

GM Science Review Open Meeting

GM animal feed: safety implications for the food chain

Tuesday 11 March 2003
Agriculture & Food Science Centre
Newforge Lane
Belfast

Introduction

This open meeting was organised to explore publicly the science associated with genetically modified (GM) animal feed and safety implications for the food chain. It is one of a series of nationwide meetings, each contributing to the GM Science Review, part of the three-strand national dialogue on genetic modification.

Present were three members of the GM Science Review Panel, Professor Bert Rima of the Medical and Biological Centre, Queens University, Belfast; Professor Philip Dale of the John Innes Centre, Norwich; and Dr Chitra Bharucha, Chair of the Advisory Committee on Animal Feedingstuffs. The members of the Panel listened to and questioned the evidence of the scientific experts, and listened to and noted questions and comments made by members of the public attending the meeting.

Each of the scientific experts gave short presentations on their own areas of expertise, including:

- Manipulation of nutritional properties of animal feed and the effects of food processing on DNA (Professor Mike Forbes, Faculty of Biological Sciences, University of Leeds);
- Metabolic fate of transgenic nucleic acids and novel proteins in farm livestock (Professor David Beever, School of Agriculture, Policy & Development, University of Reading);
- Fate of GM feed DNA fragments in animals (Professor Ralf Einspanier, Institute of Physiology, Technical University, Munich, Germany); and
- Future GM crops: edible vaccines (Dr Colin Fleming, Department of Applied Plant Science, Queen's University, Belfast).

Interested members of the public, interest groups, scientists and government officials were present at the meeting and contributed to a wide-ranging discussion.

Themes and issues

This report organises the dialogue that took place during the meeting thematically, rather than chronologically. The themes that emerged from the presentations given by the scientific experts are grouped under the headings of economics and the manipulation of output traits, DNA: from feeds to animal products, and future GM crops and animal vaccines. The final section, consequence analysis and safety implications for the food chain, summarizes questions from the Panel and the audience, and the answers given by the scientific experts.

In summary, the use of GM animal feed raises two major issues. The first is associated with modifications in crop plants: do metabolic changes have the potential to be anti-nutritional or to increase the allergenicity of plant material, and will gene flow occur to distribute such changes within or between species? The second is associated with the health of animals eating modified crop plants and potential 'knock-on' effects on secondary products such as meat, milk and eggs, and on the health of humans who consume them.

Economics and the manipulation of output traits

Although the subject of this public meeting was to review the *science* of GM, basic economic issues were included in Professor Forbes' short discussion about the differences between the supply chain for GM crops with modified input traits (e.g. herbicide resistance) and for those with modified output traits (i.e. improved nutrition). Professor Forbes stated that the production of GM crops with modified output traits only makes economic sense if genes are stacked, that is, if modification of more than one trait occurs in the same crop plant.

Dr Fleming also discussed output traits in the form of edible vaccines (see below) and pointed out that this type of GM could represent an important new crop for farmers interested in diversifying—particularly in a location such as Northern Ireland.

DNA: from feeds to animal products

Effects of feed processing on DNA

It is important to examine the effects of feed processing on DNA if it is of concern that modified DNA remains intact and moves into the food chain. Fragments of DNA that are smaller than 200 base pairs are generally considered to be too small to transmit genetic information. Based on this level of DNA fragmentation, Professor Forbes' research team carried out several experiments to find out if the processing of animal feeds makes the transmission of genetic information impossible.

Animal feeds are produced in a variety of ways. For example, oil is extracted from rape seed or soya beans, sugar beet pulp is dried, crops are made into silage, and grains are heat treated. In many cases, raw plant material is simply fed to animals without any processing.

Two sorts of experiments were carried out by the research team. The first was to measure the size of the DNA fragments that remained after different treatments. The second was to look for the survival of specific sequences of plant genes following processing. The results from both of these experimental approaches were in agreement. DNA was not fragmented to any great extent in raw plant material and silage (as it is made under typical UK conditions), but remained intact. However, later Professor Beever referred to research undertaken in Germany indicating that ensiling of maize silage does lead to the partial fragmentation of plant DNA. When wheat grains were subjected to 95 degrees Celsius for at least 5 minutes, the DNA was completely fragmented.

This means that some animal feed production processes may fragment DNA to a level that is considered to be safe. Under such treatments, the DNA would not enter the food chain. However, cereals and other plant materials are not normally exposed to 95 degrees Celsius for 5 minutes, and DNA may not be fragmented at all in other production processes, so Professor Forbes concluded that if GM crops were grown to feed animals, animals would be likely to be eating modified DNA.

Tracing DNA fragments and protein in animal products

Any investigation into whether modified DNA or novel proteins consumed by animals have the potential to affect animal health, or to enter the food chain, should consider the fate of these molecules within the animal.

Professor Beever was keen to put into context the actual amounts of DNA entering the digestive system of a typical dairy cow. Dairy cows can consume 24 kg of dry matter per day. If a dairy cow's ration includes 60% GM maize (as silage or grain) per day, then that cow is eating approximately 60 grams of total DNA a day, 54 micrograms of which is transgenic DNA.

A clear and simple biochemical explanation of the digestion process was provided. Digestion of nucleic acids (DNA and RNA) occurs through the action of nucleases present in the mouth, the pancreas and the intestinal secretions. In ruminants, additional microbial and physical degradation of food occurs, and a great deal of nucleic acid in the rumen and in the small intestine is actually of microbial origin—synthesized by the microbes present. Evidence suggests that more than 95% of DNA and RNA is completely broken down within the digestive system.

In addition, research carried out on the digestion of transgenic proteins in *in vitro* culture has shown nearly complete digestion occurring within 5 minutes in the

presence of pepsin. This is one way to test whether novel transgenic proteins are likely to move into the food chain.

A great deal of evidence was put forward by Professor Beever from the work of his research team, and of other teams, which does not support any differences in lactation performance, dry matter intake, fat composition or protein content in dairy cows fed with GM plants. A review of studies of dairy cattle, beef cattle, swine and chickens found no evidence for differences when the animals were fed GM crop material in terms of animal health. Milk from dairy cattle fed GM plants has been found to contain no modified protein or transgenic DNA fragments.

The research carried out by Professor Einspanier and his research team has concentrated on the fate of DNA fragments in animals. He began by explaining that there are two sources of DNA in the plant cell: nuclear and chloroplast. Because the chloroplast contains highly enriched genomic material, more of this sort of DNA is taken up by the animal; therefore, it is easier for scientists to detect these highly abundant genes in tissues and cells of animals and in animal products. Professor Einspanier made it clear that it is only due to this relatively easy detection that chloroplast DNA was used as an indicator in their experiments. As yet, no new transgenic plants are being constructed with the insertion of modified chloroplast genes, although this may be the case in the future.

Small lengths of non-GM plant DNA were found in the lymphocytes but only occasionally in the milk of cows fed exclusively with transgenic BT maize but no transgenic DNA was found. Plant DNA was found in the organs of chickens, but only in the beginning of the digestive tract in the pig and not in the lymph nodes or the blood. Neither Bt maize DNA, nor proteins specific to transgenic soya beans, have been found in animal tissues or products.

A number of studies were proposed by Professor Einspanier that could be carried out to help to fill in knowledge gaps, including: long-term studies focusing on the fertility of livestock; whether transgenic DNA or novel proteins have an effect on rumen bacteria in cattle; what mechanism is involved in the transfer of plant DNA across the intestinal wall; and studies involving different animal developmental stages.

The PCR¹ technology used by Professor Einspanier's group can be used to detect around five copies of a gene in a sample. As it is likely that fewer than five copies of *nuclear* transgenes would appear in a sample, it is not possible currently to detect whether secondary products, such as meat, eggs and milk, are derived from GM-fed or non-GM-fed livestock. However, if it is modified highly enriched *chloroplast* DNA that is of interest, then PCR will be able to detect transgenes

¹ Polymerase chain reaction (PCR) is a rapid and specific method for amplifying (making many copies of) a DNA base sequence; it can also be used to detect the existence of a defined sequence in a DNA sample.

and, in theory, a test kit could be developed to detect modified DNA, for example in chicken products on supermarket shelves.

There are difficulties in measuring the absolute amount of plant DNA passing through an animal as there is no numerical basis for extraction efficiencies in such different samples. Rough measurements can only be taken in a relative manner, for example, the numbers of copies of a single gene present in fresh plant material compared to silage may be reduced to one in thirty, and from silage to where the gene appears in the lymphocytes of a cow there may be a reduction to one in fifty thousand.

It is nearly impossible with current technology to trace the fate of DNA in human subjects. If plant DNA is of interest, human subjects would have to eat a large amount of plant material for several weeks, but even the ingestion of several grams of plant DNA per day would not be achieved. If homologous sequences in plant and animal food are of interest, the subject's own DNA can also be detected, and precise detection is impossible.

Future GM crops and animal vaccines

A new class of GM crops under development—plants designed to deliver edible vaccines—was discussed by Dr Fleming. This technology was conceived in response to the idea that economic and practical problems associated with vaccination programmes in under-developed regions could be solved with vaccines delivered through the diet. Veterinary science would also benefit from edible vaccines to treat animal diseases.

Vaccination currently involves a process of challenging the immune system with a weakened live virus or bacterium, or with a sub-unit or piece of the pathogen (which is considered to be safer). However, sub-unit vaccines require refrigeration and sometimes the use of injections, while edible vaccines would not. In addition, edible vaccines could be produced locally, would be more cost-effective, and have the potential to generate a greater level of mucosal immunity.

Functional antigens have been produced experimentally in transgenic plants. These antigens are not broken down in the gut and even small amounts of ingested antigen appear to be effective in creating an immunological response.

Consequence analysis and safety implications for the food chain

This section summarises the issues raised by members of the Panel and the audience, and answers given by the scientific experts. It was not always the case that issues were addressed by a single expert or immediately after they were raised, so this section endeavours to draw similar elements together.

One of the main concerns of the Panel members was what Professor Rima termed 'consequence analysis'. By this, he meant safety assessment of the consequences of modified DNA within the animal. Does this DNA move through the animal intact? What is the fate of DNA in the animal? Can it move through abrasions in the mouth of the animal? Is there an effect on the health of the animal? Does DNA enter the food chain? Does it have any effect on the environment and/or in terms of human health?

As discussed above, fragments of DNA smaller than 200 base pairs are considered to be too small to transmit genetic information. One issue surrounding the use of GM crops has been that many were modified using antibiotic resistance genes as markers, and it was not known whether this antibiotic resistance was transmissible to microbes in the gut of the animal or to the genome of the animal itself. The use of antibiotic resistance genes is being phased out, and it has been postulated that such genes found in humans are actually the result of widespread use of antibiotics in human medicine and animal agriculture.

Although Professor Forbes stated at the beginning that he would not be talking directly about safety issues, when questioned by the Panel, he did discuss some work that he had been involved in investigating the transfer of antibiotic resistant DNA from GM crops into gut and oral microorganisms. They found that no transformed material survived in the digestive system beyond the true stomach in the chicken, nor beyond the rumen in the sheep.

A potential consequence that Professor Dale enquired about was whether the regulatory process was robust enough to cope with many different transgenes inserted into a single crop plant (gene stacking) and the possible interactions between them. Professor Forbes answered this question by talking about the interactions between genes that might occur with gene stacking. He felt that interactions were less likely to occur if genes were modified for dissimilar traits within the same crop plant (e.g. for both herbicide resistance and for increased levels of a protein), and that testing for safety where there are multiple modified genes would not necessarily be any more complicated than in cases of a single gene modification. As to whether transgene interactions should be viewed and assessed differently to interactions between non-transformed genes, Professor Forbes replied that he did not know.

When asked whether any other studies could be carried out to assess negative impacts on animal welfare, Professor Beever replied that every new and novel GM crop or case of stacked genes does need to be tested rigorously, but he did not know what other studies needed to be done. Although it can never be proved absolutely that GM animal feed will not change the composition of the product, adversely affect the welfare of animals, or produce human consumables containing functional transgenic material, research has so far given no indication that this is the case.

There have been no indications, in extrapolation from tests on laboratory animals, that modification of the metabolic process in animal feeds might have immunological and nutritional impacts on the crops, which could have a 'knock on' effect on livestock products consumed by humans. As it would be very difficult to introduce 100 times the dose of modified protein into a dairy cow, pharmacological-style testing in livestock animals is impossible.

A point that was made by different speakers during the meeting is that experiments have not been carried out on animals under different levels of stress, but Professor Beever pointed out that, in dairy cows at least, the production of 8–9000 L of milk per lactation is probably a stress in itself. It was suggested that the gut of animals stressed just prior to slaughter are more permeable and it is possible that material (e.g. bacteria) might pass into the circulatory system at this time. If this was found to be the case and GM material was more likely to pass into the circulatory system, then GM feed could be withheld from the animals in the same way that growth promoters are withheld for a period before slaughter.

Crops modified to produce edible vaccines have similar risks and safety issues as other GM crops. For example, there is a risk that gene flow could occur between GM plants and non-GM plants, although it is unlikely that the production of edible vaccines will ever occur out of a greenhouse setting. One approach to minimizing or eliminating the potential for gene flow would be to use sterile species, such as the banana, to deliver the vaccines.

Another potential risk is that the antigens present in the plant material may have an unanticipated detrimental effect on the animals eating them. Dr Bharucha questioned the lack of information about other immunological consequences that might be associated with edible vaccines. Dr Fleming replied that testing for secondary effects is currently being developed with the aim of working out regulatory schemes for certification in the USA. He thought that current and developing legislation would be sufficient to provide checks and balances in regard to animal welfare.

In a discussion about the experiments carried out by Professor Einspanier's group, an audience member made the comment that the finding 'not detected' should include clear information about the limits of detection. Professor Einspanier replied that these limits are set out in the scientific publications, but in the case of his research, if there are five or more copies in a sample, they can be detected using current technology. Variation in the ability to detect copies of DNA will nevertheless occur due to differences in the size of the gene of interest, the amount and type of DNA consumed by an animal (and produced by microbes in the gut of the animal), and where the sample is taken from. In the future, improvements in technology will most likely mean that even smaller amounts of DNA will be able to be detected.

Another question was whether findings of plant DNA fragments in the muscle of chicken is a result of the DNA being present in the circulation and somehow

moving through the muscle, rather than being present actually inside the muscle cells. Professor Einspanier reassured the questioner that it would be very unlikely to be the result of environmental contamination arising from laboratory practices, but that he could not say whether some other sort of contamination was taking place within the animal itself.

Although the development of chloroplast engineering has the potential to reduce the likelihood of gene flow between plants, one member of the audience wondered whether this same technology might mean that the novel insert would be more likely to get into the food chain, given the results of Professor Einspanier's research. Because such plants are not yet available for experimental purposes, it can only be postulated that if novel chloroplast inserts are as highly abundant as the gene Professor Einspanier has amplified and traced, then they will also be detected.

There was a general discussion about the fact that in the US and China, people have been eating GM food as a part of their diet for some years now, without any reported adverse effects. In addition, within the context of a normal diet, animals eat a great deal of DNA from all kinds of sources—their own, and from microbes, plants and other animals. In addition, many transgenes are actually present in the environment. Therefore, the general questions to emerge were: 'what is different about GM crops?' and 'what are the implications for safety assessment?'

The two questions were answered in a variety of ways. One comment focused on DNA, such that in essence modified DNA is no different from unmodified DNA as it is made up of the same four base pairs that make up the DNA of every living thing, although it was recognized that concern has arisen because it is not known whether novel *sequences* of DNA are a threat to animal or human health. Another series of comments focused on proteins: first, that plants under stress produce different types and amounts of proteins compared to non-stressed plants and that these can be toxic, and second, that if novel proteins are produced by GM technology, they must be tested rigorously for potential allergic reactions and toxicity.

In addition, a member of the audience suggested that science at its most responsible must recognize cases of high uncertainty, and that this is particularly relevant when scientific discourse overlaps with economics and regulation as it did during this meeting.

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