A Safety Evaluation of Genetically Modified Feedstuffs for Livestock Production; the Fate of Transgenic DNA and Proteins

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ABSTRACT : Two genetic constructs used to confer improved agronomic characteristics, namely herbicide tolerance (HT) in maize and soyabean and insect resistance (Bt) in maize, are considered in respect of feeding to farm livestock, animal performance and the nutritional value and safety of animal products. A review of nucleic acid (DNA) and protein digestion in farm livestock concludes that the frequency of intact transgenic DNA and proteins of GM and non-GM crops being absorbed is minimal/non existent, although there is some evidence of the presence of short fragments of rubisco DNA of non-GM soya in animal tissues. It has been established that feed processing (especially heat) prior to feeding causes significant disruption of plant DNA. Studies with ruminant and non-ruminant farm livestock offered GM feeds demonstrated that animal performance and product composition are unaffected and that there is no evidence of transgenic DNA or proteins of current GM in the products of animals consuming such feeds. On this evidence, current HT and Bt constructs represent no threat to the health of animals, or humans consuming the products of such animals. However as new GM constructs become available it will be necessary to subject these to rigorous evaluation. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 5 : 764-772)***

Key Words : Genetically Modified Crops, DNA, Proteins, Feed Processing, Digestion, Ruminants, Non-ruminants, Animal Performance, Product Composition

INTRODUCTION

The last decade has witnessed a major development with respect to several agricultural crops with the introduction of new varieties produced as a result of targeted plant breeding. The ability to insert specific genes has allowed crops with new and specific characteristics to be developed which would not have been possible with conventional breeding, and genetic engineering in this century offers the same hope to increased food security as did the 'Green revolution' in the last, a comparison which has been amplified by several authors (Borlaug and Dowsewell, 2001). Maize, soya, rape, potato, rice and sugar beet represent the major crops of interest to date, with the introduction of herbicide tolerance (maize, soya, rape, rice and sugar beet) and insect resistance (maize, potato) being most relevant to the remit of this paper. Both the nature of the processes involved and the subsequent comercialisation of these crops have been extensively assessed (Beringer, 1999; James, 1999; FAO/WHO, 2000) and such crops are now being successfully grown (40×10^6 hectares). Many are fed to both ruminant and non-ruminant farm livestock, principally in USA (72%), Argentina (17%), Canada (10%) and Australia and China (both 1%) (all 1999 data; James, 1999). In contrast, member countries of the European Union have an agreed moratorium on the commercial use of such crops, pending the outcome of further safety assessments involving farm scale evaluations. The current moratorium is due to expire in 2003.

Many of the origins of the cautious approach adopted by the EU are related to several well publicised food scares which have occurred over the last decade or so. The main issues of concern regarding plant biotech products are, in summary:

Could the DNA of inserted or modified genes and/or their products (proteins) cause health problems in animals consuming GM crops?

Could DNA fragments or biotech proteins be transferred to and accumulate with animal products?

Will the consumption of crop materials or animal products derived from GM crops cause adverse effects to human health?

It is noteworthy, however, that as evidence to refute the above mentioned issues has accumulated, the focus of those opposed to the introduction of GM technology has moved on to newly created issues including escape of pollen to non-GM crops and especially organically grown relatives (Masood, 1998) and the possibility and implications of horizontal gene transfer.

DIGESTIVE FATE OF FEED DNA AND PROTEINS

Man and animals both have a historic consumption of DNA and proteins of plant and animal origin, without any substantial evidence of associated health problems. Within the context of the modern high yielding dairy cow, it was

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estimated (Beever and Phipps, 2001) that an average daily consumption of 24 kg feed dry matter would result in a daily intake of 57 g DNA, and assuming 60% of the ration to be provided as either maize silage or maize grain, with all of this being from GM crops, it was concluded that transgenic DNA would represent 54 μg or 9.4×10^{-5}% of total DNA intake. On the basis of this evidence it was concluded that this was a small, almost negligible amount although it was accepted that it could not be ignored if the transgenic DNA survived digestion and retained full functional integrity.

Nucleic acid digestion in non-ruminants is primarily associated with processes occurring in the mouth, stomach, and intestines. Food is subjected to a number of enzymes immediately following ingestion, with salivary secretions containing both RNA and DNA nucleases, in addition to more recognised enzymes such as α-amylase. The stomach is a major site of acid, pepsin and mucin secretion before digesta passes onto the intestines where it is subjected to a multitude of enzymes, including pepsin, α-amylase, proteases, lipases and nucleases, the latter being produced exclusively by the pancreas. On the basis of this evidence it is concluded that the processes of RNA and DNA digestion to constitutive nucleotides and nucleic acids are well developed in non-ruminants, and in the healthy animal there would appear to be evidence of intact RNA and DNA being absorbed into the animal's blood stream. Earlier studies with pigs (Newport and Keal, 1973) demonstrated extensive catabolism of nucleic acids with no evidence of these or their breakdown products (bases) contributing to tissue synthesis. Equally, the presence of adenine and guanine deaminases has been demonstrated in the tissues of non-ruminants (Henderson and Patterson, 1973) whilst other studies (D'Mello, 1982) have shown extensive salvage and reutilisation of purine and pyrimidine nucleotides with adenine incorporation into tissue adenine and guanine, but even in such situations the opportunity to transfer a piece of DNA of sufficient size to encode a functional protein of either transgenic (or indeed conventional) feed origin into the animal's genome is most improbable. Indeed, the pharmaceutical industry is struggling to overcome the natural digestive, metabolic and cellular barriers that protect against transfer of genetic material into the genome as they attempt to develop viable gene therapeutics.

Whilst nucleic acid digestion in the small intestines of ruminants is broadly similar to that which occurs in non-ruminants, the processes of microbial digestion in the rumen are known to have a major impact on both the amount and origin of nucleic acids being presented for digestion in the post-ruminal section of the gastro-intestinal tract. Ruminant livestock produce copious amounts of saliva especially on high forage diets and as in non-ruminants, this is a source of nucleases capable of initiating the digestion of recently ingested nucleic acids. However it is the rumen, with its resident population of bacteria, protozoa and fungi, that is the major contributor to total tract digestion. After ingestion, feed accumulates in the rumen for varying periods of time, according to the composition and level of feeding, and it is during this time that microbial cellulases and hemicellulases affect the principal purpose of rumen digestion, namely the digestion of ingested plant fibre. Other microbial enzymes will digest significant amounts of the dietary protein and starch, whilst microbial nucleases will affect extensive degradation of ingested nucleic acids. At the same time however, the microbes will utilise a significant proportion of the nutrients arising from these degradation processes for the synthesis of microbial biomass, principally proteins (from amino acids and ammonia), nucleic acids (from ammonia with hexose providing the important carbon skeleton) lipids (direct incorporation of free fatty acids and *de novo* synthesis from hexose) and polysaccharides, namely as a storage product (from hexose). Subsequently the microbes will pass from the rumen, either free floating in the liquid phase of the digesta or intimately bound to undigested/partially digested feed particles. Thus it follows that with most diets where the extent of rumen digestion may account for as much as 900 g true digestion/kg total tract digestion, albeit only 600 g apparent digestion/kg when the net synthesis of microbial biomass is taken into account, the contribution of microbial nucleic acids to total small intestinal load of nucleic acids will be high. In experimental studies, the rapid digestion of plant nucleic acids has been demonstrated (Razzaque and Topps, 1972) followed by extensive deamination of the resulting purine and pyrimidine bases. Other studies (Van Nevel and Demeyer, 1977) have shown little evidence of direct incorporation of nucleotides by rumen microbes, whilst it was suggested (Smith, 1975) that microbial nucleic acids of rumen origin may account for as much as 850 g/kg of total nucleic acids entering the small intestine. In subsequent studies (McAllan; 1980, 1982) apparent digestibility of nucleic acids in the small intestine prior to the terminal ileum was estimated to be >800 g/kg, with an estimated true digestibility of >970 g/kg. In a study involving growing cattle fed hay and concentrates, and designed to examine the fate of nucleic acids in the post-ruminal section of the tract (McAllan, 1982), the cattle were serially slaughtered and the intestines were separately ligatured into 8 approximately equal lengths starting with the abomasum. This data have been re-calculated and are included in Figure 1. Expressing abomasal levels of RNA and DNA as unity, it can be seen that by the end of the third section of the alimentary tract, RNA levels were reduced to <150 g/kg abomasal levels whilst the comparable value for DNA was 350 g/kg. By the final section of the small intestine, RNA

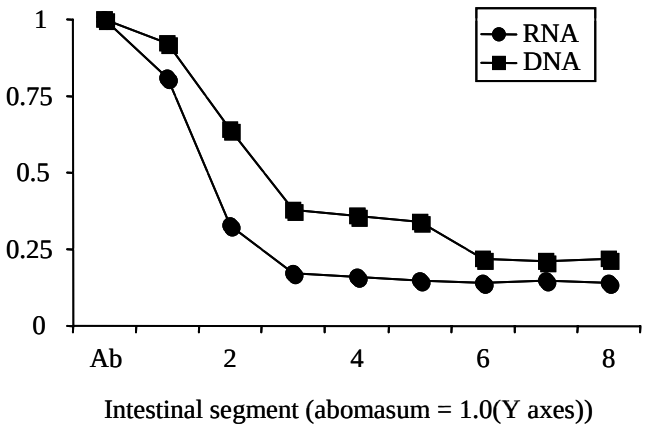

Figure 1. Fate of RNA and DNA in intestines of cattle (McAllan, 1982).

and DNA levels were further reduced to approximately 150 and 200 g/kg abomasal levels respectively.

In Figure 2, data from the same study are presented on the production and removal of adenine, guanine and pyrimidine nucleotides in the intestines of ruminants. As expected, no free adenine, guanine or pyrimidine nucleotides were detected in the abomasum but levels increased substantially thereafter with maximum amounts being detected in the first segment of the intestines. Levels then fell progressively with approximately 67% of the pyrimidine nucleotides being removed by segment 3 and complete absence by segment 8. Rate of removal of guanine was slightly slower with 50% lost by segment 3 and complete absence by segment 8, whilst 40% of abomasal adenine levels were removed by segment 3 and once again, total absence by segment 8.

In both ruminants and non-ruminants the processes of protein digestion are well documented, although relatively little attention has been paid to the fate of individual proteins. In monogastrics the small intestine is the principal site of protein digestion, whilst ruminants are characterised by extensive microbial degradation of feed protein in the rumen with an associated synthesis of microbial protein, with free amino acids and ammonia being the major substrates (Maeng and Baldwin, 1975). With respect to individual proteins, the digestive fate of rubisco (fraction 1) protein in ruminants has been well documented (Mangen, 1982), showing that in most dietary situations the rate of degradation of this protein is often greater than the rate at which the microbes can assimilate the breakdown products into microbial protein. Under such conditions, absorption of increased amounts of ammonia from the rumen is an inevitable consequence. On the other hand, chloroplastic proteins and those proteins associated with cell membranes are usually degraded more slowly. With respect to those genetic constructs that have already been commercialised, herbicide tolerance has been achieved through insertion of the EPSPS (5-enolpyruvylshikimate-3-phosphate synthase; glyphosate tolerance) or PAT gene (phosphinothricine acetyl transferase; glufosinate tolerance) whilst insect protection been introduced through insertion of the *cry1Ab* or *cry9*c gene (Bt, *Bacillus thuringiensis*). EPSPS is similar to many proteins commonly found in plants, bacteria and fungi whilst PAT protein is produced by common soil bacterium and as a member of the acetyl transferase family of proteins ubiquitous throughout nature. The Cry genes code for Bt protein that is produced naturally by *B. thuringiensis*. The mode of action of Bt proteins involves selective binding to the mid-gut of sensitive insects whilst not affecting other insects or mammals. Interestingly, Bt microbial products have been used extensively by farmers, including those growing organic crops, for more than 40 years.

Studies to examine the *in vitro* (intestinal) degradation of PAT protein (Wehrmann et al., 1996) over a wide range of pH conditions established an optimal pH of 6.5 whilst in the absence of pepsin, PAT degradation equated to 500 g/kg after 10 minutes, taking more than 120 minutes for digestion to exceed 950 g/kg total PAT protein. In contrast, when pepsin was included in the incubation (Figure 3) the degradation of PAT protein was found to be almost complete after 5 minutes of exposure.

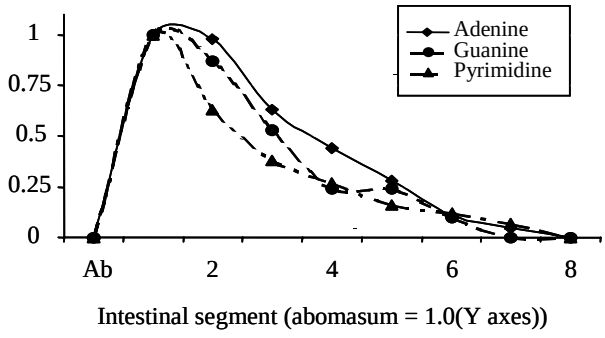

Figure 2. Production & removal of adenine guanine and pyrimidine nucleotides in small intestines (McAllan, 1982).

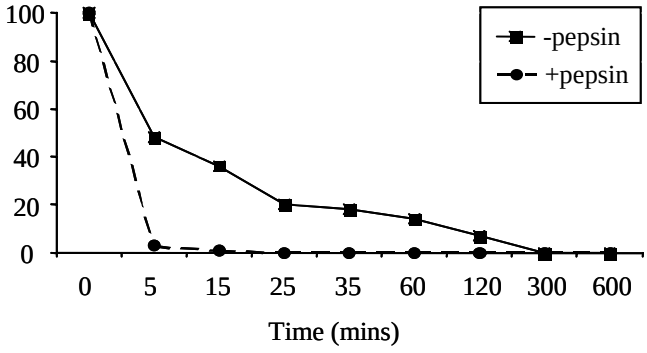

Figure 3. In vitro degradation of PAT protein; degradation (±pepsin) (Wehrmann et al., 1996).

EFFECT OF FEED PROCESSING ON DNA INTEGRITY

Almost all feeds for non-ruminant animals and many feeds for ruminants are subjected to extensive processing prior to feeding. This aspect was considered in a recent study (Forbes et al., 1998) that examined the effect of grinding, heat and pressure on the nature of the plant DNA. Using a range of feedstuffs (all non-GM) with wheat being included in several of the studies, it was concluded that neither severity nor duration of physical grinding had any effect on the structural integrity of plant DNA. Application of heat, however, showed a markedly different effect. Whilst applying heat at 90°C for varying periods of time (5 to 30 mins) had no effect on DNA structure, at 93°C partial disruption [average DNA size; 50-250 base pairs (bp)] was observed, with temperatures of over 95°C resulting in complete disruption (DNA fragments <100 bp). These results were subsequently confirmed when a range of commercially available feedstuffs were examined, without further processing in terms of heat application. Both intact soyabeans and untreated rape were found to be structurally intact with respect to DNA, with average chain lengths of >21 kbp whilst extracted soya and both expelled and extracted rape had significantly reduced DNA lengths, indicating considerable disruption of the plant DNA, presumably as a consequence of processing the feed (Forbes et al., 1998).

The studies were extended to examine the effect of high (>1.6 kg/cm^2) and low (<0.53 kg/cm^2) pressure on DNA structure and these concluded that disruption of DNA was extensive when both pressure and temperature were high but incomplete when temperatures were <95°C. Examination of forage maize however, which is likely to be subjected to only modest temperatures and pressure during ensiling, revealed no significant disruption of the plant DNA of the resulting silage. Whilst these data were largely as expected, they contrast with those from the University of Munich (Karl Heinz Engel, personal communication) that showed substantial disruption of plant DNA in ensiled maize.

ANIMAL PERFORMANCE AND PRODUCT COMPOSITION

There are two important considerations when GM crops are included in the rations of farm livestock. First, it is necessary to establish if the consumption of these feeds will affect the health and productivity of the animals as well as the composition of animal products whilst secondly, and possibly more importantly as far as the human population are concerned, will the consumption of these products cause any adverse effects on human health. In this latter regard, it is not possible within the confines of this paper to examine epidemiological evidence as this largely does not exist and the issue of allergenicity is outside of the remit of this paper. With the development of sophisticated analytical techniques however, (e.g. polymerase chain reaction -PCR, *in situ* hybridisation), it is now possible to determine if the meat, milk or eggs produced by farm livestock consuming feeds containing GM products contain any transgenic DNA, whilst several studies have also examined different animal products for the presence of introduced proteins.

Following the relatively recent introduction and commercialisation of several GM crops, a significant number of studies have been conducted to evaluate these as feeds for farm livestock. These have provided increasing evidence that the present generation of GM crops, none of which were developed to provide compositional changes, are substantially equivalent in terms of macro- and micronutrient composition. Data on carbohydrate composition (Aulrich et al., 2001), protein content, amino acid composition and lipid content (Padgette et al., 1996) for both GM maize and GM soya compared with their near isogenic equivalents is now quite convincing and has allayed many of the fears from earlier studies where some occasionally spurious differences were noted (Howard, 2002). A recent review (Clark and Ipharraguerre, 2000) of 23 different feeding studies involving dairy cows, beef cattle and poultry similarly concluded that current GM soya and maize varieties were substantially equivalent in terms of chemical composition, diet digestibility and feeding value to non-GM equivalents.

Ruminants

An early study (Hammond et al., 1996) comparing HT and non-GM soyabeans (both unprocessed) fed to lactating dairy cows [10% dry matter (DM) inclusion] reported no effects on total ration intake (23 kg DM/d), milk yield (35 kg/d) or milk fat and protein content. Studies in Germany (Daenicke et al., 1999) examined Bt and non-Bt maize silage fed to Holstein bulls during the finishing period and observed no significant effects on animal health, daily liveweight gain, carcass weight and composition, whilst parallel studies reported no effects on the apparent digestibility of the major nutrients. Feeding Bt and non-Bt maize silage (Rutzmoser and Mayer, 2000) detected no differences in diet digestibility (with sheep) and feed intake, milk yield and milk fat, protein, lactose and urea contents in dairy cows. More detailed analysis of the milk revealed no changes in the content of specific protein fractions, vitamins (A, B2 and E) and minerals (chloride, iodide). These findings were confirmed (Faust, 2000) when Bt and non-Bt maize were fed as green chop (30% DM inclusion) to lactating dairy cows, with no ill effects on animal health, productivity and milk composition whilst in a comparison

of Bt and non-Bt maize fed as grain and silage to dairy cows (Folmer et al., 2000), milk yield and composition were similarly unaffected (see table 1 below). These authors also examined the ruminal fermentation characteristics of Bt and non-Bt maize silage from either early or late maturing hybrids and did not detect any effects on *in situ* neutral detergent fibre (NDF) degradability, rumen pH or rumen volatile fatty acids (VFA) concentrations attributable to the different genetic constructs, although, as expected, differences between the two maturities were observed. Similarly when HT and conventional maize silage and grain were fed in identical mixed rations to lactating dairy cows (Donkin et al., 2000), DM intake, total milk yield, milk composition and total yield of individual milk components were unaffected, together with no differences in somatic cell counts and milk urea concentrations.

When beef cattle grazed Bt and non-Bt maize crop residues (Russell at al., 2000, 2001) over two years, no effects on animal performance were observed whilst a study (Hendrix et al., 2000) to examine the feeding of maize silage (to steers) and crop residues (to beef cows) over a similar period, (both Bt and non-Bt) found no overall effects on liveweight gain or DM intake. However feed/gain ratio was significantly increased for Bt compared with non-Bt silage whilst intake preference studies with cattle grazing crop residues revealed no differences due to genetic construct.

In a recent study (Petty et al., 2001a) Bt and near-isogenic non-Bt maize were fed as grain and silage to Angus and Simmental steers. During the growing period (average 87 days) when maize silage was a major component of the ration, no differences in daily liveweight gain and dry matter intake were noted although overall feed conversion efficiency was improved on the non-Bt ration, albeit in year 1 only. During the finishing period when dry rolled maize grain was a major part of the ration, no significant effects on feed intake, liveweight gain and feed conversion efficiency were noted together with no significant effects on carcass characteristics. In a similar study (Petty et al., 2001b) herbicide and non-herbicide tolerant maize were fed as silage followed by dry rolled grain to beef cattle and no significant effects attributable to the genetic constructs were determined with respect to feed intake, liveweight gain, feed conversion efficiency and carcass characteristics.

Non-ruminants

The study referred to earlier (Hammond et al., 1996) which found no effect of feeding HT and non-GM soyabeans to ruminants also included the same feeds in broiler rations (33% DM inclusion) and reported no significant effects on feed intake, daily liveweight gain or bird survival. Similarly, a 38 day broiler study comparing Bt and non-Bt maize grain (Brake and Vlachos, 1998) found no differences in bird mortality, feed intake or liveweight gain, but statistically significant improvements in feed conversion efficiency and breast meat yield were unexpectedly noted on the GM rations, possibly due to small improvements in overall diet composition. In a 35 day trial when the same maize types were fed (Halle et al., 1998) to broilers, no effects on feed intake, body weight gain, feed conversion efficiency and protein digestibility were noted, similar to results reported elsewhere (Sidhu et al., 2000). More recently, feeding trials with both laying hens and broilers (Aulrich et al., 1998) using Bt and non-Bt maize grain showed no differences in both protein digestibility and overall feed conversion efficiency.

Similar data are available for pigs with an earlier study (Böhme and Aulrich, 1999) showing no differences in protein digestibility and metabolisable energy content of HT and non-HT maize whilst comparison of Bt, non-Bt isogenic counterpart and commodity sourced (CS) maize (Weber and Richert, 2001) revealed no differences in average daily gain, feed intake or feed conversion efficiency. However the pigs fed CS maize had significantly heavier carcass weights due to increased killing out percentages whilst those fed the non-Bt control had reduced lean, with increased depth of backfat at the 10th rib and the P2 location compared with the other two treatments. Overall, the authors concluded that Bt maize had no adverse effects on growth performance or carcass characteristics.

More recently the results of a Canadian study to evaluate GM maize grain (var Chardon T25) and the non-GM isogenic counterpart fed to broilers has become an issue of debate between those seeking to establish the safety of this crop and those opposed, or exceedingly cautious, to the commercialisation of GM crops. Two experimental groups of 144 male birds were fed the GM or non-GM maize and the overall conclusion from the study was that feed intake, body weight gain and feed conversion efficiency were not affected. On the control diet, mortality of 3.5% was noted, and considered to be below the industry norm of 5-8% that existed at that time. In contrast, mortality was increased in the birds receiving the GM feed to 7% and attributed by the authors to normal metabolic disorders associated with rapid rates of growth. Furthermore, the difference was not statistically significant, but neither this nor the explanation provided has been recognised by those who strive to find any excuse to halt scientific progress.

Table 1. The effect of feeding Bt and non-Bt maize silage and grain to lactating dairy cows on milk yield and composition

Genetic variant	N4242		N7333	
	Non-Bt	Bt	Non-Bt	Bt
Milk yield (kg/d)	28.6	29.2	28.5	28.7
Milk yield (kg/d)	38.2	38.0	37.3	37.0
Milk yield (kg/d)	35.5	35.4	35.2	35.1

(Folmer et al., 2000)

METABOLIC FATE OF TRANSGENIC DNA AND PROTEIN

A large study undertaken in the USA with beef cattle and dairy cows fed Bt maize found no evidence of Bt DNA or Bt protein in samples (as appropriate) of muscle, spleen and whole milk. Similarly, when poultry were fed a ration containing 64% maize grain (as Bt or non-Bt) for 14 days, examination of dark and white muscle, liver, egg white and yolk failed to provide any positive detection of Bt DNA or Bt protein. A recently reported study with dairy cows (Faust, 2000) consuming GM maize also failed to detect any Bt protein in the resultant milk, but more importantly showed that when milk samples from cows consuming non-GM feeds were spiked with Bt proteins, subsequent analysis of these samples established 100% detection of the added proteins. Studies such as this are invaluable when attempting to allay concerns that the failure to detect transgenic DNA or protein in different samples may be indicative of methodological inadequacies rather than their absence from the products of animals consuming GM crops.

In a study (Ash et al., 2000) with laying hens, it was concluded that the CP4 EPSPS protein in HT soybeans could not be detected in either the liver, whole egg, egg white or faeces when using a double antibody sandwich ELISA specific for this protein, whilst examination of the raw soyabeans, soyabean meal and the complete diets used provided positive detection of the CP4 EPSPS protein. This led the authors to conclude that the digestive processes of the laying hen effectively degraded the CP4 EPSPS protein from the soybean portion of the ration such that no modified protein was detectable in animal products. A study with Starlink® maize fed to chickens (Japan MAFF, 2000) also reported no evidence of the *cry9c* gene or protein in the muscles, liver or blood of chickens that had received the experimental rations for up to 7 weeks. Meanwhile a further study with chickens fed HT soyabeans (Khumnirdpetch et al., 2001) reported no evidence of transgenic DNA in samples of muscle, skin, duodenum and liver taken after 1, 3, 5 and 7 weeks of feeding. With respect to pigs, the study involving a comparison of Bt and non-Bt maize, as referred to earlier (Weber and Richert, 2001), showed all loin muscle tissue to have no detectable levels of intact or immunologically reactive fragments of the Cry1Ab protein.

At the same time a study in the UK with lactating dairy cows (Phipps et al., 2002) examined the effect of including HT soyabean meal (CP4 EPSPS), at 26% (for 2 weeks) and 14% (6 weeks) of a total mixed ration, based on maize and grass silage and cracked wheat (all non-GM). This reduction in the inclusion rate was due to insufficient GM soyabean meal being available for the whole study period. Weekly milk samples were taken from all cows and together with weekly samples of the total mixed rations, these were subsequently analysed for transgenic DNA content using PCR. These data confirmed the absence of transgenic DNA in all milk samples of a size greater than 200 bp, establishing them to be not different in this respect from samples taken from the same cows immediately prior to introduction of the GM soyabean in their diet. In contrast, it was confirmed that all feeds taken during the experimental period were positive with respect for CP4 EPSPS soyabean meal DNA, whilst samples of feed taken immediately prior to the introduction of GM soyabean were confirmed negative.

More comprehensive studies have recently been reported from Germany (Einspainer et al., 2001) in which both Bt and non-Bt maize were fed as grain to chickens or as silage to beef cattle. In the poultry studies, maize was included at 50% of the total ration and after a suitable period of feeding the birds were slaughtered and their tissues were sampled. These were subsequently analysed for the presence of Bt gene fragments as well as the occurrence of a high abundance plant chloroplast gene, this latter approach being adopted as a marker to detect the possible transfer of DNA through the gastrointestinal wall of the animal. No Bt gene fragments were detected in any of the chicken tissues or in eggs, indicating no significant uptake of transgenic DNA and subsequent incorporation into the animal's tissues. In contrast, short (<200 bp) DNA fragments derived from plant chloroplasts were detected in liver, spleen, kidney and muscle, although no positive detections were made with respect to faeces or eggs. When the same procedures were repeated with both beef cattle and dairy cows which had received the Bt maize silage, it was similarly reported that no Bt gene fragments were detectable in any tissues whilst faeces and lymphocytes, and possibly milk, were all positive with respect to short DNA fragments of plant chloroplasts. In a parallel study these authors also reported the presence of the plant chloroplast DNA fragments in duodenal digesta obtained from fistulated cattle.

There have been other reports of ingested DNA fragments being found in mammalian tissues. In one study, microbial DNA was fed directly into the gastrointestinal lumen and tissues of mice (Schubbert et al., 1994) and fragments of this DNA were detected in some mouse white blood cells at 24 hours or more after initial exposure. The validity of this finding has however been questioned (Beever and Kemp, 2000), especially the type of DNA used, namely bacteriophage M13 DNA produced in *E coli*. It is suggested that this would not be methylated, in contrast to the DNA sequences found in normal plants and animals that are methylated. The significance of this difference is that unmethylated microbial DNA has been shown to upregulate inflammatory cell activity and stimulate a marked immune response (Sato et al., 1996).

CONCLUSIONS

Currently the only GM crops that are being grown commercially on any significant scale are those which contain genetic constructs that confer either herbicide tolerance or insect protection. With both maize (grain or forage) and soyabean, there is now considerable evidence that shows GM crops to be compositionally similar to their non-GM counterparts whilst numerous animal studies have failed to establish any significant differences in terms of gross animal performance, including voluntary feed consumption, whole tract digestibility or yield of animal product (milk, meat or eggs) per unit of feed consumed. A review of the processes of nucleic acid digestion in ruminants and non-ruminants has provided substantial evidence that the chances of intact DNA (either transgenic or native) being absorbed and incorporated into the host animal's genome are highly remote. Furthermore, studies undertaken to examine the effect of feed processing prior to consumption by the animal have shown that at temperatures of 93°C +, plant DNA has undergone substantial disruption, with the resultant DNA fragments being most unlikely to have any functional integrity. The limited amount of research that has examined the digestive fate of biotech proteins has also concluded that the normal processes of protein digestion in both ruminants and non-ruminants appear to be more than adequate to prevent any intact proteins being absorbed across the intestinal wall. There is now supporting evidence that the products from animals that have been fed GM crops do not contain any detectable amounts of transgenic DNA or biotech protein. However, one interesting observation from all of these studies has been the positive detection of DNA fragments of highly abundant plant chloroplasts derived from non-GM soya in a few animal fluids and tissues. It should be noted that these studies with the chloroplast DNA detection did not describe whether substantial care was taken to avoid accidental environmental contamination from the ubiquitous availability of this high copy plant gene. However, these results show that whilst the results might be unexpected since the processes of digestion appear to be a major route by which ingested plant DNA is degraded and rendered inert, the possibility of some DNA fragments being absorbed, albeit unlikely to be biologically active, cannot be ruled out. However it is also concluded that through the processes of pinocytosis or phagocytosis, the animal appears well placed to eliminate or degrade this DNA, thus rendering any chance of it being incorporated into the animal's genome as virtually impossible. Furthermore, it is important to state that this observation was made with a highly ubiquitous plant chloroplast gene, which is always likely to be present in much greater amounts that the amount of transgenic DNA in the diet of farm livestock, which in the case of a lactating dairy cow was estimated to be less than 0.00094% of total DNA intake.

It is concluded on the basis of evidence to date that the DNA of inserted genes or modified genes and/or their products (proteins) of those crops that are being commercially grown do not cause health problems in animals consuming GM crops and that they are not transferred to and accumulate with animal products. Thus, it is reasonable to conclude that the consumption of crop materials or animal products derived from GM crops does not pose any threat to animal or human health.

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