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TRANSGENIC FOOD ANIMALS FOR AUSTRALIA AND NEW ZEALAND

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SUMMARY
Despite 20 years of research on transgenic animals in Australia and New Zealand, there are currently no transgenic animals being grown for food or fibre production in these countries, or indeed in the developed world. This situation contrasts with the cropping industries where several transgenic plants are approved and available. This review covers some of the technical, commercial, regulatory and social issues that may impact on the application of transgenic technologies to food animal production in Australia and New Zealand.

Keywords: transgenic, livestock

THE TECHNOLOGY
The transfer of DNA into the germ-line is central to the production of a transgenic animal, requiring transport of a DNA construct across cellular and nuclear membranes, and insertion into the genome of a multipotent cell. Several approaches have been taken to mediate this transport. The original and most common method is micro-injection of DNA into a newly fertilised zygote. In livestock species, this approach is highly inefficient (0.8-1.0%) (Niemann and Kues 2000) and the animal produced is generally a somatic cell mosaic, and needs to be bred in the traditional manner to confirm the presence of the transgene in the germ-line. This adds to the expense and duration of the development process, but nonetheless, micro-injection is still commonly used. Recombinase enzymes coupled to the DNA, are reported to increase the efficiency of micro-injection (Maga *et al*. 2003). Technical improvements have led to some increases in efficiency, though the net efficiency remains low.

Nuclear transfer is another technology commonly utilised to produce transgenic animals. It involves the transfer of a nucleus from a somatic cell into an enucleated oocyte. A somatic cell is engineered and used as the source of the nucleus to generate an embryo. The reduced life expectance of cloned animals has compounded the challenges of transgenesis by limiting application of this technology. Recently cloned piglets have been produced by the injection of a whole cell into the cytoplasm of an enucleated oocyte (Lee *et al*. 2003), but these animals died from heart failure within 6 months.

Alternative methods are being developed in mice that may have an impact in this field. One such technique is sperm mediated gene transfer, which uses sperm as the carrier of transgenic DNA during the fertilisation process. Foreign DNA may be introduced by simple co-incubation with sperm (Gandolfi 2000) or by linking the DNA to sperm with antibodies, with efficiencies of up to 37% reported (Lavitrano *et al*. 2003). Electroporation and intra-testicular injection of transgene DNA are other recent advances (Rieth *et al*. 2000). Improvement in the techniques of culturing spermatogonial stem cells of livestock animals will also have impact, with engineered spermatogonial stem cells of mice used to generate transgenic sperm that were able to fertilise oocytes, resulting in 4% of the progeny being transgenic (Nagano *et al*. 2001).

Retroviral-mediated transfer of transgenes is another promising technology as these viruses are able to infect a cell and insert their nucleic acid into the host genome. Lentiviruses are a specialised type of retrovirus with a broad host range. They have been used successfully to produce transgenic mice (Rubinson *et al*. 2003) and, given their broad host range, these vectors have significant potential in livestock species. Other technologies that could combine with those above to advance our ability to generate valuable transgenic animals include: homologous recombination that may be useful for single nucleotide modification as well as for gene deletion (Denning *et al*. 2001); RNA interference (RNAi) technology for a reduction in gene expression (Rubinson *et al*. 2003); artificial chromosomes that allow the use of large transgene fragments (Robl *et al*. 2003); and transposon-mediated gene transfer that enhances the integration of gene fragments into the chromosome (Geurts *et al*. 2003).

TRANSGENIC LAND ANIMALS

Australian and New Zealand scientists have been involved in generating transgenic animals since the technology was first established. This has included applications to the dairy industry, the wool industry, the control of pest fish species, and the production of transgenic animals as donors of tissue for xenotransplantation.

Internationally, a wide variety of transgenic livestock have been generated. For example, pigs have been produced that express the enzyme, phytase, in their saliva to improve the utilisation of feed-derived phosphate, and decrease the environmental impact of their waste (Golovan *et al*. 2001). Other research includes cows with modified milk proteins, deletion of prion genes from sheep and cattle, growth hormone in sheep, insulin-like growth factor (IGF) in pigs, and various pharmaceutical proteins expressed in the milk of sheep and cattle.

Cows expressing the gene for β and κ casein (Brophy *et al*. 2003) have been generated in New Zealand, with a 20% increase in the levels of β casein, and 100% increase in the levels of κ casein. A similar project to increase the levels of Alpha S1 casein is being undertaken by the Dairy CRC in Australia. In New Zealand, transgenic sheep have also been produced that express the growth factor, IGF-1, in the wool follicle using a keratin promoter (Su *et al*. 1998). The first generation animals had up to a 17% increase in wool production compared with the control animals. However, in the F2 animals, no significant differences in wool production could be observed between control and transgenic animals despite the transgene still being expressed.

In Australia, the gene for ovine growth hormone has been over-expressed in sheep under the control of the metallothionein promoter. The transgenic animals showed improved performance in growth, body fat and wool production. More recently, transgenic rams from this experiment have been bred and the progeny assessed (Adams *et al*. 2002). Associated with the increased expression of growth hormone was a reduction in fat depth, increase in wool yield and increase in liveweight of the progeny animals. This effect was variable between strains of sheep, indicating that underlying genotypes may impact on the effect of a transgene.

At Adelaide University, transgenic sheep have been produced with the aim of generating different wool types or increasing efficiencies of wool production. Transgenic sheep that over-express sheep wool keratin genes (Bawden *et al*. 1999) have been generated. Over-expression of the type II intermediate filament gene, K2.10, resulted in changes in lustre and crimp of the wool (Bawden *et al*. 1998).

TRANSGENIC AQUACULTURE

International research on transgenics in aquaculture is growing rapidly. Fish and shellfish are highly fecund, fertilisation is often straightforward, and the fertilised eggs develop outside the body and, therefore, no extensive manipulation such as reimplantation is required. Thus, the production of transgenic fish or shellfish is quite easy. Within Australia, the focus is more on the possible use of transgenesis to control feral populations, such as the European carp.

Internationally, work on commercial aquatic species has centred on the production of freeze resistant Atlantic salmon. These fish were engineered in an attempt to extend the range of sites that the salmon could be grown (Fletcher *et al*. 1999). Other transgenic fish produced have been transgenic for growth hormone (GH). Coho Salmon (*Oncorhynchus kisutch*) transformed with a GH construct have shown an 11-fold difference in weight compared with controls 15 months post-fertilisation (Devlin *et al*. 1995). Transgenic fish research has also included attempts to improve carbohydrate metabolism to increase feed efficiencies, and to produce fish transgenic for antibacterial peptides to decrease farmed fish susceptibility to bacterial infection.

In Australia, current research is focused on the control of feral populations of fish. A project, called Daughterless Carp, is designed to produce transgenic fish that only produce male progeny on breeding. This work is predicted to lead to a population reduction, but not elimination, of the carp (http://www.marine.csiro.au/LeafletsFolder/pdfsheets/Daughterless_carp_13may02.pdf). A similar technology is being researched to control the pacific oyster (*Crassostrea gigas*).

Table 1. Survey of transgenic livestock species, existing or predicted, that should be of interest to Australian or New Zealand animal food producers in the next 5 years.

Animal	Genes introduced or deleted	Performance criteria (consumer benefit)
Bovine	β and κ casein	Increased expression of casein proteins (improved protein content of milk)
Bovine	Intestinal lactase	Reduction of lactose in the milk (lactose-intolerant people)
Bovine	Lysostaphin	Mastitis resistance (reduced use of antibiotics)
Bovine	β-lactoglobulin	Increased production of this protein in milk, as well as increased growth and disease resistance in calves feeding on the milk (reduced antibiotic use and improved health benefits)
Ovine	Growth hormone	Increased growth rates, increased feed conversion efficiency, decreased carcass fatness, and increased lactation (leaner meat)
Ovine	Myostatin	Reduced myostatin expression and increased muscle in sheep (leaner meat)
Porcine	Insulin-like growth factor 1	Increased growth rate and reduced carcass fatness (leaner meat)
Porcine	Bovine α-lactalbumin	Increased growth rate and improved health of piglets (unknown consumer value)
Porcine	Spinach Stearoyl CoA desaturase	Modified lipid composition (increased unsaturated fats)
Porcine	Phytase expressed in saliva	Utilisation of phopshorus bound to phytate by the pig, and hence a reduction in waste phosphorous (environmental impact reduced)
Caprine	Lysostaphin	Cure or prevention of *Staphlococcus. Aureus* mastitis (reduced antibiotic use)
Caprine	Rat Stearoyl-CoA desaturase	Modified milk fat composition (increased unsaturated fatty acid proportion)
Caprine	Human lysozyme	Modified milk fat composition and enhanced immune responses (reduced antibiotic use and modified milk
Common carp	Growth hormone	Increased growth rate (cheaper fish)
Chinook salmon	Growth hormone	Increased growth rate (cheaper fish)
Silver sea bream	Growth hormone	Increased growth rate (cheaper fish)
Japanese abalone	Growth hormone	Increased growth rate (cheaper fish)
Rainbow trout	Growth hormone	Increased growth rate (cheaper fish)

ISSUES AROUND APPLICATION

Whilst transgenic animals are unlikely to be part of a traditional animal production enterprise in the near future, in the longer term, they are likely to be important. The value that transgenic animals will bring is the rapid introduction of new phenotypes/genotypes into elite animals, and the generation of novel phenotypes of significant value (Table 1).

Consumer acceptance of transgenic animals is a major issue. In the cropping industry, transgenic plants have suffered opposition because of public concerns based on environmental impact. Whilst transgenic livestock are more difficult to produce than transgenic plants, they do have the advantage that it is much easier to contain the animals and, therefore, control the risk. For transgenic livestock to be accepted, there has to be a significant consumer advantage to their use and consumption (Table 1).

In recent times, there have been significant technological advances that make the production of transgenic animals more of a feasible reality. These include the advances in genomics and in our understanding of the genetic systems that work to regulate phenotype. Other advancements include improvement in the ability to transform cells, especially spermatogonial stem cells. Gene expression regulation techniques are also improving with research in the area of RNA interference. All these technologies combined provide a strong platform on which to go forward and produce transgenic livestock.

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