

## Current and Future Promises of Transgenesis for Agricultural Livestock in a Global Marketplace

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### ABSTRACT

This paper addresses the context of decisions about the application of biotechnology in livestock agriculture. It specifically addresses the role of transgenic livestock in agriculture. Key innovations in livestock genetics and reproductive technology in the last century have laid the foundations upon which genetic engineering can be applied to livestock in the post-genomics era. Transgenesis is not an end in itself; its only justification in agriculture is to benefit the quality of life of man, or animals, or to achieve better use of environmental resources. Comparisons are drawn with the successes of agronomic biotechnology and contrasts with the structure and biology of the livestock and crop industries. Tools for highly efficient gene introduction in livestock are now available. Transgenic traits can now be superimposed on elite selected livestock genetics, but will have to prove their efficacy and economic worth in the context of herds owned by diverse producers. Transgenic manipulation of livestock can facilitate precision agriculture, minimizing the use of antibiotics and pesticides, and can bring increased efficiency to the processing industry. Criteria for selection of valuable transgenes are discussed, including biologic function and compatibility with market infrastructure. Some useful transgenes will target production; others will offer new products. Transgenesis offers a new paradigm in disease control and can assist in preserving genetic diversity. Outside agriculture, transgenic livestock have an important role in biopharmaceutical production. Biotechnology, including transgenic livestock, will play a key role in meeting the challenge of feeding the world population, while creating and maintaining sustainable agricultural systems.

(**Key words:** transgenic livestock, genetic engineering)

**Abbreviation key:** NT = nuclear transfer, WWII = World War II.

### INTRODUCTION

Scientific advances have a way of sneaking up on us. During the last century the technologies we can apply to livestock have accumulated slowly. Now we are at a convergence of many tools and are poised on the threshold of a new era of rapid change. Biotechnology has developed against a backdrop of major structural change in agriculture and appears likely to function as an engine for further change. The technical and economic opportunities and the social choices are many. This paper will address the context of some of the deci-

sions that will determine which directions to take in the application of biotechnology in livestock agriculture. Implementing transgenics in agricultural livestock will be a slow process given the generational intervals involved. However, such livestock intended for agricultural use are already a reality in a research setting; additional traits are in gestation, and more are in development. This paper is not about choices to be faced *if* transgenic livestock are widely adopted, but about *when* they are adopted.

Other manifestations of biotechnology, such as building genomic libraries for domestic species, are in progress and are making an important contribution. Such efforts will provide input information that will enable genetic engineering of livestock species.

Transgenesis is not an end in itself; its only justification in agriculture is to benefit the quality of life of man, or animals, or to achieve better use of environmental resources.

### EVOLUTION OF AGRICULTURAL SYSTEMS

The evolution of livestock agriculture over several millennia has been a process of cumulative application of new technology. Starting with tethering of animals instead of following the herd, and hence the control of nutrition, mankind has applied the technology of the day to modify the quality and quantity of the products he derives from livestock and to modulate the impact livestock production systems on the environment. This has enabled our agricultural systems to evolve from herd followers, to subsistence agriculture, to today's high producing dairy herds, and intensive swine and poultry production. In parallel, we evolved complex market chains that deliver a safe and ample food supply to urban consumers.

Few of the technologies we now apply have emerged without contention, and concern about the implications of change. Vaccination, artificial insemination, machine milking in their time were all be the subject of intense debate over their safety and their societal impact.

In the last century, we saw the first steps towards intensive genetic selection with the establishment of swine breeding stations in Europe and the beginning of systematic production recording systems.

During the 1930s and 1940s, population genetics theory was developed. Lush and his colleagues (Lush, 1945) provided the basic of genetic theory that has served as the foundation of livestock population genetics implemented in the post World War II (WWII) era. Interestingly, this population genetics theory, the chemical structure of the DNA (Watson and Crick, 1953), and an understanding the role of retrotransposons in maize genetics (McClintock, 1951) were developed contemporaneously.

The ability to propagate and manipulate DNA in the laboratory became possible in the late 1970s. Now, early in 2000, almost the entire sequence of the human genome and those of

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other organisms have been determined (Office of the Press Secretary, 2000).

Improved livestock disease control has worked in parallel with the development of genetic technology. By reducing unplanned culling for disease, producers gained more latitude in selecting desirable breeding stock.

The enormous advances in reproductive technology, particularly in cattle, catalyzed genetic progress. Commercialization of artificial insemination of cattle was enabled by the development, shortly after WWII, of methods to cryopreserve semen so that it could be stored and delivered to remote locations (Polge et al., 1949). During the remainder of the century, we saw a global market develop for elite livestock traits. The ability to distribute male genetics globally was followed by the development of technologies for nonsurgical embryo transfer (Rowe et al., 1980) and the *in vitro* maturation and *in vitro* fertilization of oocytes (Leibfried and First, 1979). As it had with semen, cryopreservation of embryos also broadly enabled the distribution of high quality animals to various parts of the globe (Polge, 1977).

Once the *in vitro* culture of embryos was possible, then genetic manipulation of embryos became feasible. The ability to inject genes into the pronucleus of the fertilized embryo and, thus, the ability to insert novel genetics emerged. Pronuclear microinjection began to be used widely in biomedical research to produce transgenic mice (Palmiter et al., 1982). We anticipated that efficiencies would improve with experience. However, despite early optimism, the efficiency of pronuclear injection of genetic material in livestock species has not progressed much beyond the levels of a decade ago.

In the middle 1980s, the first nuclear transfers (NT) were done, where a diploid nucleus from an embryonic cell was placed into an (haploid, enucleated) oocyte and resulted in the birth of live calves (Prather et al., 1987). Continued work over a decade in this area provided Wilmut and his colleagues (1997) the ability to perform nuclear transfer cloning of adult somatic cells, and thus produce Dolly, the cloned sheep. The potential application of NT has stimulated an enormous amount of work in many species. At present several companies are working to commercialize applications of this NT technology. NT itself is a replicative technology. Applications in agriculture are seen as the replication of elite individuals and the potential to superimpose gene insertion into animal genomes. At present, the process still has a relatively low efficiency. Chromosomal damage and rearrangements that occur during *in vitro* manipulation of the donor cells may be responsible for the congenital defects that have been reported in animals produced by NT (Hill et al., 1999; Renard et al., 1999). Given the refinements and improvements that have occurred in other reproductive technologies, it is anticipated that similar improvements will occur in NT as well.

Bremel and coworkers at the University of Wisconsin-Madison took a different approach. They combined gene therapy and reproductive technologies, which resulted in a substantial improvement in the ability to produce transgenic animals (Chan et al., 1998). A highly engineered replication-defective retroviral vector was inserted into an oocyte arrested in the second meiotic division. This technique can produce transgenic animals at nearly 100% efficiency, with stably integrated single gene copies at multiple sites. Retroviruses are highly evolved retrotransposons. The replication incompetent retrovectors now in use in transgenic systems and in gene

therapy closely resemble the retrotransposons of McClintock. Thus, this gene transfer system probably mimics in the germline an apparently highly conserved engine of genetic diversity (Agrawal et al., 1998), which may drive evolutionary change. Besides the efficiency of transgenic production possible with these vectors, they also appear to insert stably into scaffold and matrix attachment regions of the chromatin known to give the highest level of gene expression (Schubeler et al., 1996).

**Technology convergence.** As we begin this millennium, we stand at the convergence of an incredible array of new technologies—molecular biology, bioinformatics, global communications, instrumentation to enhance our understanding of structural biology, and genomics. As this paper was drafted, the announcement was made of the publication of the first draft of the Human Genome (Office of the Press Secretary, 2000). We already refer to ourselves as living in the “post genomics era.” Our children are the first generation who will grow up understanding the chemical basis for their structure, function, and behavior. Livestock agricultural systems have an even greater potential than crop systems to benefit from the genomics effort because it will be possible to directly extrapolate many findings from human biomedical research to domestic animals. This includes both human genomic information, and research on the genomes of pathogens that impact livestock as well as human health.

## BUILDING ON THE AGRONOMIC EXPERIENCE

As we address the potential of biotechnology in livestock systems, what can we learn from the progress made in agronomy? Crop agriculture in the Western hemisphere and Asia has witnessed the rapid adoption of genetic engineering by producers. Rapid adoption would not happen if transgenic crops were not efficacious and economically advantageous for the producer. This gives the applicability of gene insertion technology credibility in agricultural systems.

Agronomic biotechnology has delivered on the promise of corn and beans that provide value to the grower and the environment. Herbicide-resistant soybeans have higher yields because of better weed control (Subcommittee on Basic Research, 2000). Reducing weed seed contamination increases the purity of the harvested crops. Overall herbicide use is reduced (Carpenter and Gianessi, 1999) Water quality is improved. No-till practices become more feasible. Less soil disturbance and compaction results in a marked improvement in soil erosion control and soil quality. The time a producer spends working a field is reduced and the fossil fuel burned to achieve crop tillage is reduced. Hedgerows and areas spared tillage by higher yields provide improved wildlife habitat. Crop biotechnologists have responded to the environmental needs and have made application of the new technology economically feasible. However, some environmentalists still fail to recognize the clear benefits of these innovations (Subcommittee on Basic Research, 2000), and high productivity in general, in minimizing the footprint of agriculture on the environment.

Crop genetics companies focused first on the addition of new single gene traits. This was driven by the economics of the traits as well as the ease of implementation. Only now is the possibility of using genomic technology to discover more complex production traits moving to the forefront of the research effort.

Crop biotechnology introduced the producer to the patented seed. Owners of genetic technology have licensed the technology widely to seed distributors. This approach has made it possible to build on customer loyalty and make the technology widely available. The license payment is packaged as a "technology fee" built into the normal price of the seeds. There is no indication that either the patented seeds or the licensing requirement have inhibited acceptance. Quite clearly, then, we have learned that farmers in a large segment of the world understand the impact of genetics on their livelihood and will accept, pay for, and rapidly adopt genetically engineered traits.

Those who would now throw up barriers to adoption of transgenic crops are often several steps removed from the economics of production agriculture and resource management. We now live in a society where less than two percent of the US population has first-hand experience of food production, and even fewer understand the delicate interplay of environmental and social factors that can disrupt the security of our food supply. We have also learned from the crop agriculture experience that we need to communicate more explicitly the issues of food security, and the benefits biotechnology offers to the consumer, the environment, and the producer. Clearly the aggregate of the benefits outlined above accrue to all citizens of the planet.

### KEY DIFFERENCES IN THE LIVESTOCK INDUSTRY

**Market structure: centers of concentration and market power.** There are similarities in livestock and crop production, especially in post-farm marketing and processing. Concentration of raw material procurement and processing into the control of fewer and fewer entities has produced pervasive concerns by producers about the oligopsonic power of processors (USDA Advisory Committee on Concentration in Agriculture, 1996). Increasingly, the inputs purchased by grain and livestock producers, as well as the processors who purchase their products, have consolidated into entities having the capacity to exert substantial power in the market over the prices paid for raw farm products.

In the case of crop production, substantial consolidation has occurred during recent years with the purveyors of seeds, pesticides, and herbicides. This consolidation has clearly been driven in large part by the opportunity to integrate marketing strategies for genetics and the other products, such as transgene-mediated herbicide tolerance and insect resistance.

Consolidation of market power in the livestock industry has taken a somewhat different tack. In the case of swine and poultry production, complete integration of production has occurred, from genetics through consumer marketing. In beef, genetic inputs remain quite fragmented, as does calf production and feeding, but beef processing has become extremely concentrated. Consumer demand for consistent product quality directly drives the genetics programs of integrated swine and poultry producers, and has impacted beef cattle acquisition and pricing practices of the processing giants.

There are some very fundamental differences between marketing of grain and animal products. These differences lead to varying degrees of difficulty (opportunity) in maintaining the identity of the composition and source of the products. Milk and eggs are commingled bulk products, similar to grain.

Individual animals, however, are sold through a marketing and animal health surveillance system that attempts, however imperfectly, to maintain sufficient identification of animals to enable tracing back to the farms of origin. The great seasonal rush to market or store grain, sometimes limited by storage and transport capacity, contrasts the year-round marketing of animals and their products. The long-term bulk storage commonly employed by grain producers and brokers has no counterpart in the marketing of unprocessed animal products. Development of source- or composition-identified marketing channels for commingled bulk products would be extraordinarily difficult. Marketing approaches must have sufficient reward to justify segregation, or sufficient penalty to justify avoiding the out-of-favor genotypes.

**Differences in reproductive biology and selection programs.** Differences in the reproductive biology of crop plants and livestock have dictated the role and market power of purveyors of genetics. Plant genetics development is effectively controlled by relatively few seed suppliers by means of their portfolios of intellectual property in quantitative and molecular genetics. In the case of livestock, selection programs managed by genetics companies are quite influential, especially in swine and dairy breeding. However, considerable autonomy and influence remains with individual producers who control their own selection and control the herds of breeding females.

The degree of control maintained by suppliers reflects differences in organisms' reproductive biology and existing intellectual property law. Many crop plant varieties are proprietary, and their asexual propagation is restricted by the Plant Variety Protection Act (7 US Code 2321 et seq.). In most instances, commercially important cultivars are hybrids, i.e., progeny of crossed inbred parents, and are not useful as parental stock for subsequent breeding. In contrast, there is no counterpart to the Plant Variety Protection Act in animal breeding. Many swine and poultry are the products of "hybrid," non-purebred breeding lines controlled by integrated production companies. However, control over most of the cattle, sheep, and swine gene pool is globally diffused among thousands of individual livestock producers.

A fundamental difference between plant and animal breeding is found in the human capacity to control the dissemination of gametes. Wind- or insect-borne dissemination of pollen has no counterpart in animal breeding. This is not to say that all livestock breeding is planned and produces offspring of known parentage. However, there is an intrinsically greater capacity to control and monitor the movement of large mammals than microscopic pollen.

The use of inbred parental strains in hybrid plant breeding makes it necessary to introduce "new" genes by returning to source genetic material in seed banks or wild relatives of domesticated cultivars. As a result, concerns have been raised, from two perspectives, about the potential for transgenes to be exchanged between domesticated crops and wild relatives (Regal et al., 1994). The first of these is fear that nondomesticated relatives might become supercompetitive in ecosystems by virtue of introduction of transgenes. The second is concern that nondomesticated genetic resources might become 'polluted' by transgenes, with consequent loss of genetic diversity.

In contrast to plant breeding, animal breeding relies primarily on outbred parental stock, utilizing a combination of quantitative selection programs in purebred animals and crossbreeding of purebreds of differing biological types, to maxi-

mize performance and heterosis (nonadditive genetic effects) in progeny intended for commercial production. Because livestock breeding works almost exclusively with domesticated parental stock, there is no significant counterpart to the practice by plant breeders of using nondomesticated gene sources.

**Application of transgenic technology.** Livestock agriculture has been constrained in the application of biotechnology principally because of the lack of an efficient means of gene transfer in mammals. New transgenic technology now makes applications in livestock an economic and biologic reality.

Livestock producers frequently produce their own female replacements and purchase breeding males or semen. Thus, novel livestock genetics will be mixed into population genetics owned by many individuals and will need to perform satisfactorily in varied genetic backgrounds. Thus, it will be essential to verify the efficacy of these traits in field trials in populations of animals. Novel traits will probably be best evaluated through adaptations of traditional progeny testing programs.

Livestock producers are knowledgeable, are willing to pay for technology, and understand the marketing of genetics. Although not always appreciated, the sophistication of the current cattle genetics far exceeds that of pre-biotechnology genetics in crop agriculture. The cattle genetics business is concentrated in relatively few major companies worldwide. In North America many of these are cooperatives. While cooperatives are not likely to lead in transgenic technology development, they can form efficient secondary marketers of key traits. Because cattle genetics are largely distributed as semen, and this is a highly controlled business, tracking transgenic intellectual property in elite cattle should be a simpler task than in plant genetics. Only about 400 proven dairy sires supply the semen used in artificial insemination in the United States at any one time. Furthermore, the presence of specific gene traits is "auditable" by tissue sampling and testing.

As with crop transgenics, the array of traits that could be added to livestock genetics is wide. The traits can be grouped into production and disease resistance traits, reducing chemical or antibiotic intervention. As is the case with crops, we expect the production traits to be harder to define, as fewer are controlled by relatively simple single or dual gene traits. In contrast, single genes can be identified which, based on solid scientific evidence, can be expected to confer specific resistance to a given infectious or metabolic disease.

The first gene introductions in plants have been synthetic transgenes that provide the plant with specific insect resistance or herbicide tolerance. In livestock, it is most likely that the genes used initially will not be transgenes but will rather be *isogenes*, or naturally occurring genes from the same species of animal, such as antibodies or milk proteins.

**Structural implications.** The livestock genetics companies that first embrace the application of transgenic technology to provide livestock with value-added traits will likely quickly dominate the market. Others will become distribution appendices to new producer organizations or producer/processor alliances. A company launching such genetics can expect to establish a "brand identity," claim a greater market share, expand the use of artificial insemination into new markets, and obtain a greater share of overall investments in livestock production.

Worldwide livestock command about 40% of the value of agriculture compared with 60% based in crops. This represents about \$90 billion in farm gate receipts annually in the United

States. The bovine genetics market is a \$400 million industry in North America. Given the product value derived from this input, the producer could sustain higher costs per unit, and thus there is potential for growth in the value of the semen market. Value added traits should be superimposed on bovine genetics that are already elite by traditional standards. Centuries of animal breeding and selection based on phenotypic traits have already raised the incremental productivity that can be achieved by such traditional selection to the upper asymptote. Application of transgenic technology in livestock will go hand in hand with more traditional approaches to animal breeding, providing a new mechanism to achieve quantum increases. Transgenic technology will also expand the genetic pool used in commercial production by incrementing the performance of less selected populations and mitigating the trend of inbreeding in US cattle populations.

Beyond the economics of the genetics industry per se lays the potential economic benefits of well-targeted transgenic traits to the livestock producer, the processing industry and to the consumer. Transgenic manipulation of livestock can form a key component of precision agriculture, can minimize use of ancillary, and sometimes deleterious substances like antibiotics and pesticides, and can bring increased efficiency to the processing industry.

### THE OPPORTUNITIES FOR GENETIC ENGINEERING IN LIVESTOCK

Agriculture is a complex economic, social, and ecologic environment. A number of overarching criteria for use of transgenes in agricultural livestock can be identified. The foremost considerations must be animal welfare, safety, and ethical acceptability (Howard et al., 2000).

The impact of specific genetic engineering interventions must be carefully evaluated before they are attempted. This is the role of oversight groups such as Institutional Animal Care and Use Committees.

Once these fundamentals have been addressed, two sets of criteria come in to play. In general, they can be segregated into those based on biology, and those based on the marketability of the output.

**Biologic criteria.** A fundamental premise in selecting traits for modification is that the functional genomics must be clearly understood. It must be possible to relate gene to protein to function before attempting to manipulate the gene in the animal. The livestock industry has historically selected for desirable traits, rather than desirable genes. Many phenotypic traits are dependent on interactions of multiple genes over time. These traits therefore do not lend themselves to easy modification. The livestock industry has become accustomed to marker-assisted selection; such markers are manifestations of linkage maps to one of a complex of genes in a phenotype.

Traits that are presently susceptible to modification by transgenes are those that require only a very small number of genes to be introduced. The capacity of current gene introduction technologies is limited to a few genes, likely less than five. Multiple genes can also be stacked by back crossing.

The function of the transgene in the individual animal must be assessed, but also its impact on the population, and the production environment. What is the risk, for instance, of stimulating pathogen evolution by creating disease-resistant animals constitutively immune to specific pathogens? Each

transgene proposed for use will need an environmental assessment. Like most new agricultural technologies, each will also require a “technology education package” approach to introduction to make sure the benefits are realized.

**Criteria of marketability.** Like crop transgenics, livestock traits must have a distinct and measurable economic impact if they are to be adopted. Experienced producers with narrow economic margins will judge them against other herd or flock management strategies. Like the use of bovine growth hormone in dairy cattle, what counts is whether they make economic sense in the management context of each farm.

For the value to be captured from traits that generate niche products that “decommoditize” agricultural markets, there must be suitable marketing infrastructure. As the economic size of dairy farms grows to 1000 cows and more, this may mean an individual herd will be dedicated to supplying product for a single processing use. Developing such infrastructure will pose challenges to the tradition of commodity markets. Transgenic technology can offer the economic incentives to change the system and the potential to share value-added gain with milk producers now trapped in the economics of commodity milk prices.

Finally, the transgenes selected for implementation must be socially acceptable. Food production for the general human population demands a different mindset than the production of biopharmaceuticals. As consumers make decisions regarding the acceptability of genetically engineered products in the marketplace, perception may be the most important factor. Only a small minority of consumers make considered choices themselves; in many cases retailers assume the role for them. Scientists must work to ensure that in either case, the perception is built upon balanced factual information and a broad view of the food system.

**Challenges in understanding the market environment.** Agriculture is a traditional and culturally complex industry. The economic drivers, the structure of the genetics component, and the culture of the producer and processor interface are unique to each species and sector in the livestock industry. Thus, beef and dairy are as different from each other as they are from the swine industry and the sheep industry. Determining the acceptability of new technology, and managing its adoption, has the same degree of cultural and environmental complexity and local variability, whether we are dealing with transgenic mastitis resistance in dairy cattle, or high oil corn, or the introduction of a new irrigation method to a developing country. We must gain an in-depth understanding of the context in which the new technology will be applied and all the secondary ramifications. Like any agricultural technology, the future of transgenic livestock will be facilitated by finding key “early adopters” and opinion leaders.

**The livestock value chain.** As we look to identifying valuable traits, it is useful to review the “value chain” of a typical commodity food item. In economic terms, “price discovery” occurs at each point in this chain and the price at the consumer level reflects the value added along the way.

At the start of the chain are the various inputs to swine production (capital, labor, facilities, nutrition, genetics, etc.), each of which has an associated cost. At each link in the chain, through nursery management, growing, processing, and retailing, value is added and the price increases accordingly until the consumer purchases the pork in the supermarket at retail price. Consumer preferences are transmitted back through the

chain as pricing signals to the producer. The producer can change input practices to better attempt to meet the consumer demand and obtain a better transfer price for their product. However, the ability to meet changing consumer preferences through genetic selection is a slow process. This could change radically with the application of genetic engineering in livestock.

Although the producer only receives about 20 cents of the food dollar, clearly, value is created at the farm producer level. Value added outside the farm gate is largely in the form of labor, packaging, marketing, and transportation, and the potential for a genetic impact in any of these areas is minimal. The greatest impact of genetic influence is at the level of the producer and to a lesser extent at the processor.

**Trait selection.** The types of traits that can be introduced can be categorized into two broad groups: input traits and output traits. Input traits are those that will impact the cost of doing business for the producer by reducing some costs associated with production. Under this scheme, a trait that reduces veterinary and drug costs through increased resistance to disease would be considered an input trait. An output trait would be one that increases the output of a normal food product, adds value to the product, or creates an entirely new food product.

Traits are being designed with capability to

- increase feed utilization,
- confer resistance to diseases and thus reduce of drug/antibiotic use,
- reduce animal waste handling and disposal problems,
- increase productivity to reduce resource use,
- diversify agricultural products providing new economic opportunities in rural areas, and
- provide new consumer products.

**Disease resistance.** Delivery of disease resistance through genetics offers many advantages. Consumers benefit because healthy animals contribute to healthy food. As on-farm antibiotic use is reduced, the risk of residues is reduced. In addition, animal welfare is benefited by disease control.

In some cases, the logistics of application of conventional vaccines precludes their efficacy. This is true when disease strikes neonatal animals. It is also true in settings where refrigeration facilities for handling live vaccines are lacking, and where round-up and handling of stock for vaccination is difficult. The broad array of production diseases that contribute most to antibiotic usage in agriculture will be most suited to constitutive control. Notable examples are enteric, respiratory, and mammary infections. Hyperendemic host-adapted infections such as salmonellosis are notoriously difficult to control by conventional means.

Reduction of disease in livestock populations inevitably enhances animal welfare. Many disease situations are triggered by man’s attempt to demand more from livestock productivity than a given environmental setting permits, or to move ill-adapted livestock breeds into alien environments. By using transgenes to promote the productivity of locally adapted, but otherwise low-producing livestock, there is less stress on the balance of environmental adaptation. Rather than move a Holstein cow to the tropics to succumb to tick-borne disease, we can contemplate simply moving her genetic potential for productivity into a locally adapted breed.

Globalization of agricultural marketplaces and the overall increase in human mobility increase the vulnerability of US livestock populations to introduction of exotic animal disease.

Sharing biotechnology, which can enable better livestock disease management, and thus food production worldwide, has obvious humanitarian benefits. It can also assist in underpinning our own food security. Once disease resistance traits are developed, their dissemination through genetics can be a fairly "low tech" process applicable in infrastructure-poor countries, where vaccination may be difficult.

Virtually any protein is a candidate transgene or, if derived from the same species, isogene. It may be possible to address the limiting aspects of animals' intermediate metabolism through application of transgenesis. Improved control of the common metabolic "production diseases" of ruminants would contribute further to reducing the use of antibiotics in animal production. Obviously, alteration of the intermediate metabolism of cattle raises issues of potential pleiotropic effects on metabolism and interactions with the animals' diets that will bear close experimental scrutiny. The capacity to alter metabolism through genetics is not new, however. Traditional breeding practices have produced very lean genotypes of cattle and swine, each well adapted to certain production systems, but unsuitable for others (R. S. Ott, 1990, Hartmann et al., 1997). Transgenesis at least provides an opportunity to rapidly evaluate the safety, production, and animal welfare implications of new genotypes before many years of selection are expended and thousands of animals are bred.

**Product composition.** Transgenesis can be used to modify product composition for several purposes. For example, milk composition, safety, nutritional value, and processing characteristics can all be fine-tuned by driving the expression of milk proteins. Such milk expression could be that of a selected, naturally occurring protein, or the addition of a novel protein. Many minor proteins in milk have intrinsic economic value but are naturally present at such low levels as to make isolation uneconomical. By increasing the relative concentration of these proteins, their isolation during dairy manufacturing can be made feasible. Consumers prefer to purchase low fat milk, but naturally secreted milk has a fixed fat to protein ratio. Consequently, at times there is a relative excess of butterfat in manufacturing channels. Driving the production of more protein can shift the protein to fat ratio.

A wide range of manipulations could be invoked to produce milks with different rheological properties, freezing characteristics, taste, and allergenicity. Some cows have milk protein polymorphisms that make their milk poorly suited for cheese making (Ikonen et al, 1999). Milk with additional kappa casein may favor some cheese-making processes (Fitz-Gerald and Hill, 1997). Many naturally occurring milk polymorphisms are recognized, but if a desirable polymorphism is present at low frequency in a population, intense selection for it may lead to inbreeding. Transgenic technologies can be used to build the advantages of such polymorphisms into the milk of breeds or breeding lines where they do not presently occur, while preserving other genetic diversity. Transgenic manipulation of milk composition may also allow dairy processors greater creativity in the design of manufactured products.

**Preservation of genetic diversity.** Despite years of selective breeding, current livestock breeds retain a high degree of genetic diversity. In addition, a wide variety of outbred populations of livestock have been selected to offer phenotypic advantages in a diverse array of production environments. Such diversity is valuable in providing adaptability to changing environments and production needs, whether driven by

market economics, feed resource availability, or disease challenge.

To the degree that transgenes may confer an added economic or biological advantage, they will do so by superimposing on the most elite genetics selected by conventional means for the specific environment in which they are to perform. It will be useful to be able to introgress transgenes into new production populations and to maintain herds with and without the transgenic features. The expression and effect of any given transgene are likely to vary depending on the genetic background against which it is superimposed. Thus, existing systems of genetic evaluation may be well suited to measure the efficacy of transgenes.

Methods of transgene introduction differ in their ability to conserve diversity. At one extreme, nuclear transfer will remove all other genetic diversity. At the other extreme, retrovector-mediated transgenesis can be applied to any mating of any existing livestock species in which in vitro fertilization can be performed.

The introduction of highly valued transgenes into a cattle population by nuclear transfer could potentially have a significant effect in narrowing the genetic pool from which subsequent generations are produced. Additive effects of embryo transfer and artificial insemination technologies, coupled with intense selection, in dairy cattle breeding programs during the past 20 yr have resulted in an increased level of inbreeding in the US dairy cattle population (USDA, Animal Improvement Programs Laboratory, and Notter, 1999). Dairy cattle genetics differs from that of swine and poultry in that selection is managed independently by a large number of individual producers. If a very popular transgene were introduced against this intense genetic background, it could have the effect of further reducing the diversity in the dairy population. Producers make selection decisions based on a number of characteristics from temperament to conformation to production. The ideal situation would thus be for a successful transgene to be combined with an array of other traits from which producers could select. Genetic diversity could also be enhanced by endowing, through transgenesis, breeding lines superior in all other respects with trait(s) sufficient to make them competitive in productivity.

While there is a huge array of potential applications of transgenesis, the caveat is to examine each application cautiously in the context of the livestock system, social context, and market infrastructure in which it will be applied.

**Biopharmaceutical applications.** Although the emphasis of this paper is agricultural applications of transgenesis, it is important to acknowledge the very important contribution to human healthcare we expect of livestock transgenics that produce biopharmaceuticals. The regulatory framework in which such production will operate precludes its complete integration into the regular agricultural infrastructure. However, the biopharmaceutical applications are important engines of research and development and have helped to pave the way to acceptance of agricultural transgenics. As the power of genomics to personalize medicine to subgroups sharing the same genetic polymorphisms unfolds, the need to create low-cost bioreactor systems will increase. Similarly as the efficacy of more recombinant protein drugs becomes known, the need increases for systems to produce complex proteins on a larger scale, but at an affordable cost to the patient. Livestock transgenics can fill this niche.

## STATUS OF THE TOOLS

There are currently three alternative methods by which genes can be inserted into genomes: pronuclear microinjection, NT—commonly called cloning—and vector mediated transgenesis.

Genomes are well protected from insertion of rogue genetic material that might have a deleterious effect on the organism. Thus, insertion of transgene DNA into zygote genomic DNA depends on function of repair enzymes. That pronuclear microinjection of DNA results in low efficiency of integration may reflect an error in repair function rather than a success in integration. Despite early optimism for increasing the efficiency of DNA insertion by pronuclear microinjection, low efficiency and mosaicism in the resulting founder animals have largely limited the utility to pharmaceutical production.

As is the case for pharmaceutical production, nuclear transfer in livestock agriculture has its primary application as a means of transgene introduction into founder animals or to replicate unique animals. Techniques commonly used in mammalian cell culture are used to introduce genes into the nuclear-donor cells and then various selection schemes are used to isolate the cells with a gene insert. Nuclear transfer production of a genetically engineered animal is then achieved by transferring the nucleus from the donor cell into an appropriately activated oocyte. Once the animal is born, appropriate temporal and spatial expression in the target animal must be evaluated and the animals bred to produce offspring. An attractive feature of NT technology is its use in doing knockout replacements in which an endogenous gene is replaced with, say, a human gene thus enabling the production of a human gene pharmaceutical product in the animal (Ayares, 1999). Knockout technology is likely to have less application in livestock but could be used to remove undesirable single gene traits.

A third approach is to use a retrovector-mediated system for production of transgenic animals. In this case RNA retrovectors are produced in very high titer and are put into the perivitelline space of an oocyte in metaphase II arrest prior to fertilization. Reverse transcription of the RNA by an enzyme carried in the vector is followed by very efficient and precise enzyme-mediated integration of single gene copies of DNA into the genome at selected sites. Insertion occurs in the oocyte, so that after fertilization the new genes behave as if they were maternal in origin, and mosaicism does not occur. A veterinarian performing embryo transfer commonly practices all other technology components used in the process. The efficiency of vector-mediated gene insertion is such that it is possible to create a number of different founder lines and then pick those for propagation based on phenotypic evaluation of the first generation. Sex selection of the transgenics can be carried out by using sexed semen for the fertilization step.

## THE CHALLENGES

The overwhelming challenge to agriculture in the next century will be to feed a huge population while creating and maintaining sustainable agricultural systems. Biotechnology, including transgenic livestock, will play a key role in meeting this challenge by allowing greater precision in resource use and product design. Utilizing the tools to their full will be possible in the context of an enabling regulatory framework

which can provide timely science based risk assessment and assurances of safety to the concerned public.

Change is inevitably threatening. The new product opportunities will demand new infrastructures, for instance to preserve food identity. There will be winners and losers among producers and processors as new economic criteria emerge. At this stage we cannot anticipate what the characteristics of each will be, except to note that the most adaptable will be the survivors.

Those of us trained in scientific logic are sometimes impatient with other mindsets and approaches to arriving at value judgments. The impact of biotechnology stretches beyond simple, factual scientific feasibility studies and risk analysis, to social judgments about the integrity of species, intervention in nature, and questions of rights in personal choice of food. Complete and balanced information will encourage complete and balanced decision-making. But we must also be mindful that special interest groups are at work to deliberately distort information to foster agendas that are often unrelated to the utility or perceived threat of the technology per se.

We face an immense array of opportunities and a bewildering complexity of applications in exercising our new technologies. Biotechnology provides us tools to respond to the demands of the planet in the third millennium. Using the toolbox wisely will require us to listen carefully to what producers, consumers, and processors want and to be guided by firm principles of safety, risk assessment and animal welfare.

In evaluating the choices, we must give equal weight to the risk of not implementing technology we have at our disposal. What is the opportunity cost of not using biotechnology to maximize our ability to create a sustainable planet? What would the costs have been of not implementing vaccination, not implementing the automobile, or not exploring space? We celebrate the visionaries in history rather than the reactionaries. As Darwin said, "It is not the strongest of the species who survive, nor the most intelligent, but the one most responsive to change."

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