DNA encoding antibiotic resistance from transgenic maize and the transformation activity of DNA in ovine saliva, ovine rumen fluid and silage effluent. *FEMS Microbiol. Letters* 191: 71-77.
- Einspanier, R., Klotz, A., Kraft, J., Aulrich, K., Poser R., Schwägele F., Jahreis, G., Flachowsky, G. (2001). The fate of forage plant DNA in farm animals: a collaborative case-study investigating cattle and chicken fed recombinant plant material. *Eur. Food Res. Technol.* 212: 129-134.
- Ewen, S., Pusztai, A. (1999). Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. *Lancet* 354: 1353-1354.
- FAO (1996). Biotechnology and food safety. Report of a joint FAO/WHO consultation. *FAO Food and Nutrition Paper* 61, Rome.
- Faust, M.A. (2000). Livestock products – Corn composition and detection of transgenic DNA/proteins. Joint ADSA/ASAS symposium on Agricultural Biotechnology in the Global Marketplace, Baltimore.
- Flachowsky, G., Aulrich, K. (2001a). Nutritional assessment of feeds from genetically modified organism. *J. Anim. Feed Sci.* 10 (Suppl.1): 181-194.
- Flachowsky, G., Aulrich, K. (2001b). Zum gentechnisch veränderter organismen (GVP) in der tierernährung. *Übers. Tierernährg.* 29: 45-79.

- Flachowsky, G., Aulrich, K., Berk, A., Daenicke, R. and Reuter, T. (2002). Nutritional assessment of feeds from genetically modified (GM) crops. *Proc. Soc. Nutr. Physiol.* 11: 183-186.
- Flint, H.J., Thomson, A.M. (1990). Deoxyribonuclease activity in rumen bacteria. *Lett. Appl. Microbiol.* 11: 18-21.
- Flint, H.J., Thomson, A.M., Bisset, J. (1988). Plasmid associated transfer of tetracycline resistance in *Bacteroides rumenicola*. *Appl. Environ. Microbiol.* 54: 855-860.
- Forano, E., Flint, H.J. (2000). Genetically modified organisms: consequences for ruminant health and nutrition. *Ann. Zootech.* 49: 255-271.
- Gawienowski, M.C., Eckhoff, S.R., Yang, P., Rayapati, P.J., Binder, T. and Briskin, D.P. (1999). Fate of maize DNA during steeping, wet-milling and processing. *Cereal Chem.* 76, 371-374.
- Gregg, K., Hamdorf, B., Henderson, K., Kopecny, J., Wong, C. (1998). Genetically modified ruminal bacteria protect sheep from fluoroacetate poisoning. *Appl. Environ. Microbiol.* 64: 3496-3498.
- Harrison, L.A., Bailey, M.R., Naylor, M.W., Padgett, S. (1996). The expressed protein in glyphosate-tolerant soybean, 5-Enolpyruvylshikimate-3 phosphate synthase from *Agrobacterium* sp. strain CP4, is rapidly digested in vitro and is not toxic to acutely gavaged mice. *J. Nutr.* 126: 728-740.
- Hohlweg, U., Doerfler, W. (2001). On the fate of plant or other foreign genes upon the uptake in food or after intramuscular injection in mice. *Mol. Gen. Genom.* 265: 225-233.
- Hupfer, C., Mayer, J., Hotzel, H., Sachse, K., Engel, K.H. (1999). The effect of ensiling on PCR-based detection of genetically modified Bt maize. *Eur. Food Res. Technol.* 209: 301-304.
- Khumnirdpetch, V., Intarachote, U., Treemanee, S., Tragoonroong, S., Thummabood, S. (2001). Detection of GMO's in the broilers that utilized genetically modified soybean meals as a feed ingredient. *Plant and Animal Genomics. Conf. San Diego, Jan. 13-17, 2001 (in press).*
- Klieve, A.V., Hudman, J.F., Bauchop, T. (1989). Inducible bacteriophages from ruminal bacteria. *Appl. Environ. Microbiol.* 55: 1630-1634.
- Klotz, A., Einspanier, R. (1998). Nachweis von "Novel-Feed" im Tier? Beeinträchtigung des Verbrauchers von Fleisch oder Milch ist nicht zu erwarten. *Mais* 26: 109-111.
- Leeson, S. (1998) The effect of corn hybrid CBH351 on the growth of male broiler chickens. Data evaluation report (MRID 44734306), United States Environmental Protection Agency.
- McAllan, A.B. (1980). The degradation of nucleic acids in, and the removal of breakdown products from the small intestines of steers. *Br. J. Nutr.* 44: 99-112.
- McAllan, A.B. (1982). The fate of nucleic acids in ruminants. *Proc. Nutr. Soc.* 41: 309-317.
- Molvig, L., Tabe, L.M., Eggum, B.O., Moore, A.E., Craig, S., Spencer, D., Higgins, T.J.V. 1992. Enhanced methionine levels and increased nutritive value of seed of transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene. *Proc. Natl. Acad. Sci. USA* 94:8393-8398.
- Munkvold, G.P., Hellmich, R.L., Rice, L.G. (1999). Comparison of fumosin concentrations in kernels of transgenic Bt maize hybrids and nontransgenic hybrids. *Plant Dis.* 83: 130-138.
- Munkvold, G.P., Hellmich, R.L., Showers, W.B. (1997). Reduced Fusarium ear rot and symptomless infection in kernels of maize genetically engineered for European corn borer resistance. *Phytopathology* 87: 1071-1077.

- Panaccione, D.G., Johnson, R.D., Wang, J., Young, C.A., Damrongkool, P., Scott, B., Schardl, C.L. (2001). Elimination of ergovaline from a grass-Neotyphodium endophyte symbiosis by genetic modification of the endophyte. *Proc. Natl. Acad. Sci. USA* 98: 12820-12825.
- Phipps, R.H., Beever, D.E., Tingey, A.P. (2001). Detection of transgenic DNA in bovine milk: Results for cows receiving a TMR containing maize grain modified for insect protection (MON 810). *J. Anim. Sci.* Vol. 79, Suppl. 1/*J. Dairy Sci.* Vol. 84, Suppl. 1/*Poult. Sci.* Vol. 80, Suppl. 1/54th Annu. Rec. Meat Conf., Vol. II, 114.
- Piva, G., Morlacchini, M., Pietri, A., Piva, A., Casadei, G. (2001). Performance of weaned piglets fed insect-protected (MON 810) or near isogenic corn. *J. Anim. Sci.* 79, suppl. 1: 106 (Abstr.).
- Peferoen, M. (1998). In vitro digestibility and heat stability of the endotoxin Cry9C of *Bacillus thuringiensis*. Data evaluation report (MRID 44258108), United States Environmental Protection Agency.
- Reuter, T., Aulrich, K., Berk, A., Flachowsky, G. (2001). Nutritional evaluation of Bt-maize in pigs. *Proc. Soc. Nutr. Physiol.* 10, 111.
- Schubbert, R., Lettman, C., Doerfler, W. (1994). Ingested foreign (phage M13) DNA survives transiently in the gastrointestinal tract and enters the bloodstream of mice. *Mol. Gen. Genet.* 242: 495-504.
- Schubbert, R., Hohlweg, U., Renz, D., Doefler, W. (1998). On the fate of orally ingested foreign DNA in mice: chromosomal association and placental transmission of the foetus. *Mol. Gen. Genet.* 259: 569-576.
- Schubbert, R., Renz, D., Schmitz, B., Doefler, W. (1997). Foreign (M13) DNA ingested by mice reaches peripheral leukocytes, spleen, and liver via the intestinal wall mucosa and can be covalently linked to mouse DNA. *Proc. Natl. Acad. Sci. USA* 94: 961-966.
- Van Wert, S., Noteborn, H. (1999a). Mouse short-term (30-day) dietary toxicity study with the protein Cry9C. Data evaluation report (MRID 44734303), United States Environmental Protection Agency.
- Van Wert, S., Noteborn, H. (1999b). Assessment of the stability to digestion and bioavailability of the LYS mutant Cry9C protein from *Bacillus thuringiensis* serovar *tolworthi*. Data evaluation report (MRID 447343-05), United States Environmental Protection Agency.
- Vilotte, J.L., Soulier, S., Persuy, M.A., Lepourry, L., Legrain, S., Printz, C., Stinnakre, M.G., L'Huilier, P., Mercier, J.C. (1997). Application of transgenesis to modifying milk protein composition. In: *Milk composition, production and biotechnology*, Eds.: R.A.S. Welch, D.J.W. Burns, S.R. Davis, A.I. Popay, C.G. Prosser, CAB International, Oxon, pp. 231-242.
- von Wettstein, D., Mikhaylenko, G., Froseth, J.A., Kannangara, C.G. (2000). Improved berley broiler feed with transgenic malt containing heat-stable (1,3-1,4)-glucanase. *Proc. Natl. Acad. Sci. USA* 97: 13512-13517.
- Wehrman, A., Van Vliet, A., Opsomer, C., Botterman, J., Schulz, A. (1996). The similarities of bar and pat gene products make them equally applicable for plant engineers. *Nature Biotech.* 14: 1274-1278.
- Yamanaka, M., Tsubota, Y., Anai, M., Ishimatsu, K., Okumura, M., Katsuki, S-O., Takagi, Y. (1974). Purification and properties of acid deoxyribonucleasas of human gastric mucosa and cervix uteri. *J. Biol. Chem.* 249: 3884-3889.

Annexes

Annex 1: Studies on the transfer of foreign DNA fragments from feed into the animal (translated from Flachowsky and Aulrich, 2001b)

Authors	DNA-source	Animal species	Results
Schubbert et al. (1994)	Phage-DNA	Mice	DNA-fragments in blood
Schubbert et al. (1997)	Phage-DNA	Mice, pregnant	DNA-fragments till 8 h in leukocytes, till 24 h in kidney and liver
Schubbert et al. (1998)	Phage-DNA	Mice, pregnant	DNA-transfer via placenta into foetus
Klotz and Einspanier (1998)	Soy beans	Dairy cows	Plant-DNA fragments in leukocytes, nothing in milk
Aeschbacher et al. (2001)	Bt-maize	Broilers	No plant-DNA fragments in the animal body
Einspanier et al. (2001)	Bt-maize (seeds and silage)	Broilers Laying hens Beef cattle Dairy cows	Plant-DNA fragments in muscle, liver, spleen, kidney of broilers and laying hens; no identification in blood, muscle, liver, spleen, kidney of bulls, eggs and excrements of broilers and layers; no identification of transgenic DNA-fragments in excrements of dairy cows
Hohlweg and Doerfler (2001)	Soy bean leaves	Mice	Plant-DNA fragments till 121 h in faeces, till 330 bp in liver and spleen samples
Khumnirdpetch et al. (2001)	Gt- soy beans	Broilers	No identification of DNA-fragments in tissue of muscles
Phipps et al. (2001)	Bt-maize	Dairy cows	No identification of transgenic DNA-fragments in the milk
Reuter et al. (2001)	Bt-maize	Pigs	Plant-DNA fragments in organs and tissues, no identification of transgenic DNA

Annex 2: Proposal for a decision tree for the nutritional assessment in combination with the safety assessment of genetically modified feeds (Flachowsky et. al., 2002)

Further questions

IMPORTANT QUESTIONS

Further questions

