Benefits and risks of genetic modification of animal feeds

D Spencer[1], CL White[2], TJV Higgins[1]

[1]CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2601
[2]CSIRO Livestock Industries, Private Bag PO Wembley, WA 6014

Summary

Genetically modified (GM) plants have been widely grown in commercial quantities in the Americas since the mid nineties. The only commercial planting of a food or feed plant in Australia is cotton. The rapid adoption of the new crops indicates that growers recognise their benefits. These are largely more sustainable production and more environmentally friendly use of land. The nutritive value of the existing GM plants are considered to be substantially equivalent to their non-modified counterparts. There are GM plants under experimental evaluation, however, that are likely to be nutritionally enhanced. Some examples include grain with reduced levels of anti-nutritional factors, increased levels of vitamins and grains with enhanced profiles of essential amino acids. There are pasture plants in development that could offer increased levels of digestible energy for grazing livestock. There is community concern that GM plants and the food or feed derived from them pose new threats to environmental sustainability and health safety. There are stringent regulations in place that will ensure that these threats are minimised and the evidence to date is that the GM plants are at least as safe as their non GM counterparts.

Introduction

The traditional method of introducing desirable new characters (traits) into a plant or animal is to seek out closely related donor lines of the target plant or animal – ones that exhibit the desired trait - and make a sexual cross between the donor and target species. The progeny of this cross will contain the new, sought-after trait, but will require a number of generations of backcrossing to the target parent line in order to select out the undesirable traits. This is the basis of traditional plant and animal breeding methods that humans have used for many thousands of years. Almost all the plants and animals that we farm today are products of this procedure.

Genetic modification (also known as gene technology, genetic engineering, genetic manipulation or recombinant DNA technology) is a much more recent addition to the options available for improving the performance of our plants and livestock. Organisms derived through the use of recombinant DNA technology are called genetically modified (GM). The techniques for genetic engineering only started to become available in the 1980s. Already, on the world scene, over 80 different plant species have been genetically modified and over 25,000 field trials have been carried out with genetically modified plants. The area planted to GM crops worldwide was 40 M ha in 1999, an increase of 44% since 1998, and a 20-fold increase since 1996. Most of the applications to date have involved genes for resistance to herbicides, insect pests and viruses in commercially important crops such as soybeans (54% GM), corn, canola, cotton (61% GM), potatoes, squash and papaya. The main countries in which GM crops are grown commercially are the US, Canada and Argentina, with increasing amounts in China and several other countries. There have been no documented reports of any adverse effects on humans or animals resulting from ingestion of commercially released GM plants.

Genes are the basic units of heredity. Plants have approximately 50,000 different genes, each one specifying a particular hereditary trait. In the sexual crossing procedures employed in plant breeding, the offspring receives half of their genes from each parent. The situation is very different in genetic modification procedures because the target plant receives only one or two new genes and these are genes that confer the trait that will bring about the desired improvement in the progeny. In other words, gene technology is a much more directed procedure. It differs from the traditional procedures of improvement in yet another major way. The genes conferring the new trait may not only come from close relatives of the target species, but also can come from any other living organism - plant, animal or microorganism. Breeders are no longer restricted to making crosses only within or between closely related species. This is possible because all living things share the same genetic code, and therefore a given gene specifies the same trait no matter in what organism it is expressed. This breakthrough has been made possible by the development of procedures to isolate specific genes from one organism and introduce them into another, unrelated organism so that they become a part of the hereditary apparatus of the recipient organism.

Gene technology has the capacity to greatly increase the efficiency of converting sunlight, water, air and minerals into high quality milk, meat and fibre for human use. It is seen by most leading scientists as an important tool to meet the needs of a rapidly expanding human population in a sustainable way. Gene technology can impact on all aspects of the production system. For feed production, advantages include lower costs through reduced fertiliser and chemical inputs, greater yields through better adapted plants and greater resistance to diseases and parasites. For animal production, advantages include higher production efficiency through the availability of feeds of higher nutritive value and reduced disease incidence through plant-borne vaccines and anti-parasitic compounds. For the environment, the application of gene technology can lead to reduced damage through reduced use of chemicals on crops and pastures and reduced emissions from animals of waste that contributes to greenhouse gases (ammonia, CO_2 and methane), acidification of soils (N) and contamination of water and soil (N and P).

In this paper we will describe some cases where gene technology is being used to improve either the productivity or the quality of plant products that are used for animal feed and discuss the benefits and risks associated with this relatively new technology.

Improving animal productivity by improving the feedstuffs

Improving the protein value of feeds

Wool is rich in the sulfur-containing amino acids (cysteine and methionine) and the growth of wool in sheep is limited by the availability of these amino acids in the diet. Because sheep can convert methionine to cysteine, but not the reverse reaction, methionine is the more critical of these two essential protein building blocks for wool growth. Plant protein ingested by sheep is largely broken down by the rumen microflora and converted into microbial protein which has a significantly lower sulfur amino acid content than the plant protein. The microbial protein then passes into the gastro-intestinal tract where it is hydrolysed into amino acids that are assimilated into the blood stream. The efficiency of this digestive process in terms of animal performance is limited by the supply of sulfur amino acids needed for wool growth, with Merino sheep typically utilising only 20% of the protein they ingest. The remainder is excreted and contributes to greenhouse gases and soil and water contamination. An ideal dietary protein for sheep, therefore,

is one that is rich in methionine and resistant to breakdown by the microorganisms in the sheep's rumen. Such protein passes from the rumen into the lower gastrointestinal tract where it is hydrolysed by proteases and the liberated amino acids, including the sulfur-amino acids, assimilated into the blood stream. A protein meeting these specifications has been identified in sunflower seeds and the gene coding for this protein has been isolated and characterised (1,2). Using plant gene technology procedures, this sunflower seed albumin (SSA) gene was introduced into lupins and a line of genetically modified lupin was developed in which 4% of the seed protein was SSA and the methionine content was increased approximately 100% (3). Feeding trials with rats showed that the genetically modified lupin seeds gave statistically significant increases in live weight gain, true protein digestibility, biological value, and net protein utilisation, compared with seeds from the non-transgenic parent line (3).

Lupins are the major pulse crop grown in Australia. Approximately one half of the Australian crop is exported and about half of the remainder is fed out to sheep during the late summer period when pasture growth is low. It is during this period of nutritional stress that wool growth is reduced, a weak zone occurs in the fibre and the overall quality of the wool is reduced. Sheep feeding trials have now been carried out to compare the nutritive value of the genetically modified, high-methionine lupins with that of the non-modified parent line. Over a six week feeding period, sheep fed high-methionine lupins had an 8% higher rate of wool growth and a 7% higher live weight gain than those fed the same amount of the non-transgenic lupins (4). The sulfur concentration of the wool and the cysteine content of the plasma were also increased in sheep fed the modified lupins. This is a clear-cut example of the use of gene technology to improve the nutritive quality of animal diets while reducing environmental damage through reduced emissions of nitrogen.

Grain legumes in general are characteristically low in the sulfur amino acids and, as a result, methionine is one of the first limiting amino acids for any animal (ruminant or non-ruminant) diet in which legume seed meal is a major source of protein. The enhanced nutritional value of genetically modified lupins containing the methionine-rich SSA gene has been demonstrated for broiler diets (5).

Subterranean clover is the major legume component of improved pastures in southern Australia and a major component of the diet of the grazing animal, particularly sheep. Current research is aimed at introducing the SSA gene into subterranean clover and in this way improving the nutritive quality of this important pasture legume. Initial attempts to achieve this goal resulted in the successful introduction of the SSA gene into the genome of the plant but the SSA protein accumulated at only very low levels. The isolated SSA gene was then modified by the addition of a short sequence coding for a targeting peptide of six extra amino acids at the C-terminus of the protein. This peptide directs the accumulation of the SSA protein to lumen of the endoplasmic reticulum rather than the vacuole of the leaf. This modification resulted in a tenfold increase in the level of SSA protein accumulated in the subterranean clover leaves. The SSA protein made up between 0.3 and 1.3% of the total leaf protein (6). Although this level is not yet sufficient to bring about a measurable increase in wool growth, this result does demonstrate, in principle, the feasibility of using this gene technology approach to improve the nutritive value of Australian pasture species. Research is continuing in an effort to increase the level of accumulation of SSA in the leaves of genetically modified pasture legumes.

Another strategy for improving the protein value of feeds for grazing livestock involves genetic modification of condensed tannins in forage legumes. This procedure protects the plant proteins against ruminal degradation, thereby increasing the flow of plant protein to the intestine (7). Genes and regulatory elements involved in the biosynthetic pathway for condensed tannins are being cloned for insertion into white clover and lucerne .

Increased digestibility of pasture plants

A senescent (mature) pasture has low feeding value and can be the major limitation to animal productivity. Digestibility declines mostly due to the loss of cell contents such as water soluble carbohydrates which are highly digestible, and to the increase in indigestible cell walls. The loss of the soluble cell contents is associated with mobilisation of this material for the reproductive phase of plant development.

A possible approach to retaining some soluble cell content in senescent pasture is to produce water soluble carbohydrates that are resistant to mobilisation during seed filling. An example is the production of a fructan that is resistant to the fructan exohydrolases (FEH) that exist in plant cells. Such a fructan could be derived from a bacterial source. This approach is being pursued using genes encoding fructosyl transferase from oral bacteria (8).

Another approach is to reduce the amount of indigestible lignin present in the plant. A naturally-occurring mutant of maize, sorghum and millet corn (brown-midrib) with low lignin content already exists, and has been demonstrated to increase intake over the normal variety by 30% in ruminants (9).

Removal of anti-nutritional substances from cereal grains

The endosperm cell walls of many cereal grains are rich in arabinoxylans and glucans, which, respectively, are polymers of xylose and glucose. Both these polymers have been found to be anti-nutritional in monogastric animals, particularly in poultry (10). This activity is thought to be due to the increased viscosity of the solubilised polymers in the small intestine leading to reduction in the rate of digestion and absorption of nutrients. This problem is currently alleviated in the feed industry by treating cereal grain with xylanases and glucanases to hydrolyse these polymers to their respective monomers. These enzymes are obtained from microbial cultures for this purpose. Recently, Patel *et al.* (11) have explored the feasibility of using gene technology to equip the microbial genes coding for these enzymes with promoters from cereal seed genes and introducing them directly into cereals where they would accumulate only in the seed. To date they have inserted the modified fungal xylanase gene into barley and found it accumulated in the endosperm of the developing seed. The enzyme retains its activity during seed maturation and desiccation and during storage at 37^0C for at least six months. In addition, it is active at the temperatures used commercially to pre-treat cereal grains. The genetically modified barley grains produce xylanase activity that is 354 times as active as the endogenous xylanase produced in non-transgenic grains and it does not appear to affect seed germination. Clearly, this strategy has great potential for using barley grains as an alternative source of feed enzymes.

Improved phosphorus utilisation from feed grains

Phytate (myo-inositol hexaphosphate) is the main storage form of phosphorus in both cereal and legume feed grains. The phosphate is re-mobilised during germination by the action of the endogenous phytases which catalyse the hydrolysis of phytate into inositol and inorganic phosphate. However the mature seed has little or no phytase activity and there are no phytases in the digestive system of monogastric animals (12). As a result, there is very poor utilisation of this dietary source of phosphorous and inorganic phosphorus must be added to stock feeds to correct this deficiency. In addition, phytate is known to render essential mineral elements, such as iron, calcium, magnesium, and zinc, unavailable. A further concern is that the undegraded phytate excreted by the animal contributes to the environmental burden of phosphate that finds its way into the waterways. To overcome this problem, it has become commercial practice to add a preparation of GM phytase from the fungus *Aspergillus niger* to stockfeed mixes. This approach has been shown to result in better phosphorus utilisation and improved growth in pigs and poultry. Pen *et al.* (13) took this approach a step further by introducing the Aspergillus phytase gene into tobacco and adding the milled transgenic tobacco seed (containing 1% of its protein as phytase) into the feed mixes. In feeding trials with chickens they found an improved growth rate, comparable to that obtained with diets supplied with either fungal phytase or inorganic phosphate. Brinch-Pedersen (12) extended this approach still further by introducing the fungal phytase gene into wheat, which is the major cereal component of feeds for non-ruminant animals.

Enhanced vitamin E content in transgenic plants

Stockfeed components such as soybean, corn and canola, are the major sources of vitamin E (α-tocopherol) in the diet of monogastric animals. However, in most oil seeds the major tocopherol component is the relatively inactive precursor γ-tocopherol rather than the more methylated end product, α-tocopherol. Recently, Shintani and DellaPenna (14) have used genetic engineering to enhance the level of α-tocopherol in seeds. They first isolated the gene for γ-tocopherol methyl transferase, an enzyme that catalysed the final step in the synthesis of α-tocopherol. They equipped this gene with a seed-specific promoter, and introduced it into *Arabidopsis thaliana* to produce a transgenic plant with >95% of its total tocopherol in the active α-tocopherol form. Although no animal feeding trials have yet been reported with oil seeds modified in this way, this work shows promise for raising the vitamin E level of oil seed crops in general. Any increase in the vitamin E content of grains should also assist grazing animals. Vitamin E deficiency occurs in grazing sheep during periods when no green feed is available and supplementary cereal or lupin grains are the main source of energy. A survey of the vitamin E status of sheep flocks showed that 54% of flocks of weaner sheep in Western Australia had biochemical evidence of vitamin E deficiency (White Muscle Disease) during autumn (15). Similarly, 30% of adult ewe flocks in the Middle East had mean levels of vitamin E in the deficient range when housed during winter (16). The deficiency results in reduced reproductive performance in ewes and sudden death from heart failure in weaner sheep.

Delivery of vaccines via transgenic plants

Recent work by Arntzen's group has explored the possibility of using genetically modified plants to deliver immunogen- or antigen-based vaccines orally (17). The gene for the immunogen

subunit of *E.coli* enterotoxin was expressed in tobacco leaves and potato tubers. In both cases, mice fed leaves or tubers from these transgenic plants developed a strong immune response to this pathogen. In a further development, it has been shown that both B-cell and T-cell epitopes of recombinant hepatitis B surface antigen are expressed in transgenic tobacco leaves (18). This work has exciting implications for the low cost delivery of vaccines to improve the health of humans and their livestock.

Improved animal feeds through improved plant production

The above examples all involve the use of genetic engineering to improve the nutritive value of animal feed by directly altering plant composition. Any modification which improves the productivity of animal feed crops also has a benefit, albeit an indirect one, for animal production. These benefits flow from reduced cost of production, higher yields, reduced dependence on chemical pesticides and reduced usage of herbicide. A few examples of the use of genetic engineering in this context are given briefly below.

Pea weevil resistant peas

Field peas are an important ingredient in stockfeed mixes. One of the two major insect pests of the pea crop is the weevil *Bruchus pisorum*. This insect lays its eggs on the immature pea pod and the emergent larvae burrow through the pod wall and into the developing seed. During their subsequent development into the adult weevil, the larvae consume a significant proportion of the seed contents. This results in a serious reduction in yield, in feed quality and in the viability of the seed. The weevil is currently controlled by chemical sprays. Schroeder *et al.* (19) used genetic engineering procedures to introduce into pea the gene for an α-amylase inhibitor protein found in the common bean seed. This inhibitor, expressed specifically in the pea seed, blocks the digestion of starch by the larvae and their development is completely halted at an early stage. Feeding trials with rats (20), and humans (21) have shown that the α-amylase inhibitor protein has no anti-nutritional activity in monogastric animals.

Better pastures, improved animal productivity

Subterranean cover and white clover are two important pasture legumes in Australia. They not only provide high quality feed for the grazing animal but also contribute to increased soil fertility because of their ability to fix atmospheric nitrogen. This increased soil fertility in turn contributes to increased growth of associated non-leguminous pasture grasses. Viruses, such as alfalfa mosaic virus (AMV) are responsible for serious reductions in yield of pasture legumes. By introducing into white clover a gene for the AMV coat protein, Kalla and colleagues (22) have been able confer protection from attack by AMV and avoid the consequent reduction in pasture yield.

The control of broad-leafed weeds is a major problem in the establishment of subterranean clover-based pastures and the options for control are limited because of the lack of selectivity of most chemical sprays. Currently, the herbicide bromoxynil is used for this purpose because subterranean clover is partially tolerant to this chemical. However, there is a penalty as the growth of subterranean clover is reduced by 30-40% by this treatment. Dear *et al.* (23) have introduced into subterranean clover a gene for the enzyme nitrilase which catalyses the breakdown of bromoxynil. The addition of this additional gene for nitrilase (isolated from a soil

bacterium) confers an added degree of tolerance to the herbicide such that clover growth is unaffected by commercial rates of the herbicide. As a result, the pastures can be treated at a much earlier growth stage when the broad-leafed weeds are more readily controlled with lower doses of the herbicide.

Risks associated with plant gene technology

There is no such thing as zero risk associated with any human activity – risks to people and risks to the environment. For most of our actions, we assess whether the benefits outweigh the risks. It can be said at the outset that whilst genetically modified food products have been on the market and consumed by humans since 1994, there has been no documented case in which any ill effects of these products have been observed. The major foods in this category are based on corn, soybeans and canola containing new genes conferring resistance to herbicides and to insect pests. Some of the concerns relating to the use of genetically modified products in feeds and foods are discussed below.

The introduction of antibiotic resistance genes into the gut microflora

In the most commonly used procedures for plant genetic engineering, the foreign gene is introduced into a fragment of plant tissue and this tissue is cultured so as to produce a new, genetically modified plant. When a new gene is introduced into a plant it is usually attached to a second gene that confers an advantage for growth of the cells that have taken up the genes. This second gene may confer resistance to an antibiotic such as kanamycin. These antibiotics, are toxic to most plants and are no longer in general use for humans. When the antibiotic is added to the tissue culture medium, only the transgenic cells in the tissue fragment (those carrying the new genes) can divide, grow and eventually form a new plant containing the introduced genes. The antibiotic resistance gene is referred to as a selectable marker because it allows us to selectively "mark" and promote the growth of the very few cells in the treated tissue fragment that have taken up the new genes. At the same time, the growth of the non-transgenic cells in the tissue fragment is suppressed by the antibiotic. As a result, the regenerated plant then contains the new genes in all its cells. There is some concern that if people or animals consume a plant product containing an antibiotic resistance gene, the gene may be transferred into the microflora in their gut and render them unresponsive to treatment with this antibiotic in the future.

It would take a series of highly improbable events for an antibiotic resistance gene ingested as a component of a transgenic plant to be incorporated into the genome of intestinal microbes. It is highly probable that the DNA (like all the other DNA in the ingested food) would be degraded in the stomach or intestine in the course of normal digestion. Further, although the antibiotic resistance transgene was originally isolated from a microorganism, it would have been modified in the test tube to ensure that it was recognised by the plant gene expression system. These changes would mean that it could not be expressed in another microorganism without reversing these modifications. This would also be a highly unlikely event in the gastrointestinal tract. The need for a series of such unlikely events means that the overall probability of a transfer of DNA from a genetically modified plant to an animal's gut microbes is vanishingly low.

Microbial geneticists studying antibiotic resistance have found that microbes with resistance to the "older" antibiotics, such as kanamycin, are widespread throughout the human and livestock population as a result of their extensive use in the 1950s and 60s. By the late 1960s, clinical

isolates of intestinal microorganisms in individuals from many parts of the world were found to be resistant to these antibiotics. The first introduction of antibiotic resistance genes into plants did not occur until the early 1980s and such plants were not grown commercially until the early 1990s.

For these reasons the regulatory authorities based in the Interim Office of the Gene Technology Regulator (IOGTR) do not place a blanket ban on the use of antibiotic resistance genes as selectable markers in plant gene technology. They have adopted a policy of considering each proposal involving the use of antibiotic resistance genes on its merits. Their aim is to prolong the useful life of the more modern antibiotics and to allow the use in plants of those antibiotic resistance genes that are known to be already widespread in humans and their livestock.

Allergens and toxins

Plants contain many compounds that have proved to be allergenic to a small proportion of the humans who consume them. People who have food allergy problems do their best to identify their particular problem allergen and adjust their diet to avoid these compounds. They are naturally concerned at the prospect of new compounds (perhaps not even of plant origin) being introduced into food plants by means of gene technology. Similarly, several of the foreign genes that are currently being engineered into plants encode proteins that are known to be anti-nutritional or are feared as being potentially toxic. For example, a gene for a bacterial toxin (Bt toxin from soil bacterium, *Bacillus thuringiensis*) has been introduced into a range of commercial crop species, including cotton and corn varieties and these latter lines are now widely grown in North America.

The possible presence of both allergens and toxins in food products from non-genetically modified plants is already considered in great detail by the regulatory bodies. This role in Australia is the responsibility of the Australian and New Zealand Food Authority (ANZFA); in the USA, it is the Food and Drug Administration (FDA). Most of the potential problems can be overcome with scientifically-based food labelling. Known allergens, such as food colouring compounds, some preservatives and wheat gluten, are already listed on prepared food packages. Allergy sufferers are well aware of the need to check new food products for potential allergens. Regulations are presently being finalised in Australia and New Zealand to ensure the same level of protection applies to food products containing ingredients from genetically modified plants.

Since allergies are very specific reactions, consumers need to be told the precise source and chemical nature of the new gene product. For example, non-GM wheat, peanuts, soybeans, tree nuts, milk, eggs, fish, and crustacea account for over 90% of documented allergies worldwide. If genes from any of these organisms were introduced into a transgenic plant, that plant would receive special scrutiny by the regulatory agency.

There are a variety of well established tests to test for the toxicity or allergenicity of any food or compound. In Australia, the regulatory authorities monitor the potential toxicity of the product of any foreign gene introduced into a plant product and approval is only granted where the compounds in question have been shown to be harmless in animal feeding trials or have a history of safe use in food crops. This has proved to be the case for the Bt toxins mentioned above, for the proteins that render plants resistant to herbicides and for an α-amylase inhibitor protein from bean which renders pea plants resistant to destruction by the pea weevil. On the other hand,

testing has identified some gene products that are allergenic or toxic and the development of these products has been discontinued. For example, a gene coding for a sulfur-rich protein from Brazil nut was transferred into soybean to improve its nutritional value. Brazil nuts are known to be highly allergenic for some people. Tests showed that the allergenicity of the Brazil nut had been transferred to the modified soybean. This was the first time that the specific allergenic protein in Brazil nut had been identified. This work on the development of a more nutritious soybean was promptly discontinued. This successful screening process has been promoted by some groups as demonstrating the dangers of gene technology. Scientists feel that it shows the strengths of the technology and the effectiveness of the screening procedures.

Food safety

There is concern that food derived from genetically modified plants is unsafe because of the technology used to develop the plants. For instance, one concern is that the fate of the new genetic information and the resultant proteins is different from existing genes and protein in food. Questions have been raised about whether the new genes could be transferred to animals and whether they could cause adverse health effects in the animals themselves or in the humans who consume the animal products. This last concern arises because of worries about the genes or their products accumulating in the meat, milk or eggs of animals fed genetically modified plants. Both the World Health Organisation and the US Food and Drug Administration have concluded that there is no inherent risk in consuming DNA, including that derived from genetically modified plants. In addition there are established regulatory requirements that must be met before release of each genetically modified plant. These requirements include a spectrum of rigorous safety tests before the plants can be grown commercially. Beever and Kemp (24) conclude that "there is a growing body of scientifically valid information that shows no significant risk associated with the consumption of the DNA or the proteins from GM crops.... Based on the safety analyses required for each crop, consumption of milk, meat and eggs produced from animals fed GM crops should be considered to be as safe as traditional practices".

The risks of not making use of gene technology

There may also be risks if the procedures of plant gene technology are not further developed and the potential benefits from this area are not fully realised. Given the inevitable increases in the world population in the next fifty years, significant increases in the total production of food and fibre will be needed to feed, clothe and house this population. To achieve this it is imperative that every means of improving the efficiency of agricultural production should be explored. The only alternative to more efficient production will be a large increase in the area of cultivated land. This will inevitably involve marginal land with a lower productive capacity. The consequences of this will be loss of tree cover, soil erosion, loss of species diversity, reduction of habitat for endangered species, increased soil salinity problems and eventually, a general reduction in living standards worldwide. Plant gene technology has the potential to make a contribution to reducing the likelihood of these consequences. Along with other measures, such as new decision support tools, better machinery, more efficient use of water resources and fertilisers and continuing improvements in plant breeding, the use of genetically engineered plants may in fact help to preserve the forests, the wetlands, the arid lands and the endangered species.

References

1. Kortt AA, Caldwell JB, Lilley GG, Higgins TJV. Amino acid and cDNA sequences of a methionine-rich 2S protein from sunflower seed (*Helianthus annuus* L.). Eur J Biochem 1991;195:329-334.
2. McNabb W, Spencer D, Higgins TJV, Barry, TN. In vitro rates of proteolysis of ribulose-1,5-bisphosphate carboxylase (Rubisco) from lucerne leaves, and of ovalbumin, vicilin and sunflower albumin 8 storage proteins. J Sci Food Agric 1994;64: 53-61.
3. Molvig L, Tabe LM, Eggum BO, Moore AE, Craig S, Spencer D, Higgins TJV. Enhanced methionine levels and increased nutritive value of seeds of transgenic lupins (*Lupinus angustifolius* L.) expressing a sunflower seed albumin gene. Proc Natl Acad Sci USA 1997;94:8393-8398.
4. White CL, Tabe LM, Dove H, Hamblin J, Young P, Phillips N, Taylor R, Gulati S, Ashes J, Higgins TJV. Increased efficiency of wool growth and liveweight gain in Merino sheep fed transgenic lupin seed containing sunflower albumin. J Sci Food Agric 2000;(in press).
5. Ravindran V, Bryden W, Tabe LM, Higgins TJV, Molvig L. Evaluation of high-methionine transgenic lupins in broiler diets. Proc. Aust. Poult. Sci. Symp. 1998;10:208.
6. Khan MRI, Ceriotti A, Tabe L, Aryan A, McNabb W, Moore A, Craig S, Spencer D, Higgins TJV. Accumulation of a sulphur-rich seed albumin from sunflower in the leaves of transgenic subterranean clover (*Trifolium subterraneum* L.). Transgenic Research 1996;5:179-185.
7. Tanner G, Joseph R, YuGang L, Stoutjesdijk P, Larkin P. Towards bloat-safe pastures. Feed Mix 1997;5:18-21.
8. Jenkins CLD, Simpson RJ, Higgins TJV, Larkin PJ. Transgenic white clover and lucerne containing bacterial fructan as a novel carbohydrate for grazing animals. Proc Fourth Int Fructan Symp 2000;(in press).
9. Cherney JH, Cherney DJR, Akin DE, Axtell JD. Potential of brown-midrib low-lignin mutants for improving forage quality. Advances in Agronomy 1991;46:157-198.
10. Choct M, Annison G. Anti-nutritive activity of wheat pentosans in broiler diets. Brit Poultry Sci 1990;31:811-821.
11. Patel M, Johnson JS, Brettell RIS, Jacobsen J, Xue G-P. Transgenic barley expressing a fungal xylanase gene in the endosperm of the developing grains. Molec Breeding 2000;6:113-123.
12. Brinch-Pedersen H, Olesen A, Rasmussen SK, Holm PB. Generation of transgenic wheat (*Triticum aestivum* L.) for constitutive accumulation of an *Aspergillus* phytase. Molec Breeding 2000;6:195-206.
13. Pen J, Verwoerd TC, van Paridon PA, van den Elzen PMJ, Geerse K, van der Klis J, Versteegh HAJ, van Ooyen AJJ, Hoekema A. Phytase-containing transgenic seeds as a novel feed additive for improved phosphorus utilization. Bio/Technology 1993;11:811-814.
14. Shintani D, DellaPenna D. Elevating the vitamin E content of plants through metabolic engineering. Science 1998, 282: 2098-2100.
15. White CL, Rewell L. Diagnostic indicators of deficiencies of vitamin E and selenium in sheep during autumn in south-western Australia. *In* G. Stone, et al., eds. Nutritional Ecology of Herbivores. Posters and Plenary Discussions and Papers presented at Satellite Symposia and Seminar held in conjunction with the Fifth International Symposium on the Nutrition of Herbivores. (CD-ROM), San Antonio TX, 1999.

16. White CL, Treacher T, Bahhady FA. Mineral and vitamin status of sheep in Syria, Jordan and Turkey. *In* D. G. Masters, et al., eds. Mineral problems in sheep in northern China and other regions of Asia, Proceedings of a workshop held in Beijing, September 1995. pp 61-67. 73 ed. Australian Centre for International Agricultural Research, Beijing, 1996.
17. Haq TA, Mason HS, Clements JD, Arntzen CJ. Oral immunisation with recombinant bacterial antigen produced in transgenic plants. Science 1995; 268: 714-716.
18. Thanavala Y, Yang YF, Lyons P, Mason HS, Arntzen CJ. Immunogenicity of transgenic plant-derived hepatitis B surface antigen. Proc. Natl. Acad. Sci. USA 1995; 92: 3358-3361.
19. Schroeder HE, Gollasch S, Moore A, Tabe LM, Craig S, Hardie DC, Chrispeels MJ, Spencer D, Higgins TJV. Bean α-amylase inhibitor confers resistance to the pea weevil (*Bruchus pisorum*) in transgenic peas (*Pisum sativum* L.). Plant Physiol 1995;107:1233-123.
20. Pusztai A, Bardocz GGS, Alonso R, Chrispeels MJ, Schroeder HE, Tabe LM, Higgins TJV. Expression of the insecticidal bean α-amylase inhibitor transgene has a minimal detrimental effect on the nutritional value of peas fed to rats at 30% of the diet. J Nutr 1999;129:1597-1603.
21. Garrow JS, Scott PF, Heels S, Nair KS, Halliday D. "Starch blockers" are ineffective in man. The Lancet 1983;Jan 1/8:60-61.
22. Kalla R, Chu P, Spangenberg G. Molecular breeding of forage legumes for virus resistance. Proc 2nd Int Symp Mol Breeding of Forage Crops: 2000 (in press).
23. Dear BS, Sandral GA, Spencer D, Higgins TJV. Benefits and risks of transgenic pasture plants. Agric Sci 1997;10:39-42.
24. Beever DE, Kemp CF. Safety issues associated with the DNA in animal feed derived from genetically modified crops. A review of scientific and regulatory procedures. Nutrition Abstracts and Reviews, Series B: Livestock Feeds and Feeding 2000;70:175-182.