

Who's afraid

Nowadays it seems that it is impossible to avoid some inclusion of novel genes in feed ingredients. However, there are safety measures in place during the whole development and production processes. Legislation and research should help put some fears to rest.

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In the United States, the Food and Drug Administration (FDA) has the responsibility to ensure that any human food or livestock feed derived from new plant varieties, including GM crops, is safe. Up to now, the FDA process has involved voluntary consultations with developers prior to marketing of a new product (FDA, 2001a). Even though all commercialised GM food and feed products have been subject to this consultation process, the FDA recently proposed a mandatory rule that would require developers to submit a scientific assessment 120 days prior to marketing (FDA, 2001b).

In Canada, all livestock feeds, including novel feeds derived from GM crops, are subject to mandatory review for safety and efficacy by the Canadian Food Inspection Agency under the *Feeds Act and Regulations*, prior to approval for sale and marketing.

The safety assessment of a novel livestock feed or human food looks at the molecular, compositional, toxicological and nutritional characteristics of the novel feed compared with its conventional counterpart. Safety considerations include the animal eating the feed, consumption of the animal product by consumers, worker safety and any other environmental aspects from use of the feed. The principal focus is on the protein expression product(s) of the inserted gene(s). Inserted genetic material itself is not of concern with respect to the ingestion of GM plants or their products, since DNA is the same in all living organisms and does not differ from what is already ingested from the diet.

In addition to the intrinsic properties of the introduced protein, the safety assessment also considers possible unexpected consequences arising from expression of the novel trait (eg, metabolic consequences of expressing a new enzyme, or altering the expression of an endogenous enzyme), or introduction of new genes into the plant genome (so called pleiotropic effects).

GM crops used in animal feed

As a source of livestock feed components, the relevant GM crop species include canola (rapeseed), maize (corn), soybean, cottonseed and potato. These species have been modified to express, either singly or in combination, the traits of insect resistance, herbicide tolerance, or, in the case of potatoes, resistance to virus infection (Table 1). Many of the proteins that have been expressed in GM plants in order to confer these so called "input" traits are already present in plant products or in other agricultural products.

Bt Proteins

Cry genes encode delta-endotoxins that have been expressed in many transgenic crops to protect against pests such as the European corn borer (ECB) or the Colorado potato beetle. These are the same proteins that are present in strains of *Bacillus thuringiensis* that have been registered for use as microbial insecticides since 1961 (Frankenhuyzen, 1993). Delta-endotoxins, such as the Cry1Ab, Cry1Ac, Cry9C, or Cry1F proteins expressed in lines of ECB-resistant

maize, act by selectively binding to specific receptors localised on the brush border epithelium of susceptible insect species. Following binding, pores are formed that disrupt midgut ion flow causing gut paralysis and eventual death due to bacterial sepsis. The specificity of action of these Cry proteins is directly attributable to the presence of specific binding sites in the target insects (Hofmann *et al.*, 1988). There are no binding sites for delta-endotoxins of *B. thuringiensis* on the surface of mammalian intestinal cells so livestock and humans are not susceptible to these proteins.

In 1971, the United States Environmental Protection Agency (US-EPA) established an exemption from the requirement for a tolerance for microbial spray formulations of Bt products. This means that there is no limit placed on the amount of Bt that humans or animals may safely consume. A history of familiarity and the acknowledged safety of registered *B. thuringiensis* products by regulatory agencies, such as the US-EPA (EPA, 1998; McClintock *et al.*, 1995), together with an understanding of their mode of action, have been used as evidence in support of the safety of these proteins when expressed in GM plants.

Herbicide tolerance proteins

In plants, the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (abbreviated EPSPS) plays a key role in the biochemical pathway that results in synthesis of phenylalanine, tyrosine, and tryptophan. This enzyme is not present in animals and humans (Levin & Sprinson, 1964; Steinrucken & Amrhein, 1980). In the early 1970s, it was discovered that the simple amino acid analogue, glyphosate, could selectively inhibit the activity of the EPSPS enzyme, thus shutting off aromatic amino acid synthesis, quickly resulting in plant death (Kishore & Shah, 1988). The introduction of a glyphosate-tolerant EPSPS gene, derived from the common soil bacterium, *Agrobacterium tumefaciens* strain CP4, forms the basis of



of GM feed?



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glyphosate herbicide tolerance when expressed in GM plants. The amino acid sequence of CP4 EPSPS shows the same level of relatedness to endogenous EPSPS in foods as the various food EPSPS proteins show with each other (eg, the phylogenetic distance between CP4 EPSPS and EPSPS from *Bacillus subtilis*, baker's yeast or food plants, is the same as that between endogenous soybean EPSPS and *B. subtilis* EPSPS (Padgett *et al.* 1996).

Virus resistance

Varieties of transgenic potato have been produced that display resistance to plant viruses. Resistance to potato virus Y (PVY) and potato leafroll virus (PLRV) has been introduced by inserting DNA sequences corresponding to the virus coat protein (CP) or the viral

Table 1 - Expressed traits and associated genes that have been incorporated into animal feed crops

Trait	Genetic element(s)
Insect resistance	Cry1Ab; cry1Ac; cry9C; cry3A; cry1F
Glufosinate herbicide resistance	Phosphinothricin N acetyltransferase
Glyphosate herbicide tolerance	5-enolpyruvylshikimate-3-phosphate synthase (EPSPS)
Male sterility	Barnase ribonuclease
Sulphonyl urea herbicide tolerance	Variant form of acetolactate synthase
Oxynil herbicide tolerance	Nitrilase
Modified seed fatty acid profile	Delta-12 desaturase
Virus resistance	Coat protein; helicase/replicase

Table 2 - Toxicity of novel proteins expressed in GM plants, based on no observed effect level (NOEL)

Protein	Crop
Cry1Ac	Cotton, tomato
Cry1Ab	Maize
Cry2Aa	Cotton
Cry2Ab	Maize, cotton
Cry3A	Potato
CP4EPSPS	Soybean, cotton, canola, sugarbeet, maize
mzEPSPS	Maize
NPTII	Cotton, potato, tomato
GUS	Sugarbeet
GOX	Canola
ACC deaminase	Tomato

replicase, respectively. Plants expressing viral CPs exhibit "pathogen-derived resistance" through a process that is related to the natural phenomenon of viral cross-protection. Although the exact mechanism by which the viral protection occurs is unknown, most evidence suggests that expression of viral CP by a plant interferes with one of the first steps in viral replication, uncoating (removal of CP) from the incoming virus (Register & Nelson, 1992). Other modes of action of cross-protection have also been suggested (Matthews, 1991). The resistance to PLRV is through a partially understood process that has been termed "replicase-mediated resistance", which may involve silencing of viral gene translation.

In non-transgenic potatoes, the presence of viral CPs is due to natural viral infection. This is a common occurrence and there is a long history of safe human and livestock animal consumption of these proteins. Plant viral proteins (whether from PVY or any other plant virus) have never been associated with toxicity or cases of allergic reaction in humans. The expression of plant virus derived proteins in transgenic crops is not different than the production of these same proteins in virus-infected crops.

Toxicity studies

In addition to demonstrating a history of safe use and/or a relation with "naturally occurring" food proteins, GM plant products are normally subjected to compositional comparison with non-GM counter-

Table 3 - Novel v. conventional feeds- livestock studies

Animal	GM crop component	Study	Reference
Poultry	Insect-resistant maize	5-day study with laying hens- no differences in nutrient composition, body weight, digestible organic matter, protein or metabolisable energy 38-day broiler study- no differences in mortality, body weight, feed intake. Slight improvement in feed conversion and breast meat yield 35-day broiler study- no difference in body weight gain, feed intake, feed conversion or protein digestibility Two studies to compare nutrient composition and availability in broilers- no differences in weight gain and feed composition	Aulrich <i>et al.</i> (1998) Brake and Vlachos, (1998) Halle <i>et al.</i> (1998) Mireles <i>et al.</i> (2000)
	Herbicide-tolerant soybean	Nutritional composition and feeding trials with broilers, catfish and dairy cows- no significant differences in any measured parameters between glyphosate-tolerant and conventional soybean meal	Pageette <i>et al.</i> (1995); Hammond <i>et al.</i> (1996)
Dairy cows	Insect-resistant maize	14-day trial with green chop- no differences in feed intake, milk yield, milk composition or udder health No differences in rumen fermentation characteristics, milk production or milk composition	Faust and miller (1997) Folmer <i>et al.</i> (2000b)
	Herbicide-tolerant maize	Glyphosate-tolerant maize silage- no differences in dry matter intake, milk production, milk protein, lactose or milk fat yield; no differences in milk composition (% fat, protein, lactose, solids non-fat) somatic cell count or urea nitrogen	Donkin <i>et al.</i> (2000)
Beef cattle	Insect-resistant maize	Two-year animal performance study grazing Bt or non-Bt maize residue- no differences in either year of study Two trials to evaluate utilisation of maize silage by steers- no difference in grain intake, intake on pasture, nor grazing preference between Bt and non-Bt maize silage. Silage from Bt maize performed equally to or slightly better than that from non-Bt maize with respect to weight gain and feed conversion efficiency Study conducted over two years to examine the effects of grazing crop residues from Bt maize hybrids on performance of pregnant beef cows	Russell and Peterson (1999); Russell <i>et al.</i> (2000) Folmer <i>et al.</i> (2000a) Russell <i>et al.</i> (2001)
	Herbicide-tolerant maize	85-day study with 56 Angus and Simmental steers fed whole plant silage and dry rolled grain from conventional and glyphosate-tolerant maize- no differences in average daily weight gain, dry matter intake or feed conversion efficiency	Petty <i>et al.</i> (2001)
Pigs	Insect-resistant maize	Compared grower-finisher performance and carcass characteristics- no differences in average daily weight gain, feed intake or feed efficiency, no adverse effects on growth performance or carcass characteristics	Weber <i>et al.</i> (2000)

parts, feeding trials, and acute toxicity studies with laboratory animals. The mode of action of many known protein toxins is to act through acute mechanisms (Sjoblad *et al.*, 1992; Hammond & Fuchs, 1998) and oral administration of high doses of purified transgenic protein, either from bacterial or plant sources, has been considered as sufficient to evaluate the toxic potential of new proteins.

Acute oral toxicity studies with purified CP4 EPSPS (Harrison *et al.* 1996) or Cry3A protein at concentrations that exceeded the anticipated consumption level by 1000 or 1,000,000- fold, respectively, have not shown any deleterious effects. As part of the safety assessment for Colorado potato beetle resistant potatoes, high doses of purified Cry3A protein (5200 mg / kg body weight) were administered to laboratory mice. Based on the average human consumption of potatoes and the level of expression of the Cry3A protein in transgenic tubers, this dosage represented a 2.5 million-fold safety factor. Adverse effects were not observed in the test animals, nor were there any effects on food consumption, weight gain or gross pathology (Lavrik *et al.* 1995).

Proteins are not normally considered to have any potential mutagenic, teratogenic, or carcinogenic activity (Hammond *et al.*, 1996a; Hammond, 1997; Hammond & Fuchs, 1998; FAO/WHO, 2000), nor is there any data to indicate that proteins are capable of interactions with DNA that would give rise to mutagenic effects (Dean, 1997).

Feed and digestive defences

Comparisons of nutritional composition and wholesomeness between animal feeds containing transgenic and non-transgenic components have been the subject of numerous studies using insect-resistant maize and cottonseed, or herbicide tolerant soybean. These studies, which have been carried out with beef and dairy cattle; broiler and layer chickens; swine; sheep; and catfish have consistently demonstrated no significant differences in nutritional composition or animal performance due to consumption of the novel feed compared to conventional counterparts (Table 3).

Quite apart from any direct effects on livestock animals associated with the consumption of GM crop-containing feeds, are questions concerning indirect effects, such as: could the DNA of inserted or modified genes, or their protein products, be transferred to and accumulate in the food products (milk, meat, eggs) of animals fed feeds derived from GM crops; and will the consumption of animal products derived from livestock fed GM feeds lead to adverse health effects in humans.

These concerns have been addressed by considering the normal digestive fate of DNA and proteins present in all foods, the digestibility of new proteins expressed in GM plants, and investigating the occurrence of transgenic DNA and proteins in food products of animal origin.

Low risk DNA doses

All whole foods and feedstuffs contain DNA, and animals and people have always consumed significant quantities of DNA from a wide variety of sources, including plants, animals, bacteria, parasites and viruses. The UN Food and Agriculture Organisation (FAO), the World Health Organisation (WHO; FAO/WHO, 1991), the US FDA (FDA, 1992) and the US EPA (EPA, 2000) have each stated that the consumption of DNA from all sources, including GM crops, is safe.

Although it is difficult to provide realistic estimates of DNA intake for typical livestock diets, it has been estimated that the DNA content of most food crops is less than 0.02% (wt/wt dry matter), with values of 0.005% reported for some crops (Watson & Thompson, 1988). In the case of dairy cows, where maize silage and maize grain may account for 40% and 20%, respectively, of total dry matter intake (eg, 60% of total ration), a 600kg animal might have a total dietary DNA intake of 608mg per day. For GM maize, where it is assumed that the maize genome (about 2.5 billion base pairs in size) contains a 4000 base pair transgene insert, the fraction of GM DNA in the diet represents 1:234,000, or 0.00042%, of the total dietary DNA intake (Beever & Kemp, 2000). On this basis, there is a negligible exposure to introduced DNA of GM crop material compared to the normal exposure to non-GM DNA.

The normal metabolic fate of ingested DNA is enzyme (eg, DNase I, DNase II) and acid catalysed hydrolysis to small molecular weight fragments and ultimately reduction to individual nucleotides - the building blocks of DNA. It has been estimated that more than 85% of the plant DNA consumed by ruminants is reduced to nucleotides before entering the duodenum (McAllan, 1982).

Not surprisingly, GM DNA suffers a similar metabolic fate. In a recent study, Rasche (1998) investigated the stability of transgenic DNA encoding the phosphinothricin N-acetyltransferase enzyme from glufosinate herbicide tolerant canola in digestive fluids isolated from swine, chickens and cows. It was observed that the DNA was completely degraded to nucleotides within one hour at body temperature and pH 1.5. Similar studies using sensitive polymerase chain reaction (PCR) amplification techniques have been unable to detect even small fragments of CP4 EPSPS or Cry1Ab encoding genes in animal products obtained from cows (Klotz & Einspanier, 1998), chickens (Einspanier *et al.*, 2001), or pigs (Weber & Richert, 2001) fed diets containing herbicide tolerant soybean or insect resistant maize.

Metabolic fate of dietary proteins

Generally, ingested proteins are broken down to increasingly smaller fragments through the combined action of acid and enzymatic hydrolysis occurring in the mammalian digestive tract. Except for milk-borne immunoglobulins (IgA) that are designed to be absorbed in order to provide passive immunity for newborns (Gardner, 1988), and small amounts of intact proteins or large polypeptides taken up by mononuclear leukocytes as part of the immune system surveillance of the gut (Tsume *et al.*, 1996), proteins are not absorbed across the gut wall. Nutritionally, dietary proteins are either non-digestible, being excreted in the faeces, or are absorbed as small peptides and amino acids.

The digestive fate of new proteins introduced into GM crops has been evaluated by examining the *in vitro* digestibility of purified proteins in simulated gastric and intestinal fluids, and by testing for the occurrence of these proteins (or their fragments) in food products from livestock animals. With the exception of Cry9C protein, which is stable to pepsin digestion at pH 2.0 for up to 4 hours, all of the novel proteins expressed in GM crops used in livestock feed are rapidly degraded under *in vitro* conditions simulating the gastric environment (Figure 1, Table 4).

Studies have also been carried out to demonstrate that transgenic proteins present in livestock feed are not detectable in food products derived from these animals. Cows that were fed a diet containing one of two glufosinate herbicide tolerants plus insect resistant corn hybrids showed no adverse effects and produced the same volume and composition of milk as cows fed a control diet (Faust & Miller, 1997). There was no evidence of the transgenic proteins, Cry1Ab or phosphinothricin-N-acetyltransferase (PAT), in the milk. Similar studies have also investigated the occurrence of CP4 EPSPS protein in whole egg, egg white, liver and faecal samples from laying hens fed a diet containing glyphosate-tolerant soybean. Enzyme linked immunosorbent assay (ELISA) failed to detect the transgenic protein in any of these samples (Ash *et al.*, 2000).

A recent study by the Japanese government also failed to detect Cry9C protein in samples of muscle, liver, or blood from chickens fed a diet containing StarLink® maize (Japan MAFF, 2001). Weber & Richert (2001) reported that all samples of pork loin muscle tissue from grower-finisher pigs fed insect-resistant maize had no detectable levels of intact or immunologically reactive fragments of the Cry1Ab protein.

Conclusions

Protein and DNA contained in foods and feeds, whether obtained from non-GM or GM crops, are typically degraded upon consumption by the normal digestive processes. For those commercially available GM crops that are components of livestock feeds, there is no evidence of significantly altered nutritional composition, deleterious effects, or the occurrence of transgenic DNA or proteins in subsequent foods of animal origin. This data, together with the history of safe usage of the transgenic proteins in agriculture and/or their similarity to already occurring constituents, provide a substantial assurance of safety. ●

Table 4 - Stability of introduced proteins to digestion in simulated gastric fluid

Protein	Relative abundance (% total protein)	Stability (sec)
Cry1A	<0.01	30
Cry2	<0.01	<15
Cry3A	<0.01	<15
CP4 EPSPS	<0.1	<15
Mz EPSPS	<0.05	<15
GOX	<0.01	<15
GUS	0.01	<15
NPTII	<0.01	<10

Figure 1 - The novel protein CP4 EPSPS is rapidly degraded under *in vitro* conditions simulating the gastrointestinal environment (Seiichiro Yamane, personal communication)

