Precision genetics for complex objectives in animal agriculture

Precision genetics for complex objectives in animal agriculture

S. C. Fahrenkrug, A. Blake, D. F. Carlson, T. Doran, A. Van Eenennaam, D. Faber, C. Galli, Q. Gao, P. B. Hackett, N. Li, E. A. Maga, W. M. Muir, J. D. Murray, D. Shi, R. Stotish, E. Sullivan, J. F. Taylor, M. Walton, M. Wheeler, B. Whitelaw and B. P. Glenn

J ANIM SCI 2010, 88:2530-2539.
doi: 10.2527/jas.2010-2847 originally published online March 12, 2010

Precision genetics for complex objectives in animal agriculture

S. C. Fahrenkrug,*†‡§[1] A. Blake,# D. F. Carlson,*†‡ T. Doran,‖ A. Van Eenennaam,¶ D. Faber, C. Galli,†† Q. Gao,‡‡ P. B. Hackett,†§§ N. Li,## E. A. Maga,‖‖ W. M. Muir,¶¶ J. D. Murray,¶*** D. Shi,§ R. Stotish,*** E. Sullivan,††† J. F. Taylor,‡‡‡ M. Walton,§§§ M. Wheeler,### B. Whitelaw,‖‖‖ and B. P. Glenn¶¶¶**

*Department of Animal Science, University of Minnesota, St. Paul 55108; †Center for Genome Engineering, University of Minnesota, Minneapolis 55455; ‡Recombinetics Inc., Minneapolis, MN 55418; §Animal Reproduction Institute, Guangxi University, Nanning, P.R. China 530000; #Yorktown Technologies, Austin, TX 78750; ‖CSIRO Livestock Industries, Australian Animal Health Laboratory, Geelong, VIC 3220 Australia; ¶Department of Animal Science, University of California, Davis 95616; **Trans Ova Genetics, Sioux Center, IA 51250; ††Laboratorio di Tecnologie della Riproduzione, Instituto Sperimentale Italiano Lazzaro Spallanzani, Cremona, Italy 26100; ‡‡Jiangsu Animal Husbandry and Veterinary College, Taizhou, P.R. China 225300; §§Department of Genetics and Cell Biology, University of Minnesota, Minneapolis 55455; ##State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing, P.R. China 100094; ‖‖Department of Animal Science, Purdue University, West Lafayette, IN 47907; ¶¶Department of Population Health and Reproduction, University of California, Davis 95616; ***Aqua Bounty Technologies, Waltham, MA 02451; †††Hematech Inc., Sioux Falls, SD 57104; ‡‡‡Division of Animal Sciences, University of Missouri, Columbia, MO 65201; §§§ViaGen Inc., Austin, TX 78727; ###Department of Animal Sciences, University of Illinois, Urbana-Champaign 61801; ‖‖‖The Roslin Institute, The University of Edinburgh, Roslin, Midlothian EH25 9PS Scotland, UK; and ¶¶¶Biotechnology Industry Organization, Washington, DC 20024

ABSTRACT: Indirect modification of animal genomes by interspecific hybridization, cross-breeding, and selection has produced an enormous spectrum of phenotypic diversity over more than 10,000 yr of animal domestication. Using these established technologies, the farming community has successfully increased the yield and efficiency of production in most agricultural species while utilizing land resources that are often unsuitable for other agricultural purposes. Moving forward, animal well-being and agricultural sustainability are moral and economic priorities of consumers and producers alike. Therefore, these considerations will be included in any strategy designed to meet the challenges produced by global climate change and an expanding world population. Improvements in the efficiency and precision of genetic technologies will enable a timely response to meet the multifaceted food requirements of a rapidly increasing world population.

Key words: cross-breeding, genetic engineering, genetic technology, hybridization, phenotypic diversity, selection

J. Anim. Sci. 2010. 88:2530–2539
doi:10.2527/jas.2010-2847

INTRODUCTION

Agricultural science has been enormously successful in providing an inexpensive supply of high-quality and safe foods to developed and developing nations. These advancements have largely come from the implementation of technologies that focus on efficient production and distribution systems as well as selective breeding and genetic improvement of cultured plants and animals. Although population growth in developed nations has reached a plateau, no slowdown is predicted in the developing world until about 2050, when the population of the world is expected to reach 9 billion (United Nations, 2008). To meet the global food demand will require nearly double the current agricultural output, and 70% of that increased output must come from existing or new technologies (United Nations, 2002).

The global demand for animal products is also substantially growing, driven by a combination of population growth, urbanization, and rising incomes. However, at present, nearly 1 billion people are malnourished (United Nations, 2008). Animal products contain

[1]Corresponding author: fahre001@gmail.com
Received January 21, 2010.
Accepted February 26, 2010.

concentrated sources of protein, which have AA compositions that complement those of cereal and other vegetable proteins, and contribute calcium, iron, zinc, and several B group vitamins. In developing countries where diets are based on cereals or bulky root crops, eggs, meat, and milk are critical for supplying energy in the form of fats. In addition, animal-derived foods contain compounds that actively promote long-term health, including bioactive compounds such as taurine, L-carnitine, creatine, and endogenous antioxidants such as carosine and anserine (Chan and Decker, 1994; Williams, 2007). Furthermore, those foods are a rich source of CLA, forms of which have anti-cancer properties (Kelley et al., 2007), reduce the risk of cardiovascular disease (Tricon et al., 2004), and help fight inflammation (Zulet et al., 2005).

A MANDATE FOR ANIMAL SCIENTISTS

Animal production will play a pivotal role in meeting the growing need for high-quality protein that will advance human health. Our technological prowess will be put to the test as we respond to a changing world and increasingly diverse stakeholders. Intensifying food production likely will be confounded by declining feedstock yields due to global climate change, natural resource depletion, and an increasing demand for limited water and land resources (Foley et al., 2005). Additionally, whereas the moral imperative to feed the malnourished people of the world is unequivocal, a well-fed, well-educated, and vocal citizenry in developed nations places a much greater emphasis on the environmental sustainability of production, the safety of food products, and animal welfare, often without regard for impact on the cost of the food. These diverse priorities will place important constraints on animal agriculture in the coming decades. Despite these daunting challenges, the sheer magnitude of potential human suffering calls on us to assume the reins from our recently lost colleague, Norman Borlaug, to harness technological innovation within our disciplines to keep world poverty, hunger, and malnutrition at bay. As was the case during the Green Revolution, advancements in genetics and breeding will provide a wellspring for a needed revolution in animal agriculture. Indeed, we have entered the era of the genome for most agricultural animal species. Genetic blueprints position us to refine our grasp of the relationships between genotype and phenotype and to understand the function of genes and their networks in regulating animal physiology. The tools are in hand for accelerating the improvement of agricultural animals to meet the demands of sustainability, increased productivity, and enhancement of animal welfare (Green et al., 2007).

A FUTURE WITH GENETIC SELECTION

The goals of animal genetic improvement are firmly grounded in the paradigm of animal production, which naturally refers to concepts of efficiency, productivity, and quality. Sustainability and animal welfare are central considerations in this paradigm; an inescapable principle is that the maximization of productivity cannot be accomplished without minimizing the levels of animal stress. Furthermore, the definition of efficiency (product per unit input) requires sustainability. Unnecessary compromises to animal well-being or sustainability are morally reprehensible and economically detrimental to consumers and producers alike.

The vast majority of outcomes from genetic selection have been beneficial for animal well-being. Geneticists try to balance the enrichment of desirable alleles with the need to maintain diversity (Lacy, 1997; Notter, 1999) because they are keenly aware of the vulnerability of monoculture to disease. Genetic improvement programs must always conserve genetic diversity for future challenges, both as archived germplasm and as live animals (Blackburn, 2004). However, unanticipated phenotypes occasionally arise from genetic selection for 2 reasons. First, every individual carries deleterious alleles that are masked in the heterozygous state but can be uncovered by selective breeding. Second, the linear organization of chromosomes leads to certain genes being closely linked to each other on the DNA molecules that are transmitted between generations. Thus, blind selection for an allele that is beneficial to 1 trait also enriches for all alleles that are closely linked to it and either through pleiotropy (genes influencing more than 1 trait) or linkage disequilibrium, undesirable correlated responses in other traits may occur. Geneticists are aware of this and closely monitor the health and well-being of populations that are under selection (e.g., Eitan and Soller, 2004) to ensure that any decrease in fitness is detected and that ameliorative actions are taken to correct problems either by the elimination of carriers from production populations, altering the selection objective to facilitate improvement in the affected fitness traits, or by introducing beneficial alleles by crossbreeding. Increasingly precise molecular tools now allow the rapid identification of genetic variants that cause single-gene defects and facilitate the development of DNA diagnostics to serve in genetic management plans that advance the production of healthy animals. Whole-genome genotyping with high-density, SNP assays will enable the rapid determination of the overall utility of parental lines in a manner that is easily incorporated into traditional quantitative genetic improvement programs (Meuwissen et al., 2001). The approach is known as genomic selection (**GS**) and essentially allows an estimation of the genetic merit of an individual by adding together the positive or negative contributions of alleles across the genome that are responsible for the genetic influence on the trait(s) of interest. Under GS, genetic improvement can be accelerated by reducing the need for performance testing and by permitting an estimation of the genetic merit of animals outside currently used pedigrees. Genomic selection also provides for development of genetic di-

agnostics using experimental populations, which may then be translated to commercial populations, allowing, for the first time, the opportunity to select for traits such as disease resistance and feed efficiency in extensively managed species such as cattle. The presence of genotype × environment interactions will also require the development of experimental populations replicated across differing environmental conditions to enable global translation of GS.

The speed with which the performance of animals can be improved by GS is determined by generation interval, litter, or family size, the frequency of desirable alleles in a population (rare variants are likely to be missed in the development of genetic prediction models), and the proximity (and phase relationship) on chromosomes of good and bad alleles. Although predicting genetic merit using DNA diagnostics may be less precise than directly testing the performance of every animal or their offspring, the reduction in generation interval by far offsets this. For example, in dairy populations, the rate of genetic improvement is expected to double with the application of GS (Hayes et al., 2009). Preliminary results from the poultry industry suggest that GS focused on leg health in broilers and livability in layers can rapidly and effectively improve animal welfare (Cheng et al., 2010). Although price constraints currently limit the widespread adoption of high-density SNP genotyping assays (≥50,000 SNP) in livestock species, low-cost, reduced-subset assays containing the most predictive 384 to 3,000 SNP are under development in sheep, beef, and dairy cattle. These low-cost assays are expected to be rapidly adopted and will be expanded in content as the price of genotyping declines. Animal selection based on GS is also expected to reduce the loss of genetic diversity that occurs in traditional pedigree-based breeding because the ability to obtain estimates of genetic merit directly from genotypes avoids the restriction of selection to the currently used parental lineages. Also, despite the increase in the rate of genetic improvement, selection for complex traits involving hundreds or thousands of genes will not result in the rapid fixation of desirable alleles at all of the underlying loci.

A FUTURE WITH GENETIC ENGINEERING

Whereas GS will accelerate animal improvement in the postgenomic era, parallel and overlapping efforts in animal improvement based on genome-informed genetic engineering (**GE**) must ensue to ensure that productivity increases at pace with the expanding world populations. The tools of functional genomics and the availability of genome sequences provide detailed information that can be used to engineer precise changes in traits, as well as monitor any adverse effects of such changes on the animal (Miller et al., 2006; Doyon et al., 2008; Geurts et al., 2009). These tools are also enabling a deeper understanding of gene function and the integration of gene networks into our understanding animal physiology (Schadt, 2009). This understanding has begun to identify major effect genes and critical nodes in genetic networks as potential targets for GE.

TECHNOLOGICAL REVOLUTION IN GENETIC ENGINEERING

The genomics revolution has been accompanied by a renaissance in GE technologies. Novel genes can be introduced into a genome (Clark et al., 2007), and existing genes can either be inactivated or their expression tuned to desirable levels using recently developed RNA interference (Fire et al., 1998; NPG, 2005). The specificity and efficiency of these approaches is expected to continue to improve. The technical advancements in GE are so significant that Greger (2009) advocated that scrutiny of the procedures for generating transgenic farm animals is undeserved and that discussion should focus on the welfare implications of the desired outcome instead of unintended consequences of GE. This position is also reflected by the rigorous regulatory mechanism established by the FDA for premarket approval of GE animals (FDA, 2009), which considers the risks of a given product to the environment and the potential impact on the well-being of animals and consumers. Indeed, this review mechanism was recently adopted as an international guideline by Codex Alimentarious (2008), which has already found GE to be a safe and reliable approach to the genetic improvement of food animals (US Code of Federal Regulations, 2009). In addition, guidelines that promote good animal welfare, enhance credibility, and comply with current regulatory requirements, for the development and use of GE animals have been developed as a stewardship guidance (Biotechnology Industry Organization, 2009). The stewardship guidance assists the industry and academia in developing and adopting stewardship principles for conducting research and developing and commercializing safe and efficacious agricultural and biomedical products from GE animals for societal benefit.

SELECTION AND ENGINEERING ARE COMPLEMENTARY

Both GS and GE are viable, long-term approaches to genetic improvement, but when should one approach be employed over the other? Genes are not all equal in their effects upon changes in phenotype. The products encoded by some genes have major effects on biochemical pathways that define important characteristics or reactions in an organism. Other genes have lesser, but sometimes still important, effects. In general, genetic modification by GE is used to add major-effect genes, whereas genetic selection is applied to all genes, including the far larger number of lesser-effect genes that appear to be responsible for about 70% of the genetic variation within a given trait (Cole et al., 2009). One of the most significant advantages of GE is the abil-

ity to introduce new alleles that do not currently exist within a population, in particular, where the allele substitution effect would be very large. This approach can include gene supplementation and genome editing, the latter enabling the precise transfer of an alternative allele without any other changes to the genome of an animal (i.e., without selection markers, or even the genome-wide changes caused by crossbreeding). In this case, molecular methods can be used to supplement or replace a target allele present in one population with a preferred allele present in another.

The key issue is, of course, the identification of the genes and alleles that should be targeted for GE in livestock species. Likely, many genes for which naturally occurring variation creates subtle phenotypic effects exist, whereas GE of these genes with novel alleles might provide profound improvements in animal health and performance. Undoubtedly, the discovery of these genes will come from research with model organisms where the effects of the allelic forms on fitness can be extensively studied, emphasizing the importance of support for basic agricultural research.

ENHANCED PRODUCTIVITY AND SUSTAINABILITY

Early research efforts in livestock GE were focused on increasing the efficiency and yield of production for a diversity of species. The use of more productive GE animals [i.e., animals that produce more units of output (e.g., gallons of milk, pounds of meat) with the same or less inputs] should be given due consideration in the context of sustainability. Making a conscious choice to use less productive animals necessitates the use of more land per unit of product or the use of more animals to produce a constant amount of animal product or both. This choice could cause conflict when open land is scarce, or other uses (e.g., habitat conservation) compete with the use of land for agriculture. The use of the best available technologies and inputs (best genotypes and best ecological management) to produce greater output per unit of input offers overt sustainability advantages (Pretty, 2008). Rapid growth and increased production result in a reduced number of animals being required for a fixed amount of output. Additionally, the environmental footprint per unit of animal product is reduced for more productive animals, regardless of the agricultural production system used. Bradford (1999) estimated that a small incremental increase of 2% per year in average milk production per cow globally, with no change in cow numbers, would result in a 60% increase in the global milk supply by the year 2020.

The observation by Palmiter et al. (1982) that supplementing the mouse genome with an extra GH gene increased muscle growth greatly stimulated research into this approach for enhancing the productivity of meat animals. However, early transgenic animal GH supplementation experiments (in the late 1980s) achieved mixed results. Whereas growth enhancement was observed in experiments with both fish and mammals (Nottle et al., 1999; Zbikowska, 2003), in some experiments increased GH concentrations compromised the health of animals (Hammer et al., 1985; Pursel et al., 1989; Nancarrow et al., 1991; Dunham, 2009). From this research, much was learned about the GH axis, methods for transgenesis, the need for controlled gene expression, and best practices for transgenic animal stewardship. One successful outcome of this early work was a transgenic salmon whose genome contains an extra copy of the salmon GH gene (Table 1).

After nearly 15 years of research and development, the GH-transgenic salmon are now in an advanced stage of regulatory review by the FDA and could constitute the first transgenic animal product approved for human consumption in the United States. These fish produce the same amount and kind of circulating GH as wild-type salmon, but they produce it throughout the entire year. This modification has resulted in fish that reach market weight faster and consume less food per kilogram of product than wild-type salmon because they process their food 10 to 30% more efficiently. The more efficient utilization of protein in the diet of an animal leads to a reduction in the excretion of nitrogenous waste (Coffey, 1996; Sillence, 1996). Concerns over the potential environmental impact of feral transgenic fish have been studied extensively (Devlin et al., 2004, 2009; Sundstrom et al., 2007) and practically addressed by eliminating the possibility of gene flow from transgenic to wild salmon by implementing a redundant biological and physical containment production system, which exclusively utilizes sterile female fish. These fish are reared in land-based facilities with multiple redundant physical containment features. Because of the controlled production environment, the fish are neither exposed to disease challenges nor are they reservoirs for disease transmission as they might be in conventional salmon net pen aquaculture. The ability to grow these salmon in land-based facilities closer to population (consumption) centers dramatically reduces transportation costs compared with conventional salmon aquaculture, affecting the economics and environmental footprint of salmon production. Finally, because these fish can be raised in inland fisheries, they represent a unique food security opportunity, potentially rejuvenating a nearly extinct US Atlantic salmon industry.

To address the sustainability of pork production, a line of GE pigs was developed with the ability to digest and metabolize natural P in their feed, which non-GE pigs cannot accomplish. These pigs referred to as Enviropigs, have a genome supplemented with a gene from *Escherichia coli* that produces phytase exclusively in their salivary glands (Golovan et al., 2001). This genetic modification reduces the excretion of undigested P in pig feces by 30 to 60%, which will ameliorate surface water eutrophication from swine production, as well as eliminate the environmental footprint of phytase production as a feed supplement (Forsberg et al., 2003). Clinical analysis of the health of Enviropigs using hematology,

Table 1. Current and envisioned genetically engineered (GE) livestock applications for agriculture[1]

Current GE application	Species	Gene	Approach	Reference
Productivity				
Enhanced growth rate	Various	GH	Transgene	Hammer et al., 1985; Vize et al., 1988; Pursel et al., 1989; Nancarrow et al., 1991; Pursel et al., 1997; Rahman et al., 1998; Cook et al., 2000; Martinez et al., 2000; Nam et al., 2001; Aerni, 2004; Bessey et al., 2004
Enhanced milk production	Swine	α-Lactalbumin	Transgene	Wheeler et al., 2001; Marshall et al., 2006
Enhanced growth rate	Swine	*IGF1*	Transgene	Pursel et al., 2004
Disease resistance				
Bovine spongiform encephalopathy resistance	Cattle	Prion protein PrP	Knockout	Richt et al., 2007a,b
Mastitis resistance	Cattle	Lysostaphin	Transgene	Wall et al., 2005
Mastitis resistance	Cattle	Lactoferrin	Transgene	van Berkel et al., 2002
Mastitis resistance	Goats	Lysozyme	Transgene	Maga et al., 2006a,c
Visna virus resistance	Sheep	Visna virus envelope gene	Transgene	Clements et al., 1994
GCH virus resistance	Grass carp	Lactoferrin	Transgene	Zhong et al., 2002
Bacterial resistance	Channel catfish	Cecropin B gene	Transgene	Dunham et al., 2002
Environmental				
Decreased P in manure	Swine	Phytase	Transgene	Golovan et al., 2001
Nutritional value				
Humanization	Cattle	Human α-lactalbumin	Transgene	Wang et al., 2008
Humanization	Cattle	Human lactoferrin	Transgene	Yang et al., 2008
Fat content	Swine	Spinach Δ^{12} fatty acid desaturase	Transgene	Saeki et al., 2004
Fat content	Swine	n-3 fatty acid desaturase	Transgene	Lai et al., 2006
Fat content	Goats	Stearoyl-CoA desaturase	Transgene	Reh et al., 2004
Protein content	Cattle	β-Casein, κ-casein	Transgene	Brophy et al., 2003
Envisioned GE applications	**Species**	**Gene**	**Proposed approach**	**Background information**
Allele replacement	Various	Various	Homologous recombination	Miller et al., 2006; Geurts et al., 2009
Increased lean-muscle growth	Various	Myostatin	Dominant negative/RNAi/knockout	McPherron and Lee, 1997
Increased postnatal growth	Various	*Socs2*	RNAi/knockout	Horvat and Medrano, 2001
Enhanced mammary gland development	Various	*Socs1*	RNAi/knockout	Lindeman et al., 2001
Sex selection	Various	*DMRT1*, sex-specific gamete enrichment	Dominant negative/RNAi	Herrmann et al., 1999; Smith et al., 2009
Suppressing infectious pathogens	Various	RNA viruses (e.g., foot and mouth, fowl plague, swine fever)	RNAi	Clark and Whitelaw, 2003; Whitelaw and Sang, 2005
Coronavirus resistance	Swine	Aminopeptidase N	RNAi/knockout	Schwegmann-Wessels et al., 2002
Avian flu resistance	Poultry	Avian influenza	RNAi	Sang, 1994; Tompkins et al., 2004
Low lactose milk	Cattle	Lactase	Transgene	Stacey et al., 1995
Low lactose milk	Cattle	α-Lactalbumin	RNAi/knockout	Jost et al., 1999
Humanization	Cattle	β-Lactoglobulin	RNAi/knockout	Wang et al., 2008
Increased ovulation rate	Sheep	*GDF9*, *BMP15*, *ALK6/BMPR1B*	RNAi/knockout	Melo et al., 2007
High n-3 fatty acid milk	Cattle	n-3 and n-6 fatty acid desaturase	Transgene	Morimoto et al., 2005
Resistance to brucellosis	Cattle	*NRAMP1*	Transgene	Barthel et al., 2001
Nutritional enhancement	Cattle	Human catalase	Transgene	He et al., 2008

[1]GCH = grass carp hemorrhage virus; RNAi = RNA interference.

clinical chemistry, and urology revealed no substantive difference between these GE and non-GE pigs except for a marked improvement in P retention, an outcome expected to enhance animal health by improving nutrient utilization and bone strength. Proteomic analysis of liver and muscle tissues from Enviropigs demonstrated no significant difference in the proportions of the major proteins as compared with those of age-matched non-GE pigs (Golovan et al., 2008; Hakimov et al., 2009).

ENHANCING ANIMAL WELFARE

As mentioned, a focus on animal welfare positively influences productivity, and, therefore, indirectly enhances the sustainability of animal production. Numerous targets of GE aim to deliver even more direct, simultaneous improvements in animal welfare and sustainability (Table 1). For example, GE could provide a humane method for sex selection in dairy and egg industries, where cows and hens provide the animal product. The development of male animals could be avoided ab initio and eliminate inefficiencies in animal production and welfare concerns associated with sex selection and castration. Gene supplementation that feminizes male embryos (Smith et al., 2009) or eliminates the production of male sperm in sires (Herrmann et al., 1999) is technically feasible; the latter approach has the desirable outcome that the animals that are produced are not themselves genetically engineered.

Based on the global importance of pork, researchers have developed GE pigs to improve the sustainability of production, enhance animal welfare, and add nutritional value (Table 1). For example, the expression of bovine α-lactalbumin and IGF in the mammary gland of lactating sows results in increased milk production, which directly enhances animal welfare as demonstrated by improved growth, intestinal development, and overall survival of piglets at weaning (Wheeler et al., 2001). In a striking example of the value of GE in enhancing animal welfare, Wall et al. (2005) at the USDA-ARS engineered Jersey cattle to express the antibacterial protein lysostaphin in their milk, an accomplishment that dramatically enhanced the resistance of these cows to infection by *Staphylococcus aureus*, the most common and most difficult to treat cause of mastitis. This genetic improvement, could not only improve the well-being of around 2 million dairy cattle per year in the United States alone, but also could decrease the economic costs of mastitis, which are currently estimated to exceed $2 billion per year in the United States (A. Saeman, National Mastitis Council, Verona, WI, personal communication).

ENHANCING HUMAN NUTRITION AND HEALTH

One promising aspect of GE is the potential for the development of functional foods that enhance food safety, human nutrition, and health (Table 1). For example, in China the nutritional value of bovine milk has been improved by GE to express human α-lactoglobulin and human lactoferrin, proteins normally found in human milk but missing from bovine milk (Wang et al., 2008; Yang et al., 2008). Given the increasing prevalence of obesity and cardiovascular disease in developed nations, changes in product composition in conjunction with improvements in dietary practices could contribute to improvements in consumer health. The amounts and type of fats in animal products are topics of frequent public discourse, and from the perspective of sustainability, improved feed conversion efficiency increases the ratio of lean-to-fat deposition in livestock. Net benefits include reduced production costs, improved product quality, reduced excretion of nitrogenous wastes into the environment, decreased grazing pressure on fragile landscapes, and reduced pressure on world feed supplies (Sillence, 2004). A decrease in the prevalence of deleterious fats and cholesterol and an increase in the prevalence of MUFA and n-3 fatty acids are consistent with dietary recommendations for cardiovascular health and an objective difficult to achieve in the absence of GE. In fact, 3 proof-of-principle studies have been published: 1) GE goats that expressed a rat stearoyl-CoA desaturase in the mammary gland and yielded milk with a reduced saturated fatty acid content and increased content of CLA, a beneficial antioxidant fatty acid (Reh et al., 2004); 2) a GE pig made transgenic for a Δ^{12} fatty acid desaturase gene from spinach that produced the PUFA, linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), which are essential for human (and pig) nutrition (Saeki et al., 2004); and 3) a GE pig that was developed to express an n-3 fatty acid desaturase capable of converting n-6 fatty acids to n-3 fatty acids (Lai et al., 2006). Although fish provide an excellent source of dietary n-3 fatty acids, which are important for fertility, cardiovascular health, immune system health, mental health, and cancer prevention (Prather, 2006), worldwide fisheries will be challenged to sufficiently supply n-3 fatty acids to the developing world. As the most widely consumed meat, pork logically should be considered as an alternative source of n-3 fatty acids. Consistent with this strategy, the pigs developed by Lai et al. (2006) produce increased content of n-3 fatty acids from n-6 analogs, and their tissues have a reduced ratio of n-6/n-3 fatty acids. Such animals may be useful as models for human health and for providing a dietary source that could enhance the health of consumers in developed and developing countries.

The first FDA approval of a GE animal product, the anticoagulant ATryn (recombinant human antithrombin), firmly established the importance and safety of engineered mammary gland-based protein expression systems. Additional GE projects in cattle and goats have targeted the mammary gland for the expression of proteins to enhance the welfare of animals, and the safety and stability of milk products (Table 1). Bacte-

rial diarrhea, which is responsible for more than 2 million infant deaths per year in developing countries, results from campylobacter, salmonellae, shigellae, and some strains of *E. coli* infections. Transgenic goats that express the human lysozyme protein, a natural antimicrobial protein in breast milk, were developed to produce milk with an enhanced shelf life that would improve the gastrointestinal health of goat kids and children (Maga et al., 2006a,c; Brundige et al., 2008). Experiments in vitro and in vivo have established that milk from these goats has antimicrobial properties, whether pasteurized or not, and that this milk inhibits the enteric bacteria *E. coli* when fed to piglets (a nonruminant model for children; Maga et al., 2006b). Approval of this product could make a significant contribution to the alleviation of hunger and disease (Brundige et al., 2009).

FOOD AND HEALTH SECURITY

Transgenic technologies can also provide and effective means for enhancing animal health. Because hosts and pathogens have coevolved, long-term selection may not be the most effective approach for the enhancement of disease resistance. Although GS has provided enhanced disease resistance to some organisms (e.g., parasites; Stear et al., 2007), it is unlikely to generate specific resistance to microorganisms (bacteria and viruses) because they evolve more rapidly than do their hosts. Precise and efficient GE tools instead provide a route to make significant, sudden improvements in general and pathogen-specific innate and humoral immunity (Table 1). The improvement of disease prevention in livestock will increase the quality of life of production animals, contribute to the needed acceleration of food production, and serve to enhance food security worldwide. Developing animals resistant to viral pandemics (Reed et al., 2009) is in the best interest of all constituencies as a means to improve animal welfare and to enhance food and human health security.

CONCLUSIONS

The dietary needs of the world in the very near future cannot be met without the immediate and ongoing utilization of GS and GE. Obfuscation by opponents of animal genetics, and more generally by opponents of animal agriculture, will stall the delivery of solutions in the face of mounting risks (Murray and Maga, 2010). Humane applications of GS for sustainable animal production, in conjunction with genetic improvement by GE, are key technologies that will be vital for meeting the future food needs of the world. Although animal genetics alone will not solve the future food problems of the world, to fail to apply the best available technologies to the solution of contemporary and future food shortages would be morally reprehensible.

LITERATURE CITED

Aerni, P. 2004. Risk, regulation and innovation: The case of aquaculture and transgenic fish. Aquat. Sci. 66:327–341.

Barthel, R., J. Feng, J. A. Piedrahita, D. N. McMurray, J. W. Templeton, and L. G. Adams. 2001. Stable transfection of the bovine NRAMP1 gene into murine RAW264.7 cells: Effect on *Brucella abortus* survival. Infect. Immun. 69:3110–3119.

Bessey, C., R. H. Devlin, N. R. Liley, and C. A. Biagi. 2004. Reproductive performance of growth-enhanced transgenic coho salmon. Trans. Am. Fish. Soc. 133:1205–1220.

Biotechnology Industry Organization (BIO). 2009. BIO guidance on genetically engineered animal stewardship. http://www.bio.org/foodag/geanimalctr/20090814_GE_Animal_Stewardship_Guidance.pdf Accessed Dec. 17, 2009.

Blackburn, H. D. 2004. Development of national animal genetic resource programs. Reprod. Fertil. Dev. 16:27–32.

Bradford, G. E. 1999. Contributions of animal agriculture to meeting global human food demand. Livest. Prod. Sci. 59:95–112.

Brophy, B., G. Smolenski, T. Wheeler, D. Wells, P. L'Huillier, and G. Laible. 2003. Cloned transgenic cattle produce milk with higher levels of beta-casein and kappa-casein. Nat. Biotechnol. 21:157–162.

Brundige, D. R., E. A. Maga, K. C. Klasing, and J. D. Murray. 2008. Lysozyme transgenic goats' milk influences gastrointestinal morphology in young pigs. J. Nutr. 138:921–926.

Brundige, D. R., E. A. Maga, K. C. Klasing, and J. D. Murray. 2009. Consumption of pasteurized human lysozyme transgenic goats' milk alters serum metabolite profile in young pigs. Transgenic Res. doi:10.1007/s11248-009-9334-4.

Chan, K. M., and E. A. Decker. 1994. Endogenous skeletal muscle antioxidants. Crit. Rev. Food Sci. Nutr. 34:403–426.

Cheng, H. W. 2010. Breeding of tomorrow's chickens to improve well-being. Poult. Sci. 89:805–813.

Clark, J., and B. Whitelaw. 2003. A future for transgenic livestock. Nat. Rev. Genet. 4:825–833.

Clark, K. J., D. F. Carlson, and S. C. Fahrenkrug. 2007. Pigs taking wing with transposons and recombinases. Genome Biol. 8(Suppl. 1):S13.

Clements, J. E., R. J. Wall, O. Narayan, D. Hauer, R. Schoborg, D. Sheffer, A. Powell, L. M. Carruth, M. C. Zink, and C. E. Rexroad. 1994. Development of transgenic sheep that express the visna virus envelope gene. Virology 200:370–380.

Codex Alimentarius. 2008. Guideline for the conduct of food safety assessment of foods derived from rDNA animals. http://www.codexalimentarius.net/download/standards/11023/CXG_068e.pdf Accessed Dec. 17, 2009.

Coffey, M. T. 1996. Environmental challenges as related to animal agriculture—Swine. Pages 29–39 in Nutrient Management of Food Animals to Enhance and Protect the Environment. E. T. Kornegay, ed. CRC Press, Boca Raton, FL.

Cole, J. B., P. M. VanRaden, J. R. O'Connell, C. P. Van Tassell, T. S. Sonstegard, R. D. Schnabel, J. F. Taylor, and G. R. Wiggans. 2009. Distribution and location of genetic effects for dairy traits. J. Dairy Sci. 92:2931–2946.

Cook, J. T., M. A. McNiven, G. F. Richardson, and A. M. Sutterlin. 2000. Growth rate, body composition and feed digestibility/conversion of growth-enhanced transgenic Atlantic salmon *Salmo salar*. Aquaculture 188:15–32.

Devlin, R. H., M. D'Andrade, M. Uh, and C. A. Biagi. 2004. Population effects of growth hormone transgenic coho salmon depend on food availability and genotype by environment interactions. Proc. Natl. Acad. Sci. USA 101:9303–9308.

Devlin, R. H., D. Sakhrani, W. E. Tymchuk, M. L. Rise, and B. Goh. 2009. Domestication and growth hormone transgenesis cause similar changes in gene expression in coho salmon (*Oncorhynchus kisutch*). Proc. Natl. Acad. Sci. USA 106:3047–3052.

Doyon, Y., J. M. McCammon, J. C. Miller, F. Faraji, C. Ngo, G. E. Katibah, R. Amora, T. D. Hocking, L. Zhang, E. J. Rebar, P.

D. Gregory, F. D. Urnov, and S. L. Amacher. 2008. Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. Nat. Biotechnol. 26:702–708.

Dunham, R. A. 2009. Transgenic fish resistant to infectious diseases, their risk and prevention of escape into the environment and future candidate genes for disease transgene manipulation. Comp. Immunol. Microbiol. Infect. Dis. 32:139–161.

Dunham, R. A., G. W. Warr, A. Nichols, P. L. Duncan, B. Argue, D. Middleton, and H. Kucuktas. 2002. Enhanced bacterial disease resistance of transgenic channel catfish *Ictalurus punctatus* possessing cecropin genes. Mar. Biotechnol. 4:338–344.

Eitan, Y., and M. Soller. 2004. Selection induced genetic variation: A new model to explain direct and indirect effects of sixty years of commercial selection for juvenile growth rate in broiler chickens, with implications for episodes of rapid evolutionary change evolutionary theory and processes. Pages 153–176 in Papers in Honour of Eviator Nevo. S. P. Wasser, ed. Kluwer Academic Publishers, Norwell, MA.

FDA. 2009. Guidance for Industry: Regulation of Genetically Engineered Animals Containing Heritable Recombinant DNA Constructs. http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM113903.pdf Accessed Dec. 17, 2009.

Fire, A., S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, and C. C. Mello. 1998. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. Nature 391:806–811.

Foley, J. A., R. Defries, G. P. Asner, C. Barford, G. Bonan, S. R. Carpenter, F. S. Chapin, M. T. Coe, G. C. Daily, H. K. Gibbs, J. H. Helkowski, T. Holloway, E. A. Howard, C. J. Kucharik, C. Monfreda, J. A. Patz, I. C. Prentice, N. Ramankutty, and P. K. Snyder. 2005. Global consequences of land use. Science 309:570–574.

Forsberg, C. W., J. P. Phillips, S. P. Golovan, M. Z. Fan, R. G. Meidinger, A. Ajakaiye, D. Hilborn, and R. R. Hacker. 2003. The Enviropig physiology, performance, and contribution to nutrient management, advances in a regulated environment: The leading edge of change in the pork industry. J. Anim. Sci. 81(Suppl. 2):E68–E77.

Geurts, A. M., G. J. Cost, Y. Freyvert, B. Zeitler, J. C. Miller, V. M. Choi, S. S. Jenkins, A. Wood, X. Cui, X. Meng, A. Vincent, S. Lam, M. Michalkiewicz, R. Schilling, J. Foeckler, S. Kalloway, H. Weiler, S. Menoret, I. Anegon, G. D. Davis, L. Zhang, E. J. Rebar, P. D. Gregory, F. D. Urnov, H. J. Jacob, and R. Buelow. 2009. Knockout rats via embryo microinjection of zinc-finger nucleases. Science 325:433.

Golovan, S. P., H. A. Hakimov, C. P. Verschoor, S. Walters, M. Gadish, C. Elsik, F. Schenkel, D. K. Y. Chiu, and C. W. Forsberg. 2008. Analysis of *Sus scrofa* liver proteome and identification of proteins differentially expressed between genders, and conventional and genetically enhanced lines. Comp. Biochem. Physiol. Part D Genomics Proteomics 3:234–242.

Golovan, S. P., R. G. Meidinger, A. Ajakaiye, M. Cottrill, M. Z. Wiederkehr, D. J. Barney, C. Plante, J. W. Pollard, M. Z. Fan, M. A. Hayes, J. Laursen, J. P. Hjorth, R. R. Hacker, J. P. Phillips, and C. W. Forsberg. 2001. Pigs expressing salivary phytase produce low-phosphorus manure. Nat. Biotechnol. 19:741–745.

Green, R. D., M. A. Qureshi, J. A. Long, P. J. Burfening, and D. L. Hamernik. 2007. Identifying the future needs for long-term USDA efforts in agricultural animal genomics. Int. J. Biol. Sci. 3:185–191.

Greger, M. 2009. Trait selection and welfare of genetically engineered animals in agriculture. J. Anim. Sci. 88:811–814. doi:10.2527/jas.2009-2043.

Hakimov, H. A., S. Walters, T. C. Wright, R. G. Meidinger, C. P. Verschoor, M. Gadish, D. K. Chiu, M. V. Stromvik, C. W. Forsberg, and S. P. Golovan. 2009. Application of iTRAQ to catalogue the skeletal muscle proteome in pigs and assessment of effects of gender and diet dephytinization. Proteomics 9:4000–4016.

Hammer, R. E., V. G. Pursel, C. E. Rexroad Jr., R. J. Wall, D. J. Bolt, K. M. Ebert, R. D. Palmiter, and R. L. Brinster. 1985. Production of transgenic rabbits, sheep and pigs by microinjection. Nature 315:680–683.

Hayes, B. J., P. J. Bowman, A. J. Chamberlain, and M. E. Goddard. 2009. Invited review: Genomic selection in dairy cattle: progress and challenges. J. Dairy Sci. 92:433–443.

He, Z., S. Yu, G. Mei, M. Zheng, M. Wang, Y. Dai, B. Tang, and N. Li. 2008. Maternally transmitted milk containing recombinant human catalase provides protection against oxidation for mouse offspring during lactation. Free Radic. Biol. Med. 458:1135–1142.

Herrmann, B. G., B. Koschorz, K. Wertz, K. J. McLaughlin, and A. Kispert. 1999. A protein kinase encoded by the t complex responder gene causes non-Mendelian inheritance. Nature 402:141–146.

Horvat, S., and J. F. Medrano. 2001. Lack of *Socs2* expression causes the high-growth phenotype in mice. Genomics 72:209–212.

Jost, B., J. L. Vilotte, I. Duluc, J. L. Rodeau, and J. N. Freund. 1999. Production of low-lactose milk by ectopic expression of intestinal lactase in the mouse mammary gland. Nat. Biotechnol. 17:160–164.

Kelley, N. S., N. E. Hubbard, and K. L. Erickson. 2007. Conjugated linoleic acid isomers and cancer. J. Nutr. 137:2599–2607.

Lacy, R. C. 1997. Importance of genetic variation to the viability of mammalian populations. J. Mammal. 78:320–335.

Lai, L., J. X. Kang, R. Li, J. Wang, W. T. Witt, H. Y. Yong, Y. Hao, D. M. Wax, C. N. Murphy, A. Rieke, M. Samuel, M. L. Linville, S. W. Korte, R. W. Evans, T. E. Starzl, R. S. Prather, and Y. Dai. 2006. Generation of cloned transgenic pigs rich in omega-3 fatty acids. Nat. Biotechnol. 24:435–436.

Lindeman, G. J., S. Wittlin, H. Lada, M. J. Naylor, M. Santamaria, J. G. Zhang, R. Starr, D. J. Hilton, W. S. Alexander, C. J. Ormandy, and J. Visvader. 2001. *SOCS1* deficiency results in accelerated mammary gland development and rescues lactation in prolactin receptor-deficient mice. Genes Dev. 15:1631–1636.

Maga, E. A., J. S. Cullor, W. Smith, G. B. Anderson, and J. D. Murray. 2006a. Human lysozyme expressed in the mammary gland of transgenic dairy goats can inhibit the growth of bacteria that cause mastitis and the cold-spoilage of milk. Foodborne Pathog. Dis. 3:384–392.

Maga, E. A., C. F. Shoemaker, J. D. Rowe, R. H. BonDurant, G. B. Anderson, and J. D. Murray. 2006b. Production and processing of milk from transgenic goats expressing human lysozyme in the mammary gland. J. Dairy Sci. 89:518–524.

Maga, E. A., R. L. Walker, G. B. Anderson, and J. D. Murray. 2006c. Consumption of milk from transgenic goats expressing human lysozyme in the mammary gland results in the modulation of intestinal microflora. Transgenic Res. 15:515–519.

Marshall, K. M., W. L. Hurley, R. D. Shanks, and M. B. Wheeler. 2006. Effects of suckling intensity on milk yield and piglet growth from lactation-enhanced gilts. J. Anim. Sci. 84:2346–2351.

Martinez, R., J. Juncal, C. Zaldivar, A. Arenal, I. Guillen, V. Morera, O. Carrillo, M. Estrada, A. Morales, and M. P. Estrada. 2000. Growth efficiency in transgenic tilapia *Oreochromis* sp. carrying a single copy of an homologous cDNA growth hormone. Biochem. Biophys. Res. Commun. 267:466–472.

McPherron, A. C., and S. J. Lee. 1997. Double muscling in cattle due to mutations in the myostatin gene. Proc. Natl. Acad. Sci. USA 94:12457–12461.

Melo, E. O., A. M. O. Canavessi, M. M. Franco, and R. Rumpf. 2007. Animal transgenesis: State of the art and applications. J. Appl. Genet. 48:47–61.

Meuwissen, T. H., B. J. Hayes, and M. E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819–1829.

Miller, D. G., P. R. Wang, L. M. Petek, R. K. Hirata, M. S. Sands, and D. W. Russell. 2006. Gene targeting in vivo by adeno-associated virus vectors. Nat. Biotechnol. 24:1022–1026.

Morimoto, K. C., A. L. Van Eenennaam, E. J. DePeters, and J. F. Medrano. 2005. Hot topic: Endogenous production of n-3 and n-6 fatty acids in mammalian cells. J. Dairy Sci. 88:1142–1146.

Murray, J. D., and E. A. Maga. 2010. Is there a risk from not using GE animals? Transgenic Res. 19:357–361. doi:10.1007/s11248-009-9341-5.

Nam, Y. K., J. K. Noh, Y. S. Cho, H. J. Cho, K. N. Cho, C. G. Kim, and D. S. Kim. 2001. Dramatically accelerated growth and extraordinary gigantism of transgenic mud loach *Misgurnus mizolepis*. Transgenic Res. 10:353–362.

Nancarrow, C. D., J. T. A. Marshall, J. L. Clarkson, J. D. Murray, R. M. Millard, C. M. Shanahan, P. C. Wynn, and K. A. Ward. 1991. Expression and physiology of performance regulating genes in transgenic sheep. J. Reprod. Fertil. Suppl. 43:277–291.

Notter, D. R. 1999. The importance of genetic diversity in livestock populations of the future. J. Anim. Sci. 77:61–69.

Nottle, M. B., H. Nagashima, P. J. Verma, Z. T. Du, C. G. Grupen, S. M. McIlfatrick, R. J. Ashman, M. P. Harding, C. Giannakis, P. L. Wigley, I. G. Lyons, D. T. Harrison, B. G. Luxford, R. G. Campbell, R. J. Crawford, and A. J. Robins. 1999. Production and analysis of transgenic pigs containing a metallothionein porcine growth hormone gene construct. Pages 145–156 in Transgenic Animals in Agriculture. J. D. Murray, G. B. Anderson, A. M. Oberbauer, and M. M. McGloughlin, ed. CABI Publ., New York, NY.

NPG. 2005. RNA interference. www.nature.com/focus/rnai/animations/index.html Accessed Dec. 17, 2009.

Palmiter, R. D., R. L. Brinster, R. E. Hammer, M. E. Trumbauer, M. G. Rosenfeld, N. C. Birnberg, and R. M. Evans. 1982. Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. Nature 300:611–615.

Prather, R. S. 2006. Cloned transgenic heart-healthy pork? Transgenic Res. 15:405–407.

Pretty, J. 2008. Agricultural sustainability: Concepts, principles and evidence. Philos. Trans. R. Soc. Lond. B Biol. Sci. 363:447–465.

Pursel, V. G., A. D. Mitchell, G. Bee, T. H. Elsasser, J. P. McMurtry, R. J. Wall, M. E. Coleman, and R. J. Schwartz. 2004. Growth and tissue accretion rates of swine expressing an insulin-like growth factor I transgene. Anim. Biotechnol. 15:33–45.

Pursel, V. G., C. A. Pinkert, K. F. Miller, D. J. Bolt, R. G. Campbell, R. D. Palmiter, R. L. Brinster, and R. E. Hammer. 1989. Genetic engineering of livestock. Science 244:1281–1288.

Pursel, V. G., R. J. Wall, M. B. Solomon, D. J. Bolt, J. E. Murray, and K. A. Ward. 1997. Transfer of an ovine metallothionein-ovine growth hormone fusion gene into swine. J. Anim. Sci. 75:2208–2214.

Rahman, M. A., R. Mak, H. Ayad, A. Smith, and N. Maclean. 1998. Expression of a novel piscine growth hormone gene results in growth enhancement in transgenic tilapia *Oreochromis niloticus*. Transgenic Res. 7:357–369.

Reed, C., F. J. Angulo, D. L. Swerdlow, M. Lipsitch, M. I. Meltzer, D. Jernigan, and L. Finelli. 2009. Estimates of the prevalence of pandemic (H1N1) 2009, United States, April-July 2009. Emerg. Infect. Dis. 15:2004–2007.

Reh, W. A., E. A. Maga, N. M. B. Collette, A. Moyer, J. S. Conrad-Brink, S. J. Taylor, E. J. DePeters, S. Oppenheim, and J. D. Rowe., R. H. BonDurant, G. B. Anderson, and J. D. Murray. 2004. Hot topic: Using a stearoyl-CoA desaturase transgene to alter milk fatty acid composition. J. Dairy Sci. 87:3510–3514.

Richt, J. A., P. Kasinathan, A. N. Hamir, J. Castilla, T. Sathiyaseelan, F. Vargas, J. Sathiyaseelan, H. Wu, H. Matsushita, J. Koster, S. Kato, I. Ishida, C. Soto, J. M. Robl, and Y. Kuroiwa. 2007a. Production of cattle lacking prion protein. Nat. Biotechnol. 25:132–138.

Richt, J. A., P. Kasinathan, A. N. Hamir, J. Castilla, T. Sathiyaseelan, F. Vargas, J. Sathiyaseelan, H. Wu, H. Matsushita, J. Koster, S. Kato, I. Ishida, C. Soto, J. M. Robl, and Y. Kuroiwa. 2007b. Production and characterization of prion protein-deficient cattle. Transgenic Res. 16:842–843.

Saeki, K., K. Matsumoto, M. Kinoshita, I. Suzuki, Y. Tasaka, K. Kano, Y. Taguchi, K. Mikami, M. Hirabayashi, N. Kashiwazaki, Y. Hosoi, N. Murata, and A. Iritani. 2004. Functional expression of a Δ^{12} fatty acid desaturase gene from spinach in transgenic pigs. Proc. Natl. Acad. Sci. USA 101:6361–6366.

Sang, H. 1994. Transgenic chickens—Methods and potential applications. Trends Biotechnol. 12:415–420.

Schadt, E. E. 2009. Molecular networks as sensors and drivers of common human diseases. Nature 461:218–223.

Schwegmann-Wessels, C., G. Zimmer, H. Laude, L. Enjuanes, and G. Herrler. 2002. Binding of transmissible gastroenteritis coronavirus to cell surface sialoglycoproteins. J. Virol. 76:6037–6043.

Sillence, M. N. 1996. Nutrient management of food animals to enhance and protect the environment. Pages 105–125 in Nutrient Management of Food Animals to Enhance and Protect the Environment. E. T. Kornegay, ed. CRC Press, Boca Raton, FL.

Sillence, M. N. 2004. Technologies for the control of fat and lean deposition in livestock. Vet. J. 167:242–257.

Smith, C. A., K. N. Roeszler, T. Ohnesorg, D. M. Cummins, P. G. Farlie, T. J. Doran, and A. H. Sinclair. 2009. The avian Z-linked gene *DMRT1* is required for male sex determination in the chicken. Nature 461:267–271.

Stacey, A., A. Schnieke, H. Kerr, A. Scott, C. Mckee, I. Cottingham, B. Binas, C. Wilde, and A. Colman. 1995. Lactation is disrupted by alpha-lactalbumin deficiency and can be restored by human alpha-lactalbumin gene replacement in mice. Proc. Natl. Acad. Sci. USA 92:2835–2839.

Stear, M. J., M. Doligalska, and K. Donskow-Schmelter. 2007. Alternatives to anthelmintics for the control of nematodes in livestock. Parasitology 134:139–151.

Sundstrom, L. F., M. Lohmus, W. E. Tymchuk, and R. H. Devlin. 2007. Gene-environment interactions influence ecological consequences of transgenic animals. Proc. Natl. Acad. Sci. USA 104:3889–3894.

Tompkins, S. M., C. Y. Lo, T. M. Tumpey, and S. L. Epstein. 2004. Protection against lethal influenza virus challenge by RNA interference in vivo. Proc. Natl. Acad. Sci. USA 101:8682–8686.

Tricon, S., G. C. Burdge, S. Kew, T. Banerjee, J. J. Russell, E. L. Jones, R. F. Grimble, C. M. Williams, P. Yaqoob, and P. C. Calder. 2004. Opposing effects of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 conjugated linoleic acid on blood lipids in healthy humans. Am. J. Clin. Nutr. 80:614–620.

United Nations. 2002. World Agriculture: Toward 2015/2030. ftp://ftp.fao.org/docrep/fao/004/y3557e/y3557e.pdf Accessed Dec. 8, 2008.

United Nations. 2008. Population Division of the Department of Economic and Social Affairs of the United Nations Secretariat, World Population Prospects. http://esa.un.org/unpd/wpp2008/index.htm Accessed Dec. 17, 2008.

US Code of Federal Regulations. 2009. New Animal Drugs; Bc6 Recombinant Deoxyribonucleic Acid Construct, Food and Drug Administration, Health and Human Services, 21 CFR Parts 510 and 528. Final Rule No. 74:6823–6824.

van Berkel, P. H., M. M. Welling, M. Geerts, H. A. van Veen, B. Ravensbergen, M. Salaheddine, E. K. Pauwels, F. Pieper, J. H. Nuijens, and P. H. Nibbering. 2002. Large scale production of recombinant human lactoferrin in the milk of transgenic cows. Nat. Biotechnol. 20:484–487.

Vize, P. D., A. E. Michalska, R. Ashman, B. Lloyd, B. A. Stone, P. Quinn, J. R. E. Wells, and R. F. Seamark. 1988. Introduction of a porcine growth hormone fusion gene into transgenic pigs promotes growth. J. Cell Sci. 90:295–300.

Wall, R. J., A. M. Powell, M. J. Paape, D. E. Kerr, D. D. Bannerman, V. G. Pursel, K. D. Wells, N. Talbot, and H. W. Hawk.

2005. Genetically enhanced cows resist intramammary *Staphylococcus aureus* infection. Nat. Biotechnol. 23:445–451.

Wang, J., P. Yang, B. Tang, X. Sun, R. Zhang, C. Guo, G. Gong, Y. Liu, R. Li, L. Zhang, Y. Dai, and N. Li. 2008. Expression and characterization of bioactive recombinant human alpha-lactalbumin in the milk of transgenic cloned cows. J. Dairy Sci. 9112:4466–4476.

Wheeler, M. B., G. T. Bleck, and S. M. Donovan. 2001. Transgenic alteration of sow milk to improve piglet growth and health. Reprod. Suppl. 58:313–324.

Whitelaw, C. B., and H. M. Sang. 2005. Disease-resistant genetically modified animals. Rev. Sci. Tech. 24:275–283.

Williams, P. 2007. Nutritional composition of red meat. Nutr. Diet 64:S113–S119.

Yang, P., J. Wang, G. Gong, X. Sun, R. Zhang, Z. Du, Y. Liu, R. Li, F. Ding, B. Tang, Y. Dai, and N. Li. 2008. Cattle mammary bioreactor generated by a novel procedure of transgenic cloning for large-scale production of functional human lactoferrin. PLoS ONE 310:e3453.

Zbikowska, H. M. 2003. Fish can be first–advances in fish transgenesis for commercial applications. Transgenic Res. 12:379–389.

Zhong, J. Y., Y. P. Wang, and Z. Y. Zhu. 2002. Introduction of the human lactoferrin gene into grass carp *Ctenopharyngodon idellus* to increase resistance against GCH virus. Aquaculture 214:93–101.

Zulet, M. A., A. Marti, M. D. Parra, and J. A. Martinez. 2005. Inflammation and conjugated linoleic acid: Mechanisms of action and implications for human health. J. Physiol. Biochem. 61:483–494.