Introduction to transgenesis in domestic animals

Introduction

Animal biotechnology has been practiced in one form or another since the beginning of the domestication of animals. Many of the previously used tools of animal breeding, genetics, and nutrition have played and will continue to play an important role in the selection, propagation, and management of desirable and economically important characteristics in livestock. In the future, livestock production will rely even more heavily on existing and emerging biotechnological advances to produce our food. Yet, improvements are still needed in product composition and production efficiency, especially in growth, disease resistance, and reproduction. Genetically modified (transgenic) livestock, stem cells, and other emerging biotechnologies will have important roles in producing more and higher quality food derived from livestock. The production of 'transgenic animals' is one such biotechnology tool. A transgenic animal is one that has integrated a gene or DNA sequence (a 'transgene'), which has been transferred by human intervention, into the genome of a cell. For the purposes of discussion, a transgenic animal is defined as one that has stably incorporated the transgene into its germ-line and is able to pass the transgene on to its offspring.

Transgenic Livestock

The production of transgenics provides methods to rapidly introduce 'new' or modified genes and DNA sequences into livestock without crossbreeding or hybridizing. It is a more precise technique, but not fundamentally different from genetic selection or crossbreeding in its result. Much has been written about the methodologies used to produce transgenic livestock (Wall 2002, Wheeler & Walters 2001, Wheeler et al. 2003) and that aspect will not be covered in this review (Table 1). The obvious question is 'Why genetically modify livestock?' The answer is not so straightforward; however, some of the reasons are to (1) study the genetic control of physiological systems, (2) build genetic disease models, (3) improve animal production traits, and (4) produce new animal products.

Applications of Transgenic Animals in Agriculture

There are many potential applications of transgenic methodology to develop new and improved strains of livestock. Practical applications of transgenics in livestock production include enhanced prolificacy and reproductive performance, increased feed utilization and growth rate, improved carcass composition, improved milk production and/or composition (Figure 1), modification of hair or fiber, and increased disease resistance. Development of transgenic farm animals will allow more flexibility in direct genetic manipulation of livestock. Gene transfer is a relatively rapid way of altering the genome of domestic livestock. The use of these tools will have a great impact toward improving the efficiency of livestock production and animal agriculture in a timely and more cost-effective manner. With ever-increasing world population and changing climate conditions, such effective means of increasing food production are needed.

Enhanced Nutrition

Human health is directly affected by the necessity for a sustainable and secure supply of healthful food. Genetic modification of livestock holds the promise to improve public health via enhanced nutrition. For thousands of years, farmers have improved livestock in order to provide for nutritious, wholesome, and cost-effective animal products.

Transgenesis allows improvement of nutrients in animal products, including their quantity, the quality of the whole food, and specific nutritional composition. Transgenic technology could provide a means of transferring or increasing nutritionally beneficial traits. For example, enhancing the omega-3 fatty acid in fish consumed by humans may contribute to a decreased occurrence of coronary heart disease. In fact, transgenic pigs that contain elevated levels of omega-3 fatty acids have been produced (Lai et al. 2006). Furthermore, transfer of a transgene that elevates the levels of omega-3 fatty acids into pigs may enhance the nutritional quality of pork (Lai et al. 2006). The production of lower fat, more nutritious animal products produced by transgenesis could enable improvements in public health.

Reduced Environmental Impact

Over the last few years, livestock production has been under attack as being harmful to the environment. However, the production of transgenic livestock has the potential to dramatically reduce the environmental footprint of animal agriculture. Increasing efficiency and productivity through transgenesis could decrease the use of limited land and water resources while protecting the soil and ground water. One excellent example of this is the swine (the Enviro-PigTM) produced by genetic engineering (Golovan et al. 2001). Pigs do not fully utilize dietary phosphorus. Dietary supplementation results in increased production costs, and incomplete utilization results in phosphorus levels in waste products that can cause pollution problems. Golovan et al. (2001) reported the production of transgenic pigs expressing salivary phytase as early as 7 d of age. The salivary phytase provided essentially complete digestion of dietary phytate phosphorus in addition to reducing phosphorus output by up to 75%. The use of phytase transgenic pigs in commercial pork production could result in decreased environmental phosphorus pollution from livestock operations.Improved production efficiencies of milk and meat would decrease the amount of manure, slow the direct competition for human food, decrease the amount of water required for the animals and the production facilities, and decrease the land necessary for livestock operations.

Enhancing Milk

Advances in transgenic technology provide the opportunity either to change the composition of milk or to produce entirely novel proteins in milk (Table 2). The improvement of livestock growth or survivability through the modification of milk composition involves production of transgenic animals that: (1) produce a greater quantity of milk; (2) produce milk of higher nutrient content; or (3) produce milk that contains a beneficial ‘nutriceutical' protein. The major nutrients in milk are protein, fat, and lactose. By elevating any of these components, we can impact the growth and health of the developing offspring. Cattle, sheep, and goats used for meat production can benefit from increased milk yield or composition. In tropical climates, heat-tolerant livestock breeds such as Bos indicus cattle are essential for the expansion of agricultural production. However, Bos indicus cattle breeds do not produce copious quantities of milk. Improvement in milk yield by as little as 2-4 liters per day may have a profound effect on weaning weights in cattle such as the Nelore or Guzerat breeds in Brazil (Figure 2). Similar comparisons can be made with improving weaning weights in meat-type breeds like the Texel sheep and Boer goat. This application of transgenic technology could lead to improved growth and survival of offspring.The overexpression of beneficial proteins in milk through the use of transgenic animals may improve growth, development, health, and survivability of the developing offspring. Some factors that have been suggested to have important biological functions in the neonate that are obtained through milk include IGF-I, EGF, TGF-β, and lactoferrin (Grosvenor et al. 1993).

Enhancing Growth Rates and Carcass Composition

The production of transgenic livestock has been instrumental in providing new insights into the mechanisms of gene action implicated in the control of growth, (Ebert et al. 1988, Vize et al. 1988, Murray et al. 1989, Pursel et al. 1989, Ebert et al. 1990, Rexroad et al. 1991, Pursel et al. 1997). It is possible to manipulate growth factors, growth factor receptors, and growth modulators through the use of transgenic technology. Results from one study have shown that an increase in porcine growth hormone (GH) leads to enhancement of growth and feed efficiency in pigs (Vize et al. 1988). In the case of fish, there is a need for more efficient and rapid production, without diminishing the wild stocks, to provide a protein source for the increasing world population. The production of GH transgenic fish has led to dramatic (30-40%) increases in growth rates in catfish through the introduction of salmon GH into these animals (Dunham & Devlin 1999). Introduction of salmon GH constructs has resulted in a 5-11 fold increase in weight after 1 year of growth (Devlin et al. 1995, Devlin et al. 1994, Dunham & Devlin 1999). This illustrates the point that increased growth rate and ultimately increased protein production per animal can be achieved via transgenic methodology. Another aspect of manipulating carcass composition is that of altering the fat or cholesterol composition of the carcass. By altering the metabolism or uptake of cholesterol and/or fatty acids, the content of fat and cholesterol of meats, eggs, and cheeses could be lowered. There is also the possibility of introducing beneficial fats such as the omega-3 fatty acids from fish or other animals into our livestock (Lai et al. 2006). In addition, receptors such as the low-density lipoprotein (LDL) receptor gene and hormones like leptin are potential targets that would decrease fat and cholesterol in animal products.

Enhanced Animal Welfare through Improved Disease Resistance

Genetic modification of livestock will enhance animal welfare by producing healthier animals. Animal welfare is a high priority for anyone involved in the production of livestock. The application of transgenic methodology should provide opportunities to genetically engineer livestock with superior disease resistance.One application of this technology is to treat mastitis, an inflammation of the mammary gland, typically caused by infectious pathogen(s). Mastitis causes decreased milk production. Transgenic dairy cows that secrete lysostaphin into their milk have higher resistance to mastitis due to the protection provided by lysostaphin, which kills the bacteria Staphylococcus aureus, in a dose-dependent manner (Donovan et al. 2005). Lysostaphin is an antimicrobial peptide that protects the mammary gland against this major mastitis-causing pathogen. Recent progress has produced prion-free (Richt et al. 2007) and suppressed prion livestock (Golding et al. 2006). Prions are the causative agents in bovine spongiform encephalopathy (BSE) or 'mad cow disease' in cattle and in Creutzfeldt-Jacob disease (CJD) in humans. This is only a partial list of organisms or genetic diseases that decrease production efficiency and may also be targets for manipulation via transgenic methodologies.

Improving Reproductive Performance and Fecundity

Several potential genes have recently been identified that may profoundly affect reproductive performance and prolificacy. Introduction of a mutated or engineered estrogen receptor (ESR) gene could increase litter size in a number of diverse breeds of pigs. A single major autosomal gene for fecundity, the Boroola fecundity (FecB) gene, which allows for increased ovulation rate, has been identified in Merino sheep (Piper et al. 1985). Each copy of the gene has been shown to increase ovulation rate by approximately 1.5 ova (Piper et al. 1985). Production of transgenic sheep containing the appropriate FecB allele could increase fecundity in a number of diverse breeds. The manipulation of reproductive processes using transgenic methodologies is only beginning and should be a rich area for investigation in the future.

Improving Hair and Fiber

The control of the quality, color, yield, and even ease of harvest of hair, wool, and fiber for fabric and yarn production has been another area of focus for transgenic manipulation in livestock. The manipulation of the quality, length, strength, fineness, and crimp of the wool and hair fiber from sheep and goats has been examined using transgenic methods (Hollis et al. 1983, Powell et al. 1994). In the future, transgenic manipulation of wool will focus on the surface of the fibers. Decreasing the surface interaction could decrease shrinkage of garments made from such fibers. Recently, a novel approach to producing spider silk, a useful fiber, has been accomplished using the milk of transgenic goats (Karatzas et al. 1999). Spiders that produce orb-webs synthesize as many as seven different types of silk for making these webs. One of the most durable varieties is dragline silk. This material can be elongated up to 35% and has tensile properties close to those of the synthetic fiber KevlarTM. Its energy-absorbing capabilities exceed those of steel. There are numerous potential applications of these fibers in medical devices, sutures, ballistic protection, tire cord, air bags, aircraft, automotive composites, and clothing.

Pitfalls and Risks

In using any new technology, there are problems that occur and there are risks to be considered. From the technical side, these problems can be: (1) unregulated expression of genes resulting in over- or underproduction of gene products; (2) too high a copy number resulting in overexpression of products; (3) possible side effects, e.g., GH transgenic swine had arthritis, altered skeletal growth, cardiomegaly, dermatitis, gastric ulcers, and renal disease; (4) insertional mutations (inserting a fragment of DNA into an important gene) that result in some essential biological processes being altered; (5) mosaicism (only a portion of the cells incorporate the gene being transferred) in the founders, which results in transmission of the transgene to only some of the offspring; and (6) transgene integration on the 'Y' chromosome, which results in only males carrying the transgene. Many, if not all, of these problems are related to the transgene itself, integration site, copy number, and transgene expression. These issues can be addressed, at least in part, through construct design and testing. From the animal side, the welfare, biology, and health of the resulting transgenic animal must be of paramount concern. Animal care guidelines are being established for the care of clones From the consumer side, the food or agricultural product produced must be safe, wholesome, non-allergenic, nutritious, and economical. These are issues being addressed by various governmental agencies.

The genetic engineering of livestock is a difficult task, and great care must be taken before such effort begins. Serious consideration is critical because of the time, cost, welfare, ethics, risks, and benefits involved in these kinds of projects. However, farmers, consumers, and scientists all want safe food, which means that animal care, animal health, animal welfare, public concern, ethics, and societal benefit and vigilance cannot be ignored. On the contrary, these concerns should be welcomed when designing and conducting such projects. Consideration of these as well as scientific issues will lead us forward toward harvesting the bounty promised by this important technology.