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6. Genetically modified livestock and poultry and their potential effects on human health and nutrition

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6.1. Introduction

The first methods for genetic modification of animals were developed in the 1980s and many of the ideas for applications of the technology to livestock and poultry were suggested at that time. Genetic modification of animals from the main livestock and poultry species is now possible. Several different technologies have been developed, with different uses depending on the specific modifications required, but there are still significant advances required to enable efficient and sophisticated modifications to be generated. Many possible applications of transgenic technologies to livestock and poultry have been identified. The two main areas of application are in agriculture and in human healthcare. The applications discussed focus on developing alternatives to methods currently in use, for example traditional animal breeding, with the potential added value of the ability to introduce genetic changes that cannot be achieved by other methods. Transgenic technology could offer alternative production systems to fulfil unmet needs that differ across geographical and socioeconomic regions of the world. Applications relevant to human healthcare include modifications to animal food products but are primarily targeted at healthcare products. There are many similar applications in development or proposed for genetically modified (GM) animals and poultry and also some applications specific to different species. The majority of research and development has concentrated on genetic modification of mammals, often using the mouse as a valuable model system. In contrast to the major developments in use and potential uses of GM plants in agriculture and specifically for food, the main focus of applications of GM technology in livestock and poultry is in applications relevant to human healthcare. There are currently no transgenic livestock or poultry food products on the market and there are unlikely to be for some years to come. However, several companies have started to produce GM animals with the aim of bringing their products to market.

The methods used to produce GM livestock and poultry differ in risk, both to animals and to humans. The risks associated with specific modifications will require analysis on a case by case basis, depending on the use of the GM product. New food products will require assessment in much the same way as GM plant products are analysed. The risks to the environment of GM livestock and poultry are considerably less than those potentially associated with GM plants or fish. There is a substantially lower likelihood of escape and dissemination of GM livestock and poultry, owing to the lack of competition with wild and related animals and bird species. However, in comparison with other GM organisms, there are major public concerns about the acceptability of modifying domesticated animals and about the welfare effects of any modifications.

6.2. Potential contributions of transgenic technology in livestock and poultry

The possible applications of transgenic technologies to livestock and poultry fall into three main areas: animal production, human nutrition and healthcare, although there is overlap between areas for some applications. Many of the applications discussed below (Sections 6.2.2–6.2.4) are in the early stages of research and

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development. Only applications in the area of healthcare are significantly advanced to the stage where GM products will be brought to market.

6.2.1. Transgenic technology in livestock and poultry

Livestock

The majority of methods developed for genetic modification of livestock have been based on methods that have proved successful in mice (Wilmut & Clark, 1991). The first method used to generate transgenic livestock was pronuclear injection of eggs, a method first developed in mice. Fertilised eggs are recovered from donor females, a gene construct is injected into one of the pronuclei of each egg and the manipulated eggs are transferred to recipient mothers for development to birth. This method is successful in all livestock species, but the efficiency varies between species and now its use is limited. Transgenic animals produced by this method contain copies of the injected gene construct stably integrated into the host chromosomes. The integrated transgenes are often found as multicopy arrays of a small number up to hundreds of the original construct. Expression of transgenes produced by pronuclear injection varies between different transgenic lines and may vary between individual animals within a transgenic line. A significant proportion of transgenic animals (20–100%) do not express the integrated transgenes, an effect known as transgene silencing (Chicas & Machino, 2001). This method can only be used to add novel genes. Other strategies allow replacement or alteration of endogenous genes. This offers the major advantage of enabling control of specific gene expression.

In mice a major advance in transgene technology resulted from the isolation of embryonic stem (ES) cells, which can be manipulated *in vitro* and animals recovered via generation of chimeras with normal embryos. ES cells can be modified by transfection of gene constructs (gene addition) but the most significant use has been the development of gene manipulation by homologous recombination. Transgenes containing sequences homologous to the gene to be targeted, flanking the sequence to be added or modified, are transfected into ES cells, and cells in which the added sequence has replaced the endogenous sequence are identified. These selected cells are used to generate animals that contain the modification to the endogenous gene. This method can be used to add, alter, delete or replace sequences in endogenous genes. Insertion of a foreign gene into an endogenous gene results in more reliable expression of the transgene. Despite the efforts of many researchers, ES cells have not until now been isolated from any livestock species. This block to the opportunities to make more sophisticated modifications to livestock genomes was broken by the development of nuclear transfer.

Nuclear transfer involves the removal of the pronucleus from a recipient egg, its replacement by the nucleus of a donor somatic cell and activation of the egg, which is then transferred to a surrogate mother where it develops to term. The first successful nuclear transfer experiments in mammals were carried out in sheep (Campbell, McWhir, Ritchie, & Wilmut, 1996) to produce cloned lambs. Clones are not GM animals because DNA sequences have not been intentionally altered in the process. The technology has now been developed to enable nuclear transfer in cattle, pigs, goats and rabbits. Nuclear transfer can be utilised for production of GM animals if the cells used as nuclear donors are GM (Schnieke *et al*., 1997).

Poultry

Methods for genetic modification of poultry are not as advanced in development as those for livestock species. The major differences between mammalian and avian reproductive physiology and the development of the avian embryo from a large yolky egg make it impossible to manipulate the avian zygote or early embryo by simply using methods developed in mammals. Most research has been focused on manipulation of the domestic chicken but successful methods will probably be easily applicable to other poultry species (Sang, 1994). A method has been developed for manipulation of the chick zygote by DNA microinjection followed by embryo culture, which generates transgenic birds with multicopy insertions of transgenes, but the efficiency is low (Love, Gribbin, Mather, & Sang, 1994). Access to the chick embryo in the newly laid egg is much easier and manipulation of this stage is the target of several research groups. Gene transfer vectors derived from avian retroviruses were used in the first successful attempts to make transgenic birds (Bosselman *et al*., 1989; Salter *et al*., 1986). Vectors in the form of packaged viral particles were injected below the embryo developing on the surface of the yolk, and chimeric transgenic birds were produced after infection by the retroviral vector. Some of the birds transmitted the vector through the germline. A second approach has been to create chimeras by injection of embryo cells into a recipient embryo in a new laid egg. The donor cells may be grown in culture and transfected with transgene constructs prior to chimera production. The resulting birds are transgenic but there is no report that the transgene is stably inherited by the offspring of such chimeric birds. Similarly, primordial germ cells have been transferred between embryos at a slightly later stage of development but as yet no stably transfected germ cells have given rise to viable gametes (Zajchowski & Etches, 2000).

6.2.2. Applications to animal and poultry production

Many ideas have been suggested for application of transgenic technologies to modification of production traits, that is the traits that relate to food production, animal health and environmental issues, in livestock and poultry. These include manipulation of growth, resistance to disease, product quality and modification of environmental impact. Many applications are specific to the different species, although some may be applicable across several species. The mouse has been used as a model for testing many of these ideas and in some cases the experimental work has yet to progress to testing in farm animals.

Enhanced growth and feed efficiency

The first dramatic demonstration of the potential power of transgenic manipulation was the production of mice carrying the human growth hormone gene that was expressed at high levels, which resulted in development of mice that were much larger than normal (Palmiter, Norstedt, Gelinas, Hammer, & Brinster, 1983). This demonstration of an effect, particularly on muscle growth, lead to experiments to develop pigs transgenic for growth hormone. These animals, the 'Beltsville pigs', showed increases in muscle mass but also suffered from deleterious effects including development of arthritis (Pursel *et al*., 1989). The deleterious effects were suspected to be due to uncontrolled expression of growth hormone and subsequent experiments have been designed to generate transgenic pigs in which the level of growth hormone can be controlled by using an inducible promoter to control expression of growth hormone. Transgenic pigs, containing a transgene consisting of the zinc-inducible metallothionein promoter driving ovine growth hormone expression, showed significant decrease in fat and increase in muscle tissue (Pursel *et al*., 1997). Some deleterious effects on animal health were also observed, but not the major effects observed in the first experiments. Similarly, transgenic pigs have been produced that express human insulin-like-growth factor 1 (IGF1) in skeletal muscle, using a transgene incorporating the regulatory sequences of chicken skeletal alpha-actin. Female transgenic pigs had significantly less fat and were leaner than non-transgenic littermates but males were not significantly affected. There were no apparent health problems (Pursel, Coleman, & Wall, 1996). Bovine alpha-lactalbumin has been expressed in the milk of transgenic sows, enhancing the nutritional value of milk to suckling piglets (Bleck, White, Miller, & Wheeler, 1998).

The growth factor, myostatin, has been shown to be a negative regulator of muscle growth and is highly conserved in mammals and birds. Mice in which the myostatin gene has been inactivated have a major increase in muscle mass and a significant reduction in fat accumulation with age (McPherron, Lawler, & Lee, 1997, McPherron & Lee, 2002). Both these effects of down regulation of myostatin function resulted in the suggestion that myostatin manipulation via transgenesis, in both livestock and poultry, may have a beneficial phenotypic effect in terms of increase in lean meat yield. In fact, spontaneous mutations in the myostatin gene in cattle have been found to be responsible for the double-muscling phenotype in the Belgian Blue breed (Grobet *et al*., 1997) and alleles with lesser effects have been found to be present in other cattle breeds (Short, MacNeil, Grosz, Gerrard, & Grings, 2002). This illustrates the fact that major phenotypic effects, resulting from alteration of a single gene, can be obtained by conventional breeding.

Alteration in growth characteristics may also have positive effects on feed efficiency, including repartitioning between muscle and fat. Feed efficiency may also be improved by modifying the digestive capability of livestock and poultry. In non-ruminant livestock, digestion of dietary cellulose and xylan is by microbial fermentation in the hind-gut, which is relatively inefficient. In poultry, dietary cellulose is inefficiently digested and forms gel-like material that traps nutrients and inhibits their absorption. Expression of bacterial cellulase enzymes in the small intestines of these species could improve digestion of plant polysaccharides and therefore increase feed efficiency. The feasibility of this approach has been demonstrated in transgenic mice (Hall *et al*., 1993).

Disease resistance

Infectious disease has major negative effects on poultry and livestock production, both in terms of economics and on animal welfare. The costs of disease are estimated to be 35–50% of turnover in developing countries and 17% in the developed world. Application of genetic modifications to livestock and poultry to improve resistance to disease is therefore a very attractive idea but implementation of any such system is still very experimental. Disease resistance or susceptibility may be manipulated by manipulating natural genes involved or by artificial methods that can only be developed using transgenesis. Natural disease resistance is often controlled by many genes and is influenced by the environment. There are many stages during infection at which resistance genes may act, from initial infection, through multiplication of the pathogen, to release and spread to other hosts. A major goal of the genomics programmes in livestock and poultry is the identification of natural resistance genes or genes that enhance the immune response (Muller & Brem, 1998). It may be possible to transfer resistance genes between

breeds (intraspecies), between species or to modify disease resistance genes and enhance their function.

A major focus of research in chicken genomics is the mapping of genes that confer resistance to major pathogens (Burt, Bumstead, Bitgood, Ponce de Leon, & Crittenden, 1995). For example, the chicken genome is being screened for genes that confer resistance to *Salmonella* and this has lead to mapping of a novel gene, not previously identified in other species as conferring increased *Salmonella* resistance (Mariani *et al.*, 2001). The function of such a resistance gene may be investigated by classical breeding methods or by gene transfer. Such experiments will be necessary before the value, in terms of significant improvement in resistance, can be evaluated. The gene may have a different level of function in different genetic backgrounds of different chicken breeds, across poultry species or if transferred by transgenic methods to livestock species. Similarly, major programmes are underway to investigate the genetics of disease tolerance and disease resistance in livestock species.

Genetic modification may be used to introduce novel genes conferring resistance or to target expression of such genes to novel tissues. Transgenic sheep have been produced that express the visna virus envelope gene, normally expressed on the surface of the virus and not encoded by the host. These sheep provide a model to study whether expression of a viral envelope glycoprotein will prevent infection by the virus (Clements *et al.*, 1994). Mammals provide passive immunity to their young by secretion of antibodies in their milk. It has been suggested that expression of specific antibodies in the mammary gland can be produced by using transgenic methods to direct expression of antibody sequences in the mammary gland. Expression of a single neutralising antibody in the milk of transgenic mice has been shown to confer complete protection to suckling offspring against the strain of hepatic virus that the antibody recognises (Kolb, Ansell, McWhir, & Siddell, 1999). This approach could be developed further in livestock species, either in development of small herds for focused use in disease outbreaks or as production herds.

Relatively few single genes have been identified that have a major effect on disease resistance. One such gene is the Mx gene in mice that mediates resistance to influenza virus. This gene confers resistance to influenza infection when expressed in chick embryo fibroblasts in culture, suggesting that its introduction via transgenesis could be protective (Garber *et al.*, 1991). Another example of the possibility of transgenic expression of a gene from a different species providing protection from a specific disease has been modelled in mice. Kerr *et al.* (2001) have directed expression of a modified lysostaphin gene from *Staphylococcus simulans*, which has an anti-staphylococcal function, to the mammary gland. The aim is to provide protection against *Staphylococcus aureus* infection, a major mastitis pathogen in dairy cattle. The transgenic mice show substantial resistance to experimental infection by *S. aureus* but the milk protein content and profile are not affected.

Some methods proposed for application of genetic modification to enhancing disease resistance in livestock and poultry involve application of methods that are not derived from natural disease defence systems. These may be based on methods designed to reduce gene expression and may be targeted specifically at reduction of expression of particular pathogen genes after infection. Two such methods are the expression of antisense mRNAs or of ribozymes. Expression of a complementary mRNA to a specific gene product can lead to degradation of the sense mRNA (gene knock down) and therefore reduction in the targeted gene product. Ribozymes are also based on complementary mRNA expression but also include an RNA sequence that cleaves target mRNA. Both these methods have been tested in transgenic mice and may be adapted to target pathogen gene function. A demonstration of the possible application of antisense technology has been achieved in rabbits. Expression of an antisense RNA transgene to bovine leukaemia virus (BLV) conferred a measurable level of resistance to infection by BLV (Kozireva *et al.*, 1996).

Enhanced product quality

Genetic modification may be applied to improving the quality of products from livestock and poultry. These include meat from all species and species-specific products, for example milk, wool and eggs. Modifications that enhance growth, as outlined above, may also improve meat quality. Manipulation of myostatin function may produce leaner and more tender meat. Transgenesis in sheep may be used to develop novel fibre qualities and manipulate wool growth rates. Significant progress has been achieved in testing these possibilities. Gene promoters have been characterised that can be used to direct transgene expression to hair follicles and to expression of a wool intermediate filament keratin transgene shown to alter fibre structure. Many possible modifications to milk, via transgenesis, have been proposed. These include alteration of endogenous proteins and addition of new proteins, with the aim of altering milk quality for specific food products or with benefits for human health. These are discussed below (Sections 6.2.3 and 6.3.1).

Modified environmental impact

Animal agriculture has a major impact on the environment and it is possible that specific genetic modifica-

tions may be designed to reduce negative environmental effects. Some genetic modifications may have a beneficial effect on environmental impact as a secondary advantage of the modification and others may be designed specifically to tackle an environmental issue. The use of genetic methods, breeding or genetic modification, to increase disease resistance may reduce the requirement for treatment with antibiotics and as a consequence also reduce the level of antibiotics in animal products and spread of antibiotic resistance. Phosphorous pollution from manure of monogastric animals, including pigs and poultry, is a major environmental issue. These animals are unable to digest plant phytate, which comprises approximately 80% of phosphorous in common plant-derived feedstuffs. Excess phosphate in manure used as fertiliser results in eutrophication in rivers and lakes. Transgenic addition of phytase to the digestive enzymes of monogastric animals and poultry has been suggested as a route to reducing this problem, by enabling animals to utilise phosphorous in high phytate diets. This approach was first modelled in transgenic mice and has now been demonstrated in pigs. Golovan *et al.* (2001) describe production and characterisation of pigs carrying a transgene designed to express an *E. coli* phytase in saliva. These pigs require almost no inorganic phosphate supplementation to their diet and excrete up to 75% less phosphorous than non-transgenic pigs. There are concerns that this positive benefit to the environment may induce a negative effect by reducing genetic variability by limiting the number of genetic lines used. It is not likely to be a major concern if appropriate genetic management of the breeding population is applied.

6.2.3. Applications in human nutrition: beneficial modifications to food products

A range of possible targets for genetic modification of food products from livestock and poultry have been suggested. Some of these are classified as nutraceuticals, the term applied to food that provides a medical or health benefit. A major focus is the modification of milk, including addition of novel proteins in milk, via transgene expression in the mammary gland, or by transgenic manipulations that alter the components of milk. These modifications may require addition of novel components to milk or modification of endogenous milk protein genes. Milk is a major component of the diet in the western world but lactose-intolerance limits the consumption of dairy products by many people; about 50 million Americans malabsorb lactose. Jost, Vilotte, Duluc, Rodeau, and Freund (1999) have taken a transgenic route to tackling this problem. They generated transgenic mice that produced a biologically-active lactase in their milk, by targeting expression of an enzyme normally present in the small intestine to the mammary gland. The lactose content of the milk from the transgenic mice was at least halved and the milk protein levels were not significantly affected. A number of proposed transgenic modifications to cows' milk have focused on modifying milk so that it is more suitable for humans and particularly for infants and premature babies (Karatzas & Turner, 1997; Lonnerdal, 1996). Expression of bile-salt stimulated lipase in the mammary gland, an enzyme normally expressed in the digestive tract, would be beneficial to cystic fibrosis patients and premature infants who do not express the enzyme. Another proposed modification is the reduction of phenylalanine content of milk by modifying the alpha-lactalbumin protein to replace phenylalanine in the coding sequence. Milk from animals modified in this way would be suitable for patients with phenylketonuria who cannot tolerate dairy products. None of these modified milk products are currently close to market.

6.2.4. Applications in human health

The major applications of genetic modification technologies in livestock and poultry species are not in the development of food products but in developments related to human healthcare. These include production of pharmaceutical proteins by transgenic animals, modification of pigs with the aim of producing organs for transplant to humans (xenotransplantation) and providing models for human diseases.

Novel products: pharmaceutical proteins

The major possible use of transgenic technology in both livestock and poultry is the production of pharmaceutical proteins. There is a rapidly growing market for therapeutic proteins, with many pharmaceutical and biotechnology companies identifying new protein therapeutics, including many therapeutic antibodies, for treatment of a wide range of diseases. Some proteins can be manufactured by expression in bacteria and yeasts but many, particularly those that require glycosylation for their function, can only be effectively produced by synthesis in animal cells. Current bulk production systems for protein production in cells in culture are very expensive and the capacity available does not meet the growing demand. Transgenic animals offer a new production system for large quantities of biologically-active proteins. Production by transgenic animals may also be cheaper and a more flexible system. This is particularly an advantage in the production of complex proteins, such as antibodies, that will extend their use both as diagnostic tools and as therapeutics. The systems involved include transgenic expression in

the mammary gland, in sheep, goats, rabbits and cattle, and in the oviduct of laying hens.

Significant advances have been made in developing systems for production of proteins by transgenic expression targeted to different tissues. Most research and development has been focused on protein production in milk or eggs but blood, urine and seminal plasma are also being considered as routes for expression and collection of foreign proteins (Houdebine, 2000). The basic approach is the same for the different species and the different organs that naturally synthesise large amounts of protein. The sequences that control expression of the main proteins synthesised in the target organ are identified, and transgenes are developed that are designed to use these sequences to drive expression of a cloned pharmaceutical protein. A first example of this is the use of the sheep milk protein gene, beta-lactoglobulin, regulatory sequences to drive expression of the human protein alpha-1-antitrypsin in the milk of transgenic sheep (Clark *et al.*, 1989). Production of some therapeutic proteins in milk is now being advanced by biotechnology companies, which have several products in clinical trials. These products are not intended to enter the food chain.

The production of therapeutic proteins in hens eggs has potential to be a major cost-effective system with advantages over more established methods. The generation time of chickens is relatively short (six months) and a transgenic flock could be rapidly bred from a single founder cockerel. Laying hens can produce more than 250 eggs per year and collection of eggs containing transgene protein products is simple and non-invasive (Sang, 1994). Transgenic hens may be the method of choice for production of proteins that require specific glycosylation modifications, as post-translational modification in birds is reported to be very similar to that in humans (Raju, Briggs, Borge, & Jones, 2000). The well-established use of hens' eggs for production of vaccines is an additional advantage in terms of establishing regulatory approval for drugs produced in eggs.

Xenotransplantation

The possibility that xenotransplantation may offer an alternative to organ transplantation from human donors, for which demand is much greater than supply, has been considered for many years (Sachs, Sykes, Robson, & Cooper, 2001). The application of transgenic technologies to alter the donor animals so that the stimulus to induce immune rejection in the recipient patients is much reduced has resulted in some significant advances. The research in this area is directed at modifications of the pig genome, to make organs and tissues more compatible with survival within a human recipient. A major barrier to successful xenotransplantation is the hyperacute rejection response, triggered by the presence on pig cells of the disaccharide, galactose-α(1,3)-galactose. Synthesis of this epitope is catalysed by the enzyme α(1,3)galactosyl transferase, which is present in all organisms except Old World monkeys, apes and humans. Disruption of this gene by gene-targeting in pig cells and production of GM pigs by nuclear transfer has recently been achieved (Dai *et al.*, 2002). Further genetic modifications have been suggested to enhance the suitability of pigs for use as organ donors.

Animal models of human disease

A further application of transgenic technologies in livestock species with relevance to human healthcare is the production of animal models of human genetic diseases. Many GM mouse mutants have been generated carrying mutations found in human diseases and are excellent experimental material for studying the specific diseases and development of possible therapies. In some genetic diseases there are significant advantages in production of animal models in livestock species for such studies. The physiology of different livestock species may be more similar to that of humans, particularly in relation to the specific organs and tissues affected by the genetic disease. The small size of mice is also a limitation on their usefulness in terms of sampling and measuring physiological parameters. The sheep has been used as a model particularly for studies of lung physiology. This is particularly relevant to development of treatments for cystic fibrosis. It has been suggested that GM sheep, with mutations in the gene responsible for cystic fibrosis, would be a valuable tool in development of treatments, including gene therapy (Harris, 1997). The rabbit is also a possible model for this disease (Fan, Challah, & Watanabe, 1999). A pig model for the human genetic disease, retinitis pigmentosa, has been developed (Petters *et al.*, 1997) by expression of a mutated rhodopsin gene. This may prove a useful model for the study of the protracted phase of cone degeneration, which cannot be modelled in the mouse.

6.3. Potential outcomes and impacts of use of GM livestock and poultry

Outlined above are many of the possible applications of transgene technologies to livestock and poultry and some of the technological challenges that remain. Although the development of applications is still very much at a basic research level, many potential applications have been demonstrated in GM mouse models or in livestock and poultry species. However, there are currently no livestock or poultry products likely to enter the food chain. Indeed, the majority of ideas have only

been elaborated in mice. Products that are developed will require a system for case-by-case evaluation of risk, cost and benefit. A key issue to be considered is whether a transgenic route to product modification offers a cost effective challenge to other approaches that achieve the same result. The structure of the breeding industries strongly influence the calculation of economic cost of introduction of specific genetic modifications. Finally, public attitudes to genetic modification of animals will be influential in the regulation of introduction and the calculation of cost/benefit of applications of genetic modifications to livestock and poultry production.

6.3.1. Modification of food product quality: milk

It is possible to manipulate the quality of milk, to enhance nutritional qualities, eliminate allergenic factors, or alter composition. Some of the specific modifications are discussed above (Section 6.2.3). Modification of cows' milk to alter the composition and protein components is focused on making the milk more digestible and reducing the risk of allergic responses, so-called 'humanisation'. The protein beta-lactoglobulin is present in cows' milk but not in human milk and approximately 10% of consumers develop an allergic response to the protein. Transgenic removal of the protein would benefit these consumers. Modifications to milk aimed at making it more suitable for use in production of infant formula, to make its composition closer to that of human milk, would be an advantage, particularly for premature infants. The acceptability to society of the development of more sophisticated infant formulas and their sale, particularly in developing countries, raises many issues unrelated to safety of the products. The possibility of reducing the lactose content of milk by transgenic expression of lactase in the mammary gland has been demonstrated (Jost *et al.*, 1999). There are alternative, non-transgenic methods, to limit the effects of lactose intolerance, including post-harvest treatment of milk or lactase-replacement products, which are much more economic.

A range of other modifications to milk have been suggested. These are aimed at tailoring milk to make it more useful for particular types of further processing. Changes to the casein protein composition affect the size and stability of the globular protein structures, the micelles. These determine the characteristics of milk for cheese processing, and qualitative and quantitative alteration of casein proteins could result in production of milk for specific cheese-making processes. Over-expression of kappa-casein affects micelle stability and leads to more efficient absorption (Zuelke, 1998). These alterations could be useful to the milk-processing industry.

6.3.2. Modification of food product quality: meat

Several of the genetic modifications discussed above (Section 6.2.2) have the potential to improve feed efficiency and meat quality, by producing leaner animals, both livestock and poultry, with less adipose tissue. This could be beneficial in terms of reduction in animal fat consumption. Leaner animals may also be produced by changes to diet, husbandry and selective breeding. The interaction of transgenic modifications with genotype has been little explored but the finding that different breeds of cattle carry different alleles of the myostatin gene (Short *et al.*, 2002) suggests that transgene effects could vary significantly in different genetic backgrounds. This could provide a wider range of phenotypes than is currently available through conventional breeding.

6.3.3. Environmental interactions and sustainable agriculture

The transgenic 'phytase pigs' (Section 6.2.2; Golovan *et al.*, 2001) excrete dramatically less phosphorous than control animals and also require little inorganic phosphorous supplementation to their diet. This is a clear demonstration of an environmental benefit that could be obtained by a specific genetic modification. However the calculation of potential benefit requires comparison of the alternative approaches to solving this problem, for example phytase supplementation of the diet. Also, the introduction of such a genetic modification may not be economic in terms of introduction and dissemination of the transgene into breeding stock.

The environmental risk of spread of transgenes outside controlled populations is low for livestock and poultry. The likelihood of release and survival of GM animals and birds is very limited and risks of breeding with other animals negligible. In comparison to the risks from release that must be considered for GM plants and fish, these issues are insignificant for livestock and poultry. Theft of GM animals could result in spread of the transgene outside controlled populations, a risk that is also true for traditionally-selected animal breeds.

There could be environmental benefits from the introduction of specific GM livestock and poultry, in terms of supporting developments in sustainable agriculture. These include decreased environmental pollution, as in the example above, and benefits from increasing disease resistance. Increased disease resistance will have benefits in terms of reduction in use of drugs, greater longevity and animal welfare. These aspects make the use of genetic modification to improve disease resistance particularly attractive but this is probably the hardest area in which to develop effective systems. A possible risk that must be considered is that resistant animals

and poultry may harbour pathogens, and novel resistance mechanisms may introduce new selective pressures on pathogens, resulting in changes to the pathogens to which non-GM animals are vulnerable.

6.3.4. Human health

Benefits to human health could result from the use of GM animal food products, either as the main target or as a secondary benefit. Over expression of lysostaphin in cows' milk (Section 6.2.2) could protect cows against mastitis and also reduce the risk of *S. aureus* infection to humans. Expression of human lactoferrin, a natural antibiotic present in human milk, may protect infants against bacterial proliferation in the intestinal tract (Pintado & Gutierrez-Adan, 1999). Transmissable spongiform encephalopathies (TSEs) are a threat to human health through consumption of meat products from infected animals. It has been shown that if the *prp* gene has been inactivated in mice they are no longer susceptible to TSE infection and there is no major phenotypic effect from the mutation. It will be possible to inactivate the equivalent gene in livestock species, via gene targeting in somatic cells and nuclear transfer (Denning *et al.*, 2001). The long term phenotypic effects of such a modification will require close monitoring. This approach could be applied through animal breeding but the economic value of introducing the mutation into production animals will have to assessed.

Some potential risks of genetic modifications have yet to be investigated in depth. Insertion of foreign DNA into the genome can have epigenetic effects. For example, insertions into hamster cell lines have been shown to effect methylation of sequences in the genome, unrelated to the inserted sequence (Remus *et al.*, 1999). These changes have in some cases been shown to lead to activation of endogenous retroviral sequences, normally silenced by methylation (Walsh, Chaillet, & Bestor, 1999). The significance of these effects, in terms of genome stability, mutation frequency and other factors, is poorly understood. Concerns have been raised about the potential uptake from the diet of foreign DNA sequences. This was thought to be a low risk but there is increasing evidence that DNA and double-stranded RNA may be taken up when ingested. A major risk that must be considered is contamination and infection by infectious agents, particularly viruses, when cultured cells or tissues from other species are involved in the genetic modification process. Cultured cells, such as those used in nuclear transfer, can become contaminated during the culture process, for example contamination by murine parvovirus (Garnik, 1996). The risk of infection by porcine endogenous retroviruses, if pig organs are used in xenotransplantation, is very significant (van der Laan *et al.*, 2000) and is being considered in development of regulations for xenotransplantation. Development and implementation of methods for the assessment of risk of food products from GM livestock and poultry must be achieved. Some of the mechanisms that are applied to risk assessment of GM plant products will be useful in assessing animal products. Additional requirements, for example assessment of risk associated with expression of retroviral sequences, due to transgene insertion, should be considered.

6.3.5. Economic and social issues

The possible benefits and risks of applying GM technologies to livestock and poultry species have been considered above. These do not include consideration of other factors that will have a major effect on the likelihood that products from GM animals will become available. The economic factor is a main determinant of whether a GM animal food is considered for production. The methods available at present are inefficient and expensive. Also, the economic cost of introducing a specific genetic modification into breeding stock will be very high. This varies depending on the structure of the breeding industry. At present it is likely to be prohibitively expensive in the cattle breeding industry but possibly the structure of the poultry breeding industry will lend itself to introduction of specific genetic modifications if the value is great enough.

Associated with issues of the economic advantage of specific genetic modifications is the challenge of public acceptability. There are substantial public concerns about the use of GM technology in animals (Gaskell *et al.*, 2000). These include fundamental objections to the manipulation and use of animals, objections to specific possible modifications and concern, particularly for animal welfare, about the consequences of genetic modifications. The application of transgenic technologies in relation to development of products for human healthcare, for example pharmaceutical protein production, is considered much more acceptable than modifications related to food production. The issues of animal welfare are important and are equally relevant to consideration of animal welfare in conventional animal breeding and production. These issues of public concern and regulatory acceptance inevitably have a major influence on breeding companies and their calculation of the risks and benefits of the introduction of GM animals into production.

6.4. Knowledge gaps

The technologies outlined above are well advanced, but there are still significant advances required to

improve existing methods to enable more efficient production of GM animals and tightly regulated transgene function. The relatively low efficiency of the different methods developed so far is a major issue in application of these technologies to livestock and poultry, as it restricts their application to projects that have a high value. There are two main areas of research to overcome the technological gaps. The first is aimed at obtaining efficient integration of the transgene and expression of the transgene. The second is to develop methods to achieve tightly controlled transgene expression, in specific tissues and at the desired time.

6.4.1. Efficient integration and expression of transgenes

A significant problem encountered with several different methods of genetic modification is transgene silencing, in which the transgene is not expressed due to epigenetic effects, apparently triggered by the foreign sequences introduced into the genome and their location within the genome. Transgene expression may be affected by age, by germ line transmission, by genetic background effects and if the transgene is homozygous. Much of the progress in this area is driven by biomedical research to develop human somatic gene therapy methods. Many of the improvements to existing technologies and novel methods may be utilised in all species, although effectiveness may vary. Many methods continue to be developed in the mouse.

Progress in the area of increasing efficiency is focused on the development of gene transfer vectors and improvements to existing cell-based methods. Vectors derived from retroviruses have been used successfully in poultry (as above) and in cattle (Chan, Homan, Ballou, Burns, & Bremel, 1998). Avian retrovirus vectors are still being used with some success for the production of transgenic chickens (Harvey, Speksnijder, Baugh, Morris, & Ivarie, 2002). Recent publications of experiments in the mouse, suggest that more sophisticated vectors derived from lentiviruses are more efficient and avoid the problems of transgene silencing encountered with previous retroviral vectors (Lois, Hong, Pease, Brown, & Baltimore, 2002; Pfeifer, Ikawa, Dayn, & Verma, 2002). These vectors are being developed primarily for gene therapy, and concerns about safety are being addressed. Gene integration vectors derived from transposons have formed the basis of transgenic methods in model organisms, particularly *Drosophila*. Transposons from one species can function in a different unrelated species, for example the *Drosophila* transposon *mariner* will transpose into the chicken genome (Sherman *et al*., 1998). The transposon *Sleeping Beauty*, isolated from fish, has potential as an integration vector that may function in all vertebrates, as has been demonstrated in the mouse (Dupuy *et al*., 2002). Transposition frequency of transposon vectors apparently decreases with increasing length of the transgene they carry, which may prove a drawback as many transgene constructs are relatively large.

Insertions of large stretches of genomic DNA, carrying the desired transgene, are often expressed in a pattern closer to that of the endogenous gene than those introduced as short cloned regions. Large genomic clones are cloned in vectors designed for the purpose: yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs) or phage P1-derived vectors. These have been used to make transgenic animals by microinjection with some success (Giraldo & Montoliu, 2001).

6.4.2. Controlled transgene expression

Effective genetic manipulation requires the ability to direct expression of a given transgene to all the cells of a particular tissue at a specific stage of development or in such a way that expression can be regulated as required. Gene-targeting, using ES cells or somatic cells for nuclear transfer, can provide an effective route to controlled transgene expression. Major efforts have been made to isolate ES cells from livestock and from the chick. These efforts are continuing but in livestock species the current focus is on improving the efficiency of nuclear transfer. The survival to birth of embryos derived by nuclear transfer is very low and many animals develop problems after birth (Chavatte-Palmer *et al*., 2002). Research is aimed at improving the technologies involved in oocyte maturation, embryo culture and transfer to surrogate mothers. For successful nuclear transfer the somatic nucleus must be reprogrammed to direct development from the single cell stage. The developmental defects are apparently related, at least in part, to disruption of normal patterns of genetic imprinting. An understanding of the molecular and cellular processes involved in reprogramming should enable increases in efficiency of the nuclear transfer process.

Gene targeting in somatic cells, followed by recovery of correctly modified animals via nuclear transfer, is proving a useful method for ensuring transgene expression or for manipulating endogenous gene expression. Targeting of a therapeutic protein transgene into the ovine alpha (1) procollagen locus was efficient and reproducible (McCreath, Howcroft, Campbell, Colman, & Kind, 2000). Gene knockouts by homologous recombination have also been demonstrated (Denning *et al*., 2001). These results suggest that the range of sophisticated gene manipulations possible using homologous recombination should be achievable in livestock species. These include allelic replacement with excision of all auxiliary sequences using site-specific recombinases, for example the Cre/Lox system (Kolb *et al*., 1999).

Controlled induction of transgene expression will often be required, to enable expression of transgenes at restricted times of development or when required for other purposes in adult animals. Several systems have been investigated with the aim of finding a mechanism whereby transgenes can be switched on and off, with no expression in the absence of induction. The first system tested in transgenic animals utilised the metallothionein gene regulatory sequences, induced by adding zinc to the diet, but this is leaky (Pursel *et al.*, 1997). Currently the most useful systems under development are the methods based on the tetracycline repressor and on the ecdysone induction process from *Drosophila* (reviewed in Ryding, Sharp, & Mullins, 2001). In parallel, numerous other inducible expression systems are being developed, some based on mammalian transcription circuits, including the P450 gene family. The feasibility of temporal and spatial control of transgene expression has now been established in the mouse. These methods may now be applied to livestock and poultry.

6.5. Conclusions

There are no food products derived from GM livestock and poultry near to production for the market. It is unlikely that there will be any for the foreseeable future, certainly in Europe and the USA. The methods involved are inefficient and high cost. There is still a lot of research required to identify useful targets for genetic modification and to increase overall efficiency of genetic modification methods, from frequency of production of transgenic animals to effective transgene expression. There is little support from research funding agencies for development of the technology in relation to food production or from the relevant industries, as costs are too high. In addition to these factors, there are significant issues in the introduction of GM animals into the breeding stock of the animal breeding industries and their dissemination to the producers.

Potential hazards to humans, involved in production or when the products become part of the food chain, will require assessment through a regulatory process with appropriate licensing powers. These regulatory processes are already established in many countries, for example within the European Union. There are serious public concerns about the ethics of manipulating domesticated animals and about the animal welfare effects of genetic modifications. These should be considered and evaluated but in a system that can be applied equally to livestock and poultry produced by conventional breeding methods.

6.6. References

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