Transgenic plants in poultry nutrition

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Studies on genetically modified (GM) feedstuffs for poultry (and other livestock species) have not added any substance to public concerns in Europe about their safety for human or bird health. The compositions of maize lines engineered for insect resistance (*Bt*-maize) or herbicide tolerance (glyphosate) and herbicide-tolerant soybean have all proved to be essentially indistinguishable from their conventional counterparts. Consequently, and not surprisingly, comparative feeding studies with broilers and layers in which conventional maize (50 to 78%) or soybeans (27%) were replaced in feeds by transgenic varieties, also have failed to show differences of any significance in production parameters. These data indicate that feeding studies with target livestock species contribute very little to the safety assessment of crops engineered for input traits that have little or no detectable effect on chemical composition. However, comparative growth studies made with broiler chicks, particularly sensitive to any change in nutrient supply or the presence of toxic elements in their feed, can be used to screen for any unintended adverse consequence of the recombinant event not detected by compositional analysis. This does, however, depend on whether the GM plant can be matched to a parental line or another suitable control and its suitability for inclusion in broiler diets. The discovery that DNA fragments from the digestive tract can be found in the tissues of animals evoked interest in the fate of ingested transgenes. Plant DNA derived from feed has been detected in the muscle, liver, spleen and kidneys of broilers and layers, although not in eggs. However, no fragments of transgenic DNA or its expressed protein have been found to date in poultry meat or eggs or in any other animal tissues examined.

Keywords: transgenic plants; recombinant DNA; poultry feed; herbicide-tolerance; insect resistance; DNA breakdown; gene transfer

Introduction

Public concerns about the safety of recombinant DNA technologies have resulted in what is effectively an embargo on the growth of genetically modified crops in Europe. Only small areas are currently grown, usually for experimental purposes. Despite the concerns expressed in Europe the global planting of GM varieties continues to increase. The area devoted to transgenic plants in 2000 was estimated as 44.4 million hectares (109 million acres). This area increased by a further 19% in 2001 reaching 52.6 million hectares (130

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million acres). Four countries, USA, Canada, Argentina and China grew 99% of the world crop with a further nine countries accounting for the remaining 1% (James, 2001). Soybean and maize are the major transgenic crops grown (*Table 1*).

Table 1 Global plantings of the major transgenic traits in 2001 (from James, 2001).

Crop	Million hectares
Herbicide tolerant soybean	33.3
Insect resistant (*Bt*) maize	5.9
Herbicide tolerant rapeseed	5.7
Herbicide tolerant cotton	2.5
Bt and herbicide tolerant cotton	2.4
Herbicide tolerant maize	2.1
Insect resistant (*Bt*) cotton	1.9
Bt and herbicide tolerant maize	1.8
Total	52.6

In Europe much of the debate has centred on the use of ingredients derived from GM plants in food items, although latterly this has spread to sources of indirect human exposure, including products from animals fed GM feedstuffs. As is evident from *Table 1*, a major factor in determining future demand for non-GM versus GM varieties will be the feed market to which the bulk of all maize and soybean is destined.

Some supermarket chains have attempted to satisfy a perceived need amongst their customers for animal products, including eggs and poultry meat, produced only with feed declared free from transgenic ingredients. This has, in turn, put pressure on feed manufacturers and the larger poultry producers to locate sources of "GM-free" feed ingredients and to establish secure supply chains to preserve their identity. In practice, use of GM cereals in poultry production is relatively easily avoided. Transgenic varieties of wheat and barley, although now available, have yet to appear on the world market and Europe is largely self-sufficient in conventional maize. This not the case with soybeans, virtually all of which are imported. Already 74% of the American and 95% of the Argentinean crops carry herbicide resistant genes introduced by genetic engineering. Consequently, securing sources of identity-preserved, non-GM varieties necessary to meet supermarket requirements is becoming increasing more difficult and more expensive.

Outcrossing of GM plants with conventional varieties is perhaps the greatest concern voiced at present and the one with at least some justification. Under present definitions, organic farmers could have difficulty in maintaining their status if adjacent fields of the same crop were planted with a GM variety. Concerns about the possible effects of transgenic plants on human health are far more nebulous than those concerns relating to impact on the environment. This is particularly the case where routes of human exposure are indirect, as in the case of animal products. Any adverse response is far more likely to be seen in the production animals rather than the consumer of animal products. Aside from the possible undesirable effects of the deliberately introduced trait(s), which can be directly assessed, two other issues commonly raised in this context are:

- the possibility of (adverse) unintended effects accompanying the introduction of the intended trait(s);
- the possibility of gene transfer from the transgenic plant to the gut flora of the host or to the host tissues.

Comparative feeding studies

Some 40 feeding studies with GM feed ingredients with various animal species have been reported in the literature. Those involving poultry, summarised in *Table 2*, have included various lines of insect resistant (*Bt*) maize and glyphosate-resistant maize and soybean. In each case diets were formulated to allow a high proportion of the test material to be incorporated (50 to 78% maize or 27% soybean) and comparisons were made with parental or near isogenic lines.

Table 2 Comparison of chemical composition and nutritional value to poultry of GM soybean and maize kernels with conventional parental or near isogenic lines.

Transgenic feed ingredient	Results of compositional analysis	Poultry categories	Results of nutritional assessment
Herbicide-tolerant Soybean[1]	No significant differences	Broilers	No significant differences
Insect-resistant maize[2]	No significant differences	Broilers	Feed: gain ratio improved ($P<0.05$) in Bt group
Insect-resistant maize[3]	No significant differences	Broilers	No significant differences
Insect-resistant maize[4]	No significant differences	Broilers, layers	No significant differences
Insect-resistant and herbicide-tolerant maize lines[5]	No significant differences	Broilers	No significant differences
Insect-resistant soybean[6]	No significant differences	Broilers	No significant differences
Insect-resistant maize[7]	No significant differences	Broilers	No significant differences
Insect-resistant maize[8]	No significant differences	Broilers	Higher live weight gain ($P<0.05$) in Bt group
Herbicide-tolerant maize[9]	No significant differences	Broilers	No significant differences
Insect-resistant and herbicide-tolerant maize lines[10,11]	No significant differences	Broilers	No significant differences
Insect-resistant maize[12]	No significant differences	Broilers	No significant differences

References: [1]Hammond *et al.*, 1996; [2]Brake and Vlachos, 1998; [3]Aeschbacher *et al.*, 2001; [4]Aulrich *et al.*, 2001; [5]Gaines *et al.*, 2001; [6]Kan *et al.*, 2001; [7]Mireles *et al.*, 2000; [8]Piva *et al.*, 2001; [9]Sidhu *et al.*, 2000; [10]Taylor *et al.*, 2001a; [11]Taylor *et al.*, 2001b; [12]Tony *et al.*, 2002.

In each study, the chemical composition of the GM feed ingredients proved to be essentially indistinguishable from its conventional counterpart. Consequently, and not surprisingly, comparative feeding studies with broilers and layers also failed to show differences of any consequence in the various production parameters monitored. There were two studies in which significant differences were observed, but these were not considered cause for concern. Piva *et al.*, (2001), observed a higher live weight gain in the test group compared to the control group, but ascribed this to a lower mycotoxin content in the *Bt* maize compared to the conventional maize used in the diet of the control group. It has been widely recognised that better control of the European Corn Borer can result in less damage to the maize kernel reducing the opportunity for infection by the common field fungi responsible for some mycotoxin production (Munkvold and Hellmich, 1999; Valenta *et al.*, 2001). In an earlier study (Brake and Vlachos, 1998), a small but significant improvement in feed to gain ratio was observed for the test group, but the value remained well within the range typical of other maize lines. Feeding studies made with other classes of livestock have produced very similar results (see Flachowsky and Aulrich, 2001; Aumaitre *et al.*, 2002; Faust, 2002).

The insect resistance or herbicide tolerance introduced into most existing commercial GM varieties are agronomic traits and have little or no measurable effect on feed composition or the bioavailability of nutrients. As *Table 2* shows, the gross composition of such GM varieties falls within the range normally associated with conventional varieties of the same feedstuff and the evidence to date is that they behave as any other variety. This suggests that for those GM plants with modified input traits, provided that compositional equivalence can be concluded, nutritional equivalence can be assumed. For such GM varieties, routine feeding studies made with poultry or other target species would appear to add little to a safety assessment.

Few transgenic plants that address product quality or feeding value for livestock have reached the field trial stage of production, although many experimental constructs exist. Typical of these are modifications to storage protein to increase protein content or to modify the amino acid profile of the seed (Molvig *et al.*, 1997; Nikiforova *et al.*, 2002), reduction in the content of undesirable substances such as phytate (Spencer *et al.*, 2000) or introduction of novel enzymes for better nutrient use (Baah *et al.*, 2002). As changes to the composition of the transgenic plant become more extensive it becomes increasingly difficult to make comparisons with a conventional counterpart. This is particularly the case when the transformation event also affects the bioavailability of the major nutrients. For simple input traits, establishing compositional and agronomic equivalence to a conventional counterpart allows the safety assessment to focus on the deliberately introduced traits. Where compositional equivalence cannot be concluded, then the assessment must give greater emphasis to the whole plant in addition to the introduced trait(s). Under these circumstances, feeding studies with the main target species assumes a far greater importance. Any nutritional parameter is a product of many metabolic processes, changes to any one of which can influence the overall response. Consequently, obtaining the expected (predicted) production response offers evidence that the intended changes are functioning as intended and that inadvertent effects introduced by the transformation are absent or, if present, have no adverse consequences for the bird/animal. The high amylopectin starch potato is the only case to date of a transgenic plant with modifications to a major nutrient that has sought release in Europe. However, this product was directed to the starch industry rather than animal feeding. Limited feeding studies were made, but as the modification was to starch granule structure and not to total starch production and as feeding studies were made with heat-treated material, it was possible to make direct and meaningful comparisons with the parent line. Consequently, there has been no body of experience of feeding studies with nutritionally modified GM plants in which comparisons to a parental line is not possible. However, the International Life Science Institute (ILSI) is in the process of preparing guidelines for experiments of this type.

Unintended effects and their detection

Given the uncertainty associated with the introduction of new genetic material into a plant, there remains a remote possibility of unintended effects not detected by chemical analysis that could have unforeseen consequences for recipient animals or for humans. The incidence of unintended effects in conventional breeding, which escape detection during development, is very low and, as a consequence, there has been no pressure to develop more refined protocols that might allow their recognition. There is, in any event, no reason to suppose that the incidence of unintended effects is significantly greater when recombinant DNA methods are used. In time, improved molecular characterisation of recombinant events and understanding of the implications of the events for the metabolism of the plant should remove even this low level of uncertainty.

In the meantime, there is an opportunity to use the growth rate of broilers as the outcome measure to examine GM products for unintended changes. Because of their rapid weight gain, broilers are particularly sensitive to any change in nutrient supply or the presence of toxic elements in their feed. Their use is, however, dependent on being able to match the GM material to a parental line or another suitable control and is limited to those plant materials suitable for inclusion in broiler diets. For such studies, the expectation based on the preceding chemical analyses would be that the growth rate of chicks fed the control and transgenic lines would not be significantly different. In the unlikely event of any deviation, this would trigger further investigations to determine the cause and its implications.

The fate of transgenic DNA and protein in poultry

Naked DNA/RNA released from the food matrix is rapidly degraded in most compartments of the gastro-intestinal tract. However, there is a constant leaching of DNA into the gut lumen as the feed matrix is disrupted and plant genes, or at least amplifiable fragments of the genes, can be detected for many hours after feeding (Hohlweg and Doerfler, 2001). A small proportion of marker DNA (1-2% of that originally ingested) has been traced from the intestinal contents of mice (1-2%), via the intestinal wall and the peripheral white blood cells to the spleen and liver (Schubbert *et al.*, 1997). The foreign DNA was exclusively found in the nucleus of the host cells covalently linked to chromosomal DNA. Subsequent work traced the ingested marker DNA in pregnant mice to the placenta and into the foetus and the newborn animal (Schubbert *et al.*, 1998). Plant chloroplast (*rubisco*) DNA fragments similarly could be amplified from intestinal contents and cells from the spleen and liver, but there was no evidence of expression. More importantly, routine monitoring of DNA from tail tips of the living mice failed to detect *rubisco* and intramuscular injection of marker DNA demonstrated the progressive elimination of foreign DNA from somatic cells (Hohlweg and Doerfler, 2001).

As might be expected following the work of Schubbert and colleagues, fragments of plant DNA have been detected in the tissues of many livestock including poultry and in a variety of animal products. Fragments of plant chloroplast DNA were found in muscle, liver, spleen and kidneys of broilers and layers (Aeschbacher *et al.*, 2001; Einspanier *et al.*, 2001; Klotz *et al.*, 2002) but not in eggs or in litter. No transgenic DNA has been detected in birds fed transgenic maize (Faust, 2000; Einspanier *et al.*, 2001; Anon, 2001) or soybean meal (Khumnirdpetch *et al.*, 2001). The evidence to date indicates that transgenic DNA behaves as any other DNA (Chambers *et al.*, 2002) and whether any particular gene (including a transgene) is detected in livestock tissues is largely a product of the sensitivity of the detection method. Fragments of plastid (e.g. chloroplast) encoded genes are far more likely to be detected than nuclear genes because of their copy number.

The metabolic processes involved in the digestion, absorption and utilisation of amino acid and peptides by livestock species do not wholly preclude the incorporation of intact (transgenic) proteins into animal products. However, the vast majority of proteins are synthesised *de novo* from an amino acid pool. In the case of egg proteins the site of synthesis is usually the liver and they are exported as specifically tagged lipoproteins. Drug and other residues generally are transferred to eggs because of their high lipid solubility (Kan and Petz, 2000). Thus it would be very unlikely for an expressed protein of any plant gene to be found intact in poultry meat or eggs and none have been detected to-date (Faust, 2000; Ash *et al.*, 2000).

A future for transgenic feedstuffs?

Recombinant DNA technology has much to offer poultry producers ranging from transgenic feed ingredients better matched to the nutrient requirements of the bird to alternative methods for the control of *Eimeria* spp. However, before any transgenic material can introduced into the food chain it must first be demonstrated as safe for the bird and for the consumer of poultry products. Above all, it also must be acceptable to a European public increasingly concerned about the safety and means of production of its food supply. Unfortunately, there is no evidence at present that consumers in Northern Europe see any real benefit in GM technology applied to crops or are willing to accept its widespread use in food production.

This is likely to present real difficulties in the future for a poultry industry dependent on imported feed protein. As the proportion of the world soybean crop devoted to GM varieties grows, segregation becomes increasing difficult in the main producer countries. There is already evidence from the USA that many farmers no longer consider it worthwhile. An inevitable consequence will be that some major retail outlets will have to withdraw from claims that their poultry items are produced without recourse to GM feeds.

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