GENETICALLY ENGINEERED ANIMALS AND PUBLIC HEALTH

Compelling Benefits for Health Care, Nutrition, the Environment, and Animal Welfare

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GENETICALLY ENGINEERED ANIMALS AND PUBLIC HEALTH:
Compelling Benefits for Health Care, Nutrition, the Environment, and Animal Welfare

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Abstract

Genetically engineered animals embody an innovative technology that is transforming public health through biomedical, food and environmental applications. They are integral to the development of new diagnostic techniques and drugs for human disease while delivering clinical and economic benefits that cannot be achieved with any other approach. They promise significant benefits in human health and food security by enabling dietary improvements through more nutritious and healthy meat and milk. Genetically engineered animals also offer significant human health and environmental benefits with livestock which are more efficient at converting feed to animal protein, and reducing waste production. Finally, genetic engineering will improve the welfare of the animal by imparting resistance to disease and enhancing overall health and well being. These numerous benefits will be realized only when we resolve policy obstacles that are limiting investment in research and holding back product development.

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Executive Summary

Animal biotechnology, executed judiciously, will provide compelling and practical benefits to mankind, as we have seen from other fundamental advances in life science. Genetic engineering is the deliberate modification of the animal's genome using techniques of modern biotechnology. Genetically engineered agricultural animals are being developed to transform and improve public health. These public health benefits can be grouped into five broad areas of scientific development.

1. Genetically engineered animals will improve human health through production of novel replacement proteins, drugs, vaccines and tissues for the treatment and prevention of human disease.

2. Animals that are genetically engineered will have improved food production traits enabling them to help meet the global demand for more efficient, higher quality and lower-cost sources of food.

3. Genetically engineered animals will contribute to improving the environment and human health with the consumption of fewer resources and the production of less waste.

4. Genetic engineering offers tremendous benefit to the animal by enhancing health, well-being and animal welfare.

5. Finally, genetically engineered animals have produced high-value industrial products such as spider silk used for medical and defense purposes.

Today, there are more than two dozen drugs in development derived through genetic engineering of farm animals, and numerous agricultural animal applications with beneficial environmental and husbandry attributes suitable for commercialization. But so far, the practical benefits of this technology have not reached American patients and consumers, owing to regulatory and political obstacles rather than scientific limitations. The public health benefits can only be realized when we create the regulatory framework for governing how these animals can provide human health, environmental and food and agricultural benefits. Establishing a predictable, rigorous, science-based regulatory pathway is essential if this technology is going to be allowed to deliver practical benefits in the areas that the science of genetic engineering of agricultural animals is now enabling.

Introduction

The objective of this paper is to evaluate the benefits of development-stage technologies that are based on genetic engineering, review the policy and regulatory challenges and provide a recommendation that will result in benefits realized in products for consumers.

Precedents exist for understanding how a new area of beneficial science can create uncertainty and fear, and how these initial concerns can be resolved through science. In the early 1970s, unease spread through the media about a new scientific technique called recombinant deoxyribonucleic acid (DNA). The concept was easy to understand: you take a gene out of one living thing and put it in another. When scientists proposed to insert human genes into bacteria, where they could be more easily manipulated, opponents worried about unforeseen social and scientific implications. They called for legal moratoria or stringent regulation that promised to thwart any reasonable development efforts. Many envisioned evil applications—deadlier strains of old viruses or designer babies. The technology, they argued, was dangerous.

But the benefits were compelling. Before this technology came along, fundamental advances on cellular disease didn't seem possible. Recombinant DNA changed all that, and in a short time, gave rise to new medicines and insights into many common diseases. Yet in the 1970s, some polls suggested many Americans were against the research, captive to concerns about its perceived risks and willing to forgo obvious public health opportunities. Prominent critics of the technology were convinced that "recombinant" bacteria were unsafe and capable of infecting people. When they proposed a moratorium on further research, some British researchers mixed the recombinant bacteria into their milk and drank it with no ill effects. The point was made. The moratoria never passed. And medical practice has been transformed as a result. [1]

Government restrictions on scientific research are again at issue. The technology encompasses everything from the genetic modification of animals to improve their ability to produce food, to animals that acquire the capacity to produce drugs or other natural proteins in their milk. This is a science broadly referred to as genetic engineering. Genetic engineering is the deliberate modification of the animal's genome using techniques of modern biotechnology.

Some policymakers and consumer advocates have focused their concerns on the agricultural applications of genetic engineering. But the two primary applications of this science—food production and drug development—are inextricably linked. Consequences of regulatory hurdles on one area will be broadly felt across the science. Policies applied to genetically engineered animals intended for the food supply will inevitably impact the ability to develop genetic engineering as a science for improved and lower-cost drug development, as well as other benefits.

Genetic engineering is the deliberate modification of the animal's genome using techniques of modern biotechnology.

[1] Scott Gottlieb. Consequences of the Biotechnology Revolution, New York Sun, A11, May 1, 2002

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How the Science Enables Solutions

Science has given history its forward direction. There is good reason to believe that animal biotechnology will enable the kind of practical benefits we have seen from other fundamental advances in life science. While there have always been those in society who resist scientific change, the attacks against genetically engineered animals – enhanced for improved production of food, novel human drugs and for environmental protection among other purposes – have been intense and sustained.

Genetically engineered animals – which often incorporate genes from other organisms in a process called transgenesis – are being developed to transform and improve public health. The broad possibilities encompass the treatment of human disease, the production of safer or more effective human proteins, new drugs and vaccines, the easing of shortages of human tissue and organs available for transplant patients through new avenues of supply, the enhancement of the environment and sustaining food security and quality through the improved efficiency of food production, and production of more nutritious foods. [2][3][4][5]

The creation of the first genetically engineered farm animals was documented in 1985 [6] and the capability for biopharmaceutical production by these animals was demonstrated shortly thereafter. Today, there are more than two dozen drugs in development derived through transgenic methods, and numerous agricultural animal applications with beneficial environmental and husbandry attributes suitable for commercialization.

While there are fundamental misunderstandings about the potential risks from this new technology, there are also ample gaps in peoples' knowledge of its potential benefits. These public health benefits can be grouped efficiently into the following five broad areas of scientific development:

- Novel and more efficient production of replacement proteins, drugs, vaccines, and tissues for the treatment and prevention of human disease;

- Production of animals with improved food production traits enabling them to become more efficient, higher quality, and lower-cost sources of food;

- Engineering of "environmental friendly" animals capable of meeting human needs more efficiently, with the consumption of fewer resources and the production of less waste, allowing direct positive impacts on human health;

- Enhanced animal welfare and health through genetic engineering to increase resistance to disease, minimizing the need for animal care interventions; and

- Production of high-value industrial products such as spider silk used for medical and defense purposes.

Genetically engineered animals are being developed to transform and improve public health.

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[2] Fulton, S. (2000). Roundup on bioprocess validation issues: transgenic animal production of biopharmaceuticals. Genetic Engineering News Jan 1 20:36

[3] Echelard, Y. (1996). Recombinant protein production in transgenic animals. Current Opinion in Biotechnology 7: 536-540.

[4] Young, M.W., H. Meade, J. Curling, C. Ziomek, and M. Harvey. (1998). Production of recombinant antibodies in the milk of transgenic animals. Res Immunol. Jul-Aug; 149(6): 609-610.

[5] Reggio, B.C., H.L. Green, M. Sansinena, L.H. Chen, E. Behboodi, R.S. Denniston, Y. Echelard and R.A. Godke. (2002). Production of cloned transgenic goats as a potential source for human pharmaceuticals. Theriogenology 57:445.

[6] RE Hammer, VG Pursel, CE Rexroad, RJ Wall, DJ Bolt, KM Ebert, RD Palmiter, and RL Brinster. Production of Transgenic Rabbits, Sheep, and Pigs by Microinjection. Nature 1985; 315:680-683

Few efforts to date have attempted to catalogue the near- and medium-term health benefits from transgenic technology, especially when it comes to the medical applications.[7] This paper will attempt to fill that void, by evaluating the genetic engineering technologies (Tables 1–5), and providing some qualitative and quantitative measures of their potential public health impact.

The greatest obstacles to realizing these opportunities are presently not due to technical obstacles but rather due to policy limitations. While regulatory pathways for developing drug products based on genetically engineered animal methods have been generally developed,[8 9 10 11] similar regulatory pathways remain ambiguous when it comes to genetically engineered animals intended for human consumption, despite the absence of any data or experience to justify such confusion. In part, that owes to less familiarity among policymakers and consumer groups when it comes to using genetically engineered animals to produce food or industrial proteins, versus using animals as sources for drug production.

The problem is that regulations applied to either one of these two arenas—agriculture or human health—will unavoidably impact product development in the other. Regulatory uncertainty with regard to agricultural applications cannot avoid impeding development of the medical applications. The regulatory policy to govern this technology is not as dichotomous as its applications appear. In fact, genetically engineered animal applications are consistent in their significant public health implications, whether the products are targeted at agriculture or human health. It follows that policy cannot enable one application while thwarting another without greatly impeding development of both. This is, in part, because our ability to develop genetically engineered animals capable of manufacturing novel drugs, proteins, and vaccine components is dependent upon a transparent, predictable and science-based regulatory pathway to govern under what circumstances these animals can be safely used as food sources for human and animal consumption.

In the final analysis, those seeking to promote the development of genetically engineered animals because of their demonstrated ability to deliver safer, more novel, and lower cost protein drugs (or those who, at worst, take an ambivalent view of genetic engineering when it is applied to these medical purposes) cannot endorse the technology in this one context without simultaneously allowing a regulatory pathway to develop genetically engineered animals for other agricultural purposes. Yet this contradiction exists when it comes to both the perception by some, and the regulation of genetically engineered animals. Breaching this intellectual partition, and establishing a rigorous, science-based regulatory pathway, is essential if this technology is going to be allowed to deliver practical benefits in the areas science is now enabling.

Genetically engineered animal applications are consistent in their significant public health implications, whether the products are targeted at agriculture or human health.

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[7] Pew Initiative on Food and Biotechnology. 2002. Biotech in the Barnyard: Implications of Genetically Engineered Animals. The conference brought together representatives of industry, academia, consumer groups, animal welfare groups and government agencies to share information and exchange views.
http://www.pewtrusts.org/uploadedFiles/wwwpewtrustsorg/Summaries_reports_and_pubs/PIFB_Biotech_in_Barnyard.pdf

[8] Guidelines on the Use of Transgenic Animals in the Manufacture of Biological Medicinal Products for Human Use, Committee for Proprietary Medicinal Products (CPMP), 1995

[9] Points to Consider in the Manufacture and Testing of Therapeutic Products for Human Use Derived from Transgenic Animals, FDA Center for Biologics Evaluation and Research (CBER) 1995

[10] Notes for Guidance on Minimizing the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Medicinal Products, Committee for Proprietary Medicinal Products (CPMP), 1999; CPMP/BWP/1230/98

[11] William G Gavin. The Future of Transgenics. Regulatory Affairs Focus May 2001, 13-18

There are now dozens of products derived from genetically engineered animals under development that hold promise of benefit to human health.

Genetically Engineered Animals and the Improved Production of Existing Human Proteins, Drugs, Vaccines, and Tissues

For years, genetically engineered animals, particularly mice, have been used to help scientists understand how genes work and interact with one another. More recently, researchers have introduced genes coding for the production of specific protein sequences into other species in order to manufacture large quantities of those proteins for medical purposes.

In biology, genetic sequences provide the instruction set or "code" for the manufacture of specific proteins, which comprise everything from enzymes to hormones, and are themselves the vehicles for carrying out the body's many functions. Transgenic animals are so named because they contain a "transgene" from another individual or organism that codes for the production of a particular protein that scientists are interested in expressing.

While there are a number of different techniques for developing genetically engineered animals, the critical requirement is stable integration of the desired genetic sequence into the host animal's DNA, while minimizing other potentially detrimental alterations. Once this requirement is demonstrated and traditional out-breeding has begun, the next step is raw product recovery of the protein that is being developed, typically during the animal's lactation. The subsequent steps, the process for adapting and breeding these genetically engineered animals, is well understood and has been standardized across various commercial and research enterprises. Scientists continue to refine these standard approaches, drawing on developments from molecular genetics and reproductive physiology, and the new techniques offer perhaps even more potential public health opportunities. The aim of developing new approaches is to increase the efficiency of producing and reproducing useful founder animals. [12]

Transgenic animals were initially recognized as a novel platform for the production of recombinant drug products for a number of reasons. First, it was demonstrated that transgenic approaches could reliably and safely express novel proteins due to the unique nature of the mammary gland's capacity for production of complex molecules. Second, genetically engineered animals showed the ability to produce significantly greater amounts of protein with higher expression levels and volume output than the traditional protein culture systems. These culture systems are currently the dominant approach to commercial production of protein medicines across industry. Third, transgenics demonstrated the potential for a significant reduction in the cost per unit protein due to the animal being the true "biorector," requiring less complicated monitoring and industrial hardware than a traditional recombinant cell culture system. Finally, genetically engineered animals held out the possibility of developing safer and more sustainable and flexible manufacturing sources for vital human protein replacements and blood products.

As a result of these public health opportunities, there are now dozens of products derived from genetically engineered animals under development that hold promise of benefit to human health. [13] They range from therapeutic advances, such as animals that produce blood clotting proteins that are potentially safer than current plasma-derived products

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[12] Natalie S. Rudolph. Biopharmaceutical production in transgenic livestock, Tibitech September 1999, volume 17, 367-374

[13] C. L. Keefer, J. Pommer and J. M. Robl. 2007. The role of transgenic livestock in the treatment of human disease. Council on Agricultural Science and Technology Issue Paper 35: 1-11.

(being free from risk of infection or contamination) to gains in efficiency and access, for example from animals capable of producing lower cost, pharmaceuticals, tissue components and vaccines in their milk.

The most immediate medical applications of transgenics involve efforts to produce novel recombinant biological drug and blood components. Right now there are several methods traditionally used for industrial production of these proteins. For example, bacterial systems such as *Escherichia coli* are commonly used and are very efficient. These systems generally offer a low-cost route of production. But these approaches are limited to the production of simple or "non-glycosylated" proteins (meaning that the protein itself is not significantly modified by the addition of sugar subgroups, a level of complexity that usually makes proteins harder to copy or manufacture). Indeed the active forms of many important human therapeutic proteins are glycosylated in a mammalian-specific manner. Bacterial systems are also usually reserved for the production of proteins that do not require a sophisticated folding process to reach their active state.

A second approach—the production of protein drugs in fungal systems—enables efficient production of some secreted proteins. But glycosylation in these systems adds a number of unwanted subgroups which strongly affect the functional properties of the protein. Still a third approach, baculovirus systems, exploits the hugely productive capacities of certain insect viruses to produce a wide range of proteins, but these have yet to be scaled-up to industrial levels.

The prevalent method today for producing glycosylated proteins is mammalian cell culture. This approach is commonly used in the production of monoclonal antibody drugs such as the breast cancer drug, Herceptin®, or the lymphoma drug, Rituxan®. This approach enables manufacturers to produce properly shaped and active proteins, but it suffers from high costs and low yields, raising the price of the finished drugs. Manufacturing costs can account for up to a third of the cost of some complex protein drugs. Finally, genetically engineered plant systems are useful for large scale production. However, similar to the fungus-based production methods, glycosylation in plants can add a number of plant-specific sugars to which some human patients have adverse reactions.

By comparison to all these techniques, manufacturing approaches based on genetically engineered animals appear to be a desirable alternative for producing complex glycosylated proteins. These combine both the expression levels available with bacterial systems and the ability for "post-translational modifications" or, in other words, the fine tailoring that can be achieved with tissue culture. Compared to cellular expression, protein production through transgenics also enables lower product costs. Milk, egg white, blood and silk worm cocoon from genetically engineered animals are all potential sources for recombinant proteins produced at an industrial scale. [14]

Owing to these advantages, there are as many as two-dozen different human and animal drugs developed through transgenics that are in the early and mid stages of development with active Investigational New Drug Applications (INDs) or Investigational New Animal Drug Applications (INADAs) on file with the U.S. Food and Drug Administration (FDA).

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[14] Natalie S. Rudolph. Biopharmaceutical production in transgenic livestock, Tibitech September 1999, volume 17, 367-374
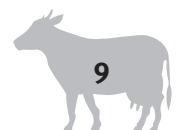
In addition to these advanced programs, there are literally hundreds of transgenic medical protein products that are in pre-clinical development. These drugs and biologics being created by genetically engineered animals can be roughly divided into four broad categories, each of which will be reviewed in greater detail in the sections that follow. These include: 1) blood products, 2) other protein-based drugs, 3) vaccine components and 4) replacement tissue products. Within each of these four categories, some examples of the protein-based medical products that are in development follow.

Blood Products

While there are no protein drugs from genetically engineered animals yet approved for medical use in the United States, a number of different proteins derived from the blood of transgenic animals are in various stages of development. In some cases, the uses of genetically engineered animals for bio-manufacturing enables scientists to develop proteins with unique attributes that might offer commercial or therapeutic advantages over compounds made through traditional production sources.

The list of products under development is broad. It includes widely used and vital blood products such as clotting factors, antithrombin, [15][16] and human albumin. [17][18] The product that is furthest along in development is ATryn®, which is in phase III clinical trials and has been granted orphan drug status by the FDA for the treatment of hereditary antithrombin deficiency, or HD, to prevent excessive bleeding in patients undergoing high-risk surgical procedures or childbirth.[19] ATryn® was recently granted fast track status by the FDA and a Biologics License Application requesting permission from the FDA to market the drug in the United States is expected to file by the end of 2008. ATryn® is already approved in the European Union for the treatment of HD patients undergoing surgical procedures. Rhucin®, a recombinant human C1 esterase inhibitor produced in the milk of transgenic rabbits is also in clinical trials in Europe. Rhucin® treats acute attacks of hereditary angioedema (HAE), a rare disease characterized by painful swelling of soft tissue. [20]

While HD is a rather rare disease in its frequency among the population, afflicted patients must receive treatment if they are to have any hope of a normal life. Low levels or inactive forms of the protein antithrombin cause the disease. As a consequence, some patients develop blood clots in their large veins, a medical condition referred to as venous thromboembolism. These blood clots can cause organ damage or even death. Sometimes the clots can

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[15] Lu, W., T.G.K. Mant, J.H. Levy and J.M. Bailey. (2000) Pharmacokinetics of recombinant transgenic antithrombin in volunteers. Anesthesia and Analgesia. 90:531-534.

[16] Zhou, Q., J. Kyazike, Y. Echelard, H.M. Meade, E. Higgins, E.S. Cole and T. Edmunds (2005). Effect of genetic background on glycosylation heterogeneity in human antithrombin produced in the mammary gland of transgenic goats. J. Biotechnology. 117:57-72.

[17] Echelard, Y., M.M. Destrempes, J.A. Koster, C. Blackwell, W. Groen, D. Pollock, J.L. Williams, E. Behboodi, J. Pommer and H.M. Meade. (2002). Production of recombinant human serum albumin in the milk of transgenic cows. Theriogenology 57:779.

[18] Bleck GT, White BR, Miller DJ, Wheeler MB. Production of bovine alpha-lactalbumin in the milk of transgenic pigs. Journal of Animal Science 1998(76) 3072-3078

[19] Information on the clinical trial can be found at: http://clinicaltrials.gov/ct2/show/NCT00110513?cond=%22Antithrombin+III+Deficiency%22&rank=1

[20] Van Doorn, M. B., J. Burggraaf, T. van Dam, A. Eerenberg, M. Levi, C. E. Hack, R. C. Schoemaker, A. F. Cohen and J. Nuijens. 2005. A phase I study of recombinant human C1 inhibitor in asymptomatic patients with hereditary angioedema. J. Allergy Clin. Immunol. 116:876-883. doe:10.1016/J.JACI.2005.05.019

form spontaneously, putting an individual at sudden and unexpected risk. Other research suggests that HD can contribute to the loss of a fetus during pregnancy. HD patients are perhaps at greatest risk during events that are independently associated with a probability of thrombosis, such as surgery and delivery. [21]

Genetically engineered animals are also being used for the development of safer and less expensive blood clotting factors for the treatment of hemophilia, with a number of these products also in advanced stages of development. Hemophilia is caused by genetic conditions in which the patients' failure to express enough coagulation factors may lead to excessive bleeding. Type A hemophilia is due to the lack of factor VIII. Type B hemophilia is due to the lack of factor IX. It is largely inherited. People with the disease are missing some or all of a vital protein needed to form blood clots. In about 30 percent of cases, there is no family history of the disorder and the condition results from a spontaneous gene mutation. Hemophilia B is far less common than Hemophilia A, occurring in about one in 25,000 male births. It affects about 3,300 individuals in the United States. All races and economic groups are affected equally.

A person with hemophilia, when injured, does not bleed harder or faster than a person without hemophilia, one bleeds longer because the blood is slower to clot. Small cuts or surface bruises are usually not a problem, but more traumatic injuries may result in serious problems and potential disability, or even death. People with severe hemophilia, about 60 percent of patients, have bleeding following an injury and may have frequent spontaneous bleeding episodes, often into the joints and muscles.

The preferred treatment is to provide supplemental coagulation factors prophylactically to prevent episodes of excessive bleeding. But the price and availability of recombinant coagulation factors often allows for use in only limited circumstances. When patients are unable to get access to sufficient replacements of these proteins, uncontrolled internal bleeding can cause pain, swelling, and permanent damage to joints and muscles.

While the missing blood-clotting protein can be produced in mechanical bioreactors, the cost of this standard treatment runs up to $200,000 per year, per patient. Right now, the only sources of replacement factor IX are the plasma of blood donors (which raises certain safety concerns, including the potential for transmission of disease) and recombinant factor IX produced in Chinese hamster ovary cells (which is expensive and of limited supply). The limited supply and high cost of both the plasma derived and recombinant factor make prophylactic treatment prohibitively expensive. [22]

Genetically engineered animals are being used for the development of safer and less expensive blood clotting factors for the treatment of hemophilia.

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[21] Donald J. Filip, John D. Eckstein, Jan J. Veltkamp. Hereditary antithrombin iii deficiency and thromboembolic disease, American Journal of Hematology, volume 1, Issue 3, July 2006: Pages 343-349

[22] R Kashyap, VP Choudhry. Indian Journal of Pediatrics 68(2001)151

This is another area where genetically engineered animals offer some significant public health opportunities. Scientists have developed genetically engineered animals, including sheep and pigs, able to produce Factor IX, a structurally complex blood clotting protein. [23][24][25] The pigs, which are perhaps closest to commercialization, produce the factor in their mammary glands at a productivity level 250-1,000 fold higher than mechanical reactors. The protein can then be extracted from their milk. The high concentration makes the protein easy and inexpensive to purify. Researchers are also using genetically engineered animals in the experimental production of factor VIII, for the treatment of Hemophilia A. [26] Using genetically engineered animals to produce these and other blood factors offers a myriad of potential medical opportunities, not only the prospect of a safer and more renewable source of clotting factors, but also the potential for a lower cost product available for more routine use, perhaps improving the standard of care.

Protein-Based Drugs

Researchers have also developed a number of genetically engineered animals capable of producing complex protein-based drugs, often at a lower cost and through perhaps more reliable and safer production means than traditional manufacturing processes. [27] Protein-based drugs differ from protein products synthesized in the blood in that they are produced in vivo by other organs. This technology is even being applied to the development of complex proteins such as monoclonal antibodies [28] as well as many other important human replacement proteins and protein drugs such as polyclonal antibodies, [29][30] plasminogen activator, [31] human

[23] K.H. Choo*, K. Raphael, W. McAdam and M.G. Peterson. Expression of active human blood clotting factor IX in transgenic mice: use of a cDNA with complete mRNA sequence. Nucleic Acids Research, 1987, vol. 15, No. 3 871-884, 1987

[24] Angelika E. Schnieke, Alexander J. Kind, William A. Ritchie, Karen Mycock, Angela R. Scott, Marjorie Ritchie, Ian Wilmut, Alan Colman, Keith H. S. Campbell. Human Factor IX Transgenic Sheep Produced by Transfer of Nuclei from Transfected Fetal Fibroblasts. Science, vol. 278. no. 5346, pp. 2130 - 2133, December 19, 1997

[25] Myles Lindsay, Geun-Cheol Gila, Armando Cadizb, William H. Velandera, 1, Chenming Zhangc and Kevin E. Van Cott. Purification of recombinant DNA-derived factor IX produced in transgenic pig milk and fractionation of active and inactive subpopulations. Journal of Chromatography Volume 1026, Issues 1-2, 13 February 2004, Pages 149-157

[26] Paleyanda RK, Velander WH, Lee TK, Seandella DH, Gwazdauskas FC, Knight JW, Hoyer LW, Drohan WN, Lubon H. Transgenic pigs produce functional human factor VIII in milk. Nature Biotechnology 1997 (15) 971-975

[27] Higgins, E. J. Pollock, P. DiTullio and H. Meade. (1996). Characterization of the glycosylation on a monoclonal-antibody produced in the milk of a transgenic goat. Glycobiology 6: 1211.

[28] DP Pollock, JP Kutzko, E Birk-Wilson, JL Williams, Y Echelard, and HM Meade. Transgenic Milk as a Method for the Production of Recombinant Antibodies. Journal of Immunological Methods 1999; 231:147-157

[29] Sullivan EJ, Pommer J, Robl JM. Commercialising genetically engineered animal biomedical products. Reprod Fertil Dev. 2008;20(1):61-6.

[30] Kuroiwa, Y., Kasinathan, P., Choi, Y., Naeem, R., Tomizuka, K., Sullivan E.J., Knott, J.G., Duteau, A., Goldsby R.A., Osborne, B.A., Ishida, I., Robl, J.M. Cloned transchromosomic calves producing human immunoglobin. *Nat. Biotech.* 20, 889-894 (2002).

[31] Denman, J., M. Hayes, C. O'Day, T. Edmunds, C. Bartlett, S. Hirani, K.M. Ebert, K. Gordon and J.M. McPherson. (1991). Transgenic expression of a variant of human tissue-type plasminogen activator in goat milk: purification and characterization of the recombinant enzyme. *Bio/Technology* 9: 839-843 and Ebert, K., P. DiTullio, C. Berry, J. Schindler, S. Ayers, T. Smith, L. Pellerin, H. Meade, J. Denman and B. Roberts. (1994). Induction of human tissue plasminogen activator in the mammary gland of transgenic goats. *Biotechnology* Jul; 12(7): 699-702 and Ebert, K.M., J. Selgrath, P. DiTullio, J. Denman, T.E. Smith, M.A. Memon, J.E. Schindler, G.M. Monastersky, J.A. Vitale and K. Gordon. (1991). Transgenic production of a variant of human tissue-type plasminogen activator in goat milk: Generation of transgenic goats and analysis of expression. *Bio/Technology* 9: 835-838 and Pittius, C.W., L. Hennighausen, E. Lee, H. Westphal, E. Nicols, J. Vitale and K. Gordon. (1988). A milk protein gene promoter directs the expression of human tissue plasminogen activator cDNA to the mammary gland in transgenic mice. *Proc. Natl. Acad. Sci.* 85: 5874-5878.

alpha-fetoprotein, [32] alpha-1-proteinase inhibitor, alpha glucosidase and others. [33 34 35 36 37] Advanced scientific techniques have been developed to help ensure the purity and safety of these proteins to levels of confidence that in many cases match or exceed traditional production techniques. [38 39]

To take just one example, researchers recently created a line of transgenic swine that produce recombinant human erythropoietin or "epo," a naturally occurring human hormone that boosts the body's production of red blood cells. The transgenic swine produced the hormone in their milk through a potentially more efficient and lower cost process than traditional methods employed by the drug's two main manufacturers. Epo is used commercially in patients with diseased kidneys no longer able to produce the protein, as well as cancer patients being treated with chemotherapy who develop anemia as a consequence of bone marrow depletion from their cancer drug regimens. Erythropoetin-based drugs are some of the most widely used protein-based drugs, and are expensive to manufacture. In advanced pre-clinical experiments, the amino acid sequence of the swine-produced form of the protein matched that of commercial Epo produced from cultured animal cells. The high yields of the swine-derived protein could offer cost-effective alternatives for clinical applications as well as providing other potential clinical advantages. [40]

The high yields of the swine-produced protein used to boost a person's red blood cell production could offer cost-effective alternatives for clinical applications.

[32] Parker, M.H., E. Birck-Wilson, G. Allard, N. Masiello, M. Day, K.P. Murphy, V. Paragas, S. Silver and M.D. Moody (2004). Purification and characterization of a recombinant version of human alpha-fetoprotein in the milk of transgenic goats. Protein Expression & Purification. 38: 177-183.

[33] Behboodi, E., L. Chen, M. Destrempes, H.M. Meade and Y. Echelard (2002). Transgenic cloned goats and the production of therapeutic proteins. in Principles of Cloning. Elsevier Science (USA).

[34] Cammuso, C., C. Porter, S. Nims, D. Gaucher, D. Melican, S. Bombard, N. Hawkins, A. O'Coin, C. Ricci, C. Brayman, N. Buzzell, C. Ziomek and W. Gavin. (2000) Hormonal induced lactation in transgenic goats. Animal Biotechnology. 11: 1-17.

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[36] Pollock, DP, JP Kutzko, E Birck-Wilson, JL Williams, Y Echelard and HM Meade, 1999. Transgenic milk as a method for the production of recombinant antibodies. Journal of Immunological Methods. 231:147-157

[37] Edmunds, T., S. Van Patten, J. Pollock, E. Hanson, R. Bernasconi, E. Higgins, P. Manavalan, C. Ziomek, H. Meade, J. McPherson and E. Cole. (1998). Transgenically produced human antithrombin: structural and functional comparison to human plasma-derived antithrombin. Blood Jun 15; 91(12): 4561-71.

[38] Ziomek, C. (1999). Validation strategies for biopharmaceuticals: viral risk minimization for transgenic proteins from milk. Genetic Engineering News Apr. 15: 54.

[39] Ziomek, C.A. (1996). Minimization of viral contamination in human pharmaceuticals produced in the milk of transgenic goats. Dev. Biol. Stand. 88: 265-268.

[40] Jin-Ki Parka, Yun-Keun Leea, Poongyeon Leea, Hak-Jae Chunga, Sungwoo Kima, Hyun-Gi Leea, Myung-Kyu Seoa, Joo-Hee Hana, Chun-Gyu Parka, Hun-Taek Kim. Recombinant human erythropoietin produced in milk of transgenic pigs. Journal of Biotechnology Volume 122, Issue 3, 10 April 2006, Pages 362-371

The application of transgenics to vaccine production has not only public health benefits, but also national security implications.

Vaccine Components

Genetically engineered animals are also being used in the manufacture of novel vaccine components. This offers the opportunity for more rapid manufacture of vaccines, perhaps enabling vaccines to be developed in direct and rapid response to viral outbreaks (for example, responding to a pandemic flu). It also offers the opportunity for vaccines to be produced at a lower cost because of the efficiency and high capacity of the transgenic methods [41] [42] [43]. Each animal is, in effect, a product-specific production plant.

For these reasons, the application of transgenics to vaccine production has not only public health benefits, but also national security implications. Our ability to respond effectively to an emerging viral or bacterial threat or a pandemic could be predicated on our ability to quickly scale up manufacturing of a novel vaccine uniquely tailored to an emerging virus or bacteria. Genetically engineered animals are uniquely suited to providing that capability.

To take just one example of where this technology is being deployed in the production of experimental vaccines, researchers have demonstrated that it may be possible to produce malaria vaccines using genetically engineered animals—at a lower cost than traditional vaccine manufacture methods, and in high volumes. A single goat producing 700 liters/year of milk at the yields researchers obtained experimentally [44] (0.9 g/liter of purified antigen) could supply enough vaccine components called antigens to vaccinate 8.4 million people annually. Thus a herd of three goats could conceivably produce enough antigen to vaccinate 20 million African children per year. Successful development of this potential requires that the antigens produced in the milk of genetically engineered animals retain biological efficacy. For vaccines, as opposed to therapeutic agents, this means that they must retain appropriate immunogenicity. Research has demonstrated that vaccine components produced in genetically engineered animals indeed retain these properties and show evidence of efficacy.

Replacement Tissues

Finally, when it comes to the direct benefits of genetic engineering to human health through improvements in medical care, another frontier of research involves the use of genetically engineered animals to produce human replacement tissues, cells or organs for human transplant. The science of using animal-derived tissues for human transplantation is referred to as xenotransplantation. Pigs have advantages over other animals as a tissue source in this context, as they are easy to breed, have anatomical and physiological characteristics compatible with humans, and are well studied for several pathogens potentially transmissible to humans. [45] Unlike most non-human primates that are known to carry diseases which are

41 Stowers, A.W., L.H. Chen, Y.L. Zhang, M.C. Kennedy, L.L. Zou, L. Lambert, T.J. Rice, D.C. Kaslow, A. Saul, C.A. Long, H. Meade and L.H. Miller. (2002). A recombinant vaccine expressed in the milk of transgenic mice protects Aotus monkeys from a lethal challenge with Plasmodium falciparum. Proceedings of the National Academy of Sciences of the United States of America. 99(1):339-344.

42 Behboodi, E., S.L. Ayres, E. Memili, M. O'Coin, L.H. Chen, H.M. Meade and Y. Echelard. (2004). Health and reproductive profiles of nuclear transfer goats producing the MSP1-42 malaria antigen. Proceedings of the Annual Conference of the International Embryo Transfer Society. 16 (1,2***): 29.

43 Rudolph, N. S. (1999) Trends Biotechnol. 17, 367-374

44 Anthony W. Stowers, Li-how ChenDagger , Yanling Zhang, Michael C. Kennedy, Lanling Zou, Lynn Lambert, Timothy J. RiceDagger, David C. Kaslow, Allan Saul, Carole A. Long, Harry MeadeDagger, and Louis H. Miller. A recombinant vaccine expressed in the milk of transgenic mice protects Aotus monkeys from a lethal challenge with Plasmodium falciparum. Proceedings of the National Academy of Sciences, December 18, 2001, 10.1073/pnas.012590199

45 Mohiuddin MM. Clinical xenotransplantation of organs: why aren't we there yet? PLoS Med. 2007 Mar 27;4(3):e75.

potentially dangerous or even fatal to humans (i.e. HIV and HTLV), caesarean-derived piglets can be maintained free from pathogens that could infect humans, when housed and grown in environmentally controlled facilities with filtered air and water supplies, and by using sterilized vegetarian feed which is validated as animal-protein-free. [46]

Xenotransplantation presents the opportunity to change completely the transplantation field by providing a vastly expanded supply of human compatible donor tissues. This will enable a solution for overcoming the worldwide organ shortage crisis, a new source for replacement tissues including heart valves, skin and orthopedic tissues. While this field took some time to mature (starting in the early 1990's), with the advent of nuclear transfer technology, [47] and the successful production of alpha 1,3 galactosyltransferase knockout (GT-KO) pigs, five years ago,[48] the critical barrier of organ rejection caused by pre-formed anti-pig (anti-Gal) antibodies was overcome. As a result, in contrast to tissues from normal, unmodified pigs which are rejected in minutes to hours, survival of transgenic GT-KO pig organs, including heart and kidneys, when transplanted into non-transgenic primates, can survive as long as six months. [49] Despite these recent advances, transgenic pig tissues are not yet ready for human clinical testing, but research aimed at further genetic modification of the donor animal, and validation of the technology is progressing rapidly.

This approach also holds out promise for more effective treatments for diabetes. Insulin-producing pancreatic islet cells from pigs are showing substantial promise, and are likely to be the first live xenograft tissues tested in human clinical trials. Using protocols similar to those optimized for human islet cell transplantation, pre-clinical studies in monkeys have demonstrated three to six months cure of diabetes. [50] Recent studies using islet cells from pigs transgenic for a human CD46 complement inhibitor gene [51] are showing even greater efficacy, and may signal the beginning of human trials for treatment of diabetes as soon as 2010.

In relation to whole organ xenografts, because the liver does not require a perfect tissue match and it is relatively resistant to antibody-mediated rejection, the liver is the organ for which there is the greatest chance of near-term success. The use of transgenic pig livers on a temporary basis (capable of functioning for as little as two weeks to a month), likely will provide opportunities for patients with acute liver failure, when used as a "bridge" to transplant until a human liver can be obtained. Timelines for human trials with bridging transgenic pig livers are similar to those indicated for pig islet transplants. Heart and kidney xenografts are somewhat further off, as they must survive longer without rejection. Due to physiological incompatibilities, heart and kidney xenografts likely will require further genetic modification of the donor pigs, including the addition of other human genes, such as

Xenotransplantation, another frontier of research involving use of genetically engineered animals, presents the opportunity to change completely the transplantation field.

[46] Lai L, Prather RS . Cloning pigs as organ donors for humans. Eng Med Biol Mag. 2004 Mar-Apr;23(2):37-42

[47] Polejaeva et. al., 2000. Cloned pigs produced by nuclear transfer from adult somatic cells. Nature 407, p. 81-90.

[48] Phelps et. al., 2003. Production of alpha1, 3-galactosyltransferase-deficient pigs. Science 299, p. 411-414.

[49] Tseng et. al., 2005. Alpha-1,3, galactosyl-transferase gene-knockout pig heart transplantation in baboons with survival approaching six months. Transplantation 80, p. 1493; Yamada et. al., 2005. Marked prolongation of porcine renal xenograft survival in baboons through the use of alpha-1,3-galactosyltransferase gene-knockout donors and the cotransplantation of vascularized thymic tissue. Nat. Med. 11, p. 32.

[50] Cardona et. Al. 2006. Long-term survival of neonatal porcine islets in non-human primates by targeting co-stimulation pathways. Nat. Med., 12, p. 304; Hering et al. 2006. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. Nat. Med., 12, p. 301.

[51] Mckenzie et.al. 2003. CD46 protects pig islets from antibody but not cell-mediated destruction in the mouse. Xenotransplantation 10, p615.

complement inhibitor genes to mop up anti-non-gal antibody reactions, anti-coagulant genes that inhibit blood clots, or genes that have properties that further suppress or modify the human immune rejection response.[52] [53] [54] [55] [56] [57]

Similar to the large unmet need for viable human-compatible cells and organs, due to the same supply constraints, processed tissues obtained from donated human cadavers, and used to make more than a hundred different types of human-derived tissue products, are also in limited supply. As a result, processed tissues including heart valves, skin, surgical mesh (derived from small intestine submucosa or SIS), and orthopedic tissues (including bone and tendons), are currently obtained from pigs and used for human therapeutic applications. The FDA regulates them as medical devices, and although they have shown efficacy in their human therapeutic applications, recently it has been demonstrated that some of these non-transgenic pig-derived products (specifically heart valves and SIS) are subject to gal-mediated immune responses that result in chronic rejection and premature failure of the devices.[58] The advent of transgenic Gal-free (GT-KO) pigs promises improved outcomes for like devices. Because these fall under the medical device regulatory umbrella (unlike live cell/organ xenotransplantation tissues), they provide near-term opportunities (possibly less than 3 years for those tissue devices that would follow a specific regulatory approval path) for commercial products derived from genetically engineered pigs. These products bring the promise of scale, safety, and improved efficacy for these tissue markets.

Despite the recent technology advances in this field, it is true that xenotransplantation still faces both technical and regulatory hurdles, as well as some criticism. But much of it is strongly reminiscent of the criticism leveled against human-to-human transplantation during the late 1960s and early 1970s. Yet with persistence, the field of human-to-human transplantation has proved highly successful. This success was the result of a stepwise increase in our understanding of the biology of rejection, improvements in immune suppression drug management, and experience.[59] Likewise, with respect to xenotransplantation, especially for whole organ pig xenografts like heart and kidney, where it's likely that xenotransplantation

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[52] Cooper et.al. 2007. a-1,3-galactosyltransferase gene-knockout pigs for xenotransplantation: Where do we go from here?, Transplantation 84, p1-7; Cooper et.al. 2008. Recent advances in pig-to-human organ and cell transplantation. Expert Opin. Biol. Ther. 8(1), p1.

[53] Schuurman HJ, Pierson RN 3rd. Progress towards clinical xenotransplantation. Front Biosci. 2008 Jan 1;13:204-20

[54] Fung J, Rao A, Starzl T. Clinical trials and projected future of liver xenotransplantation. World J Surg. 1997 Nov-Dec;21(9):956-61

[55] Cooper DK. Clinical xenotransplantation—how close are we? Lancet. 2003 Aug 16;362(9383):557-9

[56] McGregor CG, Davies WR, Oi K, Teotia SS, Schirmer JM, Risdahl JM, Tazelaar HD, Kremers WK, Walker RC, Byrne GW, Logan J
http://www.pewtrusts.org/uploadedFiles/wwwpewtrustsorg/Public_Opinion/Food_and_Biotechnology/2006summary.pdf S. Cardiac xenotransplantation: recent preclinical progress with 3-month median survival. J Thorac Cardiovasc Surg. 2005 Sep;130(3):844-51

[57] McGregor CG, Teotia SS, Byrne GW, Michaels MG, Risdahl JM, Schirmer JM, Tazelaar HD, Walker RC, Logan JS. Cardiac xenotransplantation: progress toward the clinic. Transplantation. 2004 Dec 15;78(11): 1569-1575

[58] Konakci et.al. 2005. Alpha-gal on bioprostheses: xenograft immune response in cardiac surgery. Eur. J. of Clin. Invest. 35, p17-33; Malcarney et.al. 2005. Early inflammatory reactions after rotator cuff repair with porcine small intestine submucosal implant: a report of 4 cases. Am. J. of Sports Med. 33, p907.

[59] Cooper DK, Keogh AM, Brink J, Corris PA, Klepetko W, Pierson RN, Schmoeckel M, Shirakura R, Warner Stevenson L; Xenotransplantation Advisory Committee of the International Society for Heart and Lung Transplantation. J Heart Lung Transplant. 2000 Dec;19(12):1125-65

may not be universally successful until further technologic advances occur. However, exciting pre-clinical advances in cellular transplantation for treatment of diabetes, as well as for treatment of acute liver failure, either using transgenic (ie. GT-KO) pig livers as a temporary bridge to transplant, or purified pig liver cells in bioartificial liver devices, present opportunities that could be achievable.[60] Also, the application of genetically engineered animals for producing medical device products is generating significant interest from orthopedic and pharmaceutical companies and is likely to take the lead in forging the path to commercialization of safe and efficacious xenograft tissue products.

Table 1. Genetically engineered animals will enhance public health through more abundant, affordable medicines

Trait: Produce human drugs and replacement tissues

Type of Animal: Cattle, chickens, fish, goats, pigs, sheep

- Blood products: Antithrombin, Human albumin, Factor IX
- Other protein-based drugs: Monoclonal antibodies, Polyclonal antibodies, Plasminogen activator, Human alpha-fetoprotein, Alpha-1-proteinase inhibitor
- Vaccine Components: Antigens for any viral or bacterial disease such as pandemic flu, malaria, small pox
- Replacement tissues: Pancreatic islet cells, Whole organ xenografts such as liver, heart, kidney, Heart valves, Skin, Surgical mesh from intestinal mucosa, Orthopedic tissues, Cellular transplants such as liver

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Genetic Engineering Applied to the Improved Production of Animals for Agriculture: Food, Environment and Animal Welfare

There are numerous potential applications of genetic engineering of agricultural animals to develop new or altered strains of agriculturally important livestock. The future benefits of these applications are consistently as compelling as those for the biomedical applications, as they both promise to advance public health. In addition, owing to their global role in provision of animal food products, genetic engineering promises to improve food security and production, quality and safety, while reducing the environmental footprint of livestock agriculture. In addition, the technology promises to improve animal welfare.

Enhanced Nutrition and Public Health. Human health is directly impacted in large part by the requirement for a sustainable and secure supply of healthful food. Genetic engineering of agricultural animals has the potential to provide compelling consumer benefits to public health via enhanced nutrition. For over 10,000 years, farmers and ranchers have improved the genetics of livestock and poultry to provide for nutritious, safe and economical animal protein products. Indeed it is predicted that there will be a 60 percent increase in

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[60] Fung J, Rao A, Starzl T. Clinical trials and projected future of liver xenotransplantation. World J Surg. 1997 Nov-Dec;21(9):956-61

The production of lower-fat, more nutritious foodstuffs from meat and milk produced by genetic engineering could enable potential improvements to public health.

consumption of animal protein by 2020 in developing countries. [61] It is a well-known fact that as socio-economic status of global communities rise, consumers demand more dietary animal protein as meat and milk, and that health and cognitive skills of children improve. It can be argued that the only technology that will allow such improvements in diet and health will be genetic engineering of livestock and poultry that is sustainable and available consistently worldwide.

Genetic engineering holds the promise to improve nutritional attributes of animal food products including their quantity, the quality of the whole food, and specific nutritional composition. For example, increasing lean meat may be achieved by using genetic engineering to impact growth modulators, such as growth hormone, and insulin-like growth factor. Another strategy is to introduce or regulate genes that mediate the formation of muscle tissue. In addition, introducing or altering proteins regulating lipid metabolism such as the hormone leptin or the enzyme fatty acid synthase could accomplish improvement in the percentage of lean meat to fat in whole foods. A new and promising area of genetic engineering is the development of livestock with modified lipid profiles, or "heart-healthy" fatty acids. This could be extended to other meat and milk producing species to improve and extend the health benefits of altering lipid composition to a wide variety of animal products. All of these potential interventions could result in more nutritious and healthful animal products used for food. Implications for public health through amelioration of pathologies (i.e. cardiovascular and cerebrovascular disease, cancer, diabetes, and obesity) associated with poor diet (high fat, low quality protein) could be monumental. The production of lower fat, more nutritious foodstuffs from meat and milk produced by genetic engineering could enable these potential improvements to public health.

Food borne diseases are a major global contributor to human morbidity/mortality, and genetically engineered animals can help manage and mitigate the causes in many ways. The public health benefits of improving food safety, via a more wholesome food supply, include production of genetically engineered animals that have inherent resistance to food borne pathogens. Early research has included development of poultry and livestock resistant to such organisms as *E. coli, campylobacter, clostridium* and *streptococcus.* Other genetic engineering could eliminate the animal's susceptibility to diseases, zoonotic and other, and their threat to human health, such as bovine spongiform encephalopathy or "mad cow disease" or mastitis, an inflammation of the mammary gland that reduces milk quality. Improving animal health via genetic engineering also provides the added benefit of reducing the need for veterinary interventions and use of antibiotics and other medicinal treatments. The implications for public health through improving animal welfare, and increasing the animal's disease resistance are significant.

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[61] Delgado, CM, Rosegrant, M, Steinfeld, H, Ehui, S, Courbois, C. Livestock to 2020: The next food revolution. 2020 Vision for Food, Agriculture, and the Environment International Food Policy Research Institute. 1999. Discussion Paper 28.

Practical applications of genetic engineering in livestock production include improved milk production and composition, increased growth rate, improved feed utilization, improved carcass composition, enhanced reproductive performance, increased prolificacy, and altered cell and tissue characteristics for biomedical research [62] and manufacturing. The production of swine with a growth hormone transgene serves as an excellent example of the value of this technology. Improvement of milk composition through genetic engineering has the potential to enhance the production of certain proteins and/or growth factors deficient in milk. [63] The improvement of the nutrient or therapeutic value of milk may have a profound impact on survival and growth of newborns in both humans and animals. Other animal products, such as eggs and meat could also benefit from the use of genetic engineering. Genes could be targeted that could increase egg production in chickens, and postpone reproductive senescence not only in chicken but also in other species as a result of physiologic events such as lactation, anorexia, poor nutrition and season of the year. [64]

Reduced Environmental Impact. Livestock agriculture has been targeted by some as being harmful to the environment. However, genetic engineering of agricultural animals has the potential to significantly reduce its environmental footprint. Genetic engineering of animals could make a significant impact on protecting and improving the environment, such as decreasing phosphorous and nitrogen pollution in the Chesapeake Bay watershed or in the aquifers in hog and poultry producing areas such as Minnesota, North Carolina and Arkansas. Increasing efficiency and productivity per animal through genetic engineering will lead to a decreased burden on limited land and water resources while protecting the environment by decreasing potential pollutants from entering the soil and ground water. The protection of watersheds and ground water will become an ever more pressing issue regarding human health as populations continue to grow and expand into rural environments. Ample research and development has ensued for swine (the Enviro-Pig™) produced by genetic engineering [65] that has already reduced the amount of phosphorous excreted into the environment. Increased rate of production of milk or meat will also decrease the impact on the environment by decreasing 1) the amount of manure, 2) the direct competition for human food, 3) the water requirement both for the animals and for facility hygiene, and 4) the land footprint required for livestock facilities. Also improving feed conversion efficiency, or reducing the pounds of feed required to produce a pound of meat or milk, could significantly reduce the environmental footprint of feedlot operations. Reducing feed inputs reduces manure outputs per unit of food produced. The AquAdvantage™ salmon produced by genetic engineering triples growth rate, improves feed efficiency, and will contribute to a major reduction in the environmental footprint of aquaculture while producing a safe, healthy food.

Increasing efficiency and productivity per animal through genetic engineering will lead to a decreased burden on limited land and water resources while protecting the environment by decreasing potential pollutants from entering the soil and ground water.

[62] Wheeler MB, and Choi SJ Embryonic stem cells and transgenics: recent advances. Arch. Fac. Vet. UFRGS 1997:25, 64-83

[63] Bremel RD, Yom HC, Bleck GT. Alteration of milk composition using molecular genetics. J. Dairy. Sci. 72, 2826-2833

[64] Seidel G.E. The future of transgenic farm animals. In: Genetically engineered animals in Agriculture (Murray, J.D., Anderson, G.B., Oberbauer, A.M., Mc Gloughlin, M.M., eds.), 1999 pp. 269-283. CABI Publishing, New York

[65] Golovan SP, Meidinger RG, Ajakaiye A, Cottrill M, Wiederkehr MZ, Barney DJ, Plante C, Pollard JW, Fan MZ, Hayes MA, Laursen J, Hjorth JP, Hacker RR, Phillips JP, Forsberg CW. Pigs expressing salivary phytase produce low-phosphorus manure. Nature Biotechnol 2001;19: 741-745.
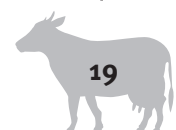
Genetic engineering stands to significantly impact the health and well being of livestock.

Table 2. Environmental impact will be reduced through genetic engineering of animals
Trait: Reduced phosphorus excretion Type of Animal: Pigs
• Improve phosphorus digestion: Salivary phytase
Trait: Enhancing efficiency of growth reduces total waste excreted Type of Animal: Cattle, crustaceans, fish, pigs
• Enhanced growth rate: Increasing growth factors, hormones, Increased muscle protein synthesis or growth rate
Trait: Fluorescence in presence of polluters as an environmental indicator Type of Animal: Fish
• Environmental detector of pollutants: Zebra danio (GloFish®)

Improved Animal Welfare. Genetic engineering of agricultural animals will improve animal welfare by producing healthier animals. Animal welfare is the top priority of anyone involved in animal husbandry and stewardship of the production of livestock. Therefore, because the technology can specifically impart resistance to a number of diseases, and improve productive characteristics, genetic engineering stands to significantly impact the health and well being of livestock. The end result of the improved health and well being from genetic engineering is to reduce frequency of veterinary interventions and use of various dietary and metabolic supplements, which have become commonly used in livestock production.

Due to the outlook for significant benefits, there is ample global research, and private development of genetically engineered animals that improve foods, are environmentally friendly, improve animal welfare, and produce industrial products. It appears that the first food application with the U.S. FDA for genetic engineering is to enhance the growth rate of commercially valuable fish such as Atlantic Salmon.[66] Other food applications are also underway.   Genetic engineering may improve several aspects of livestock production including 1) milk quality, 2) meat production as growth and carcass composition, 3) animal welfare (via disease resistance), 4) reproductive performance, and 5) quality of hair and fiber.

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[66] Fletcher GL, Shears MA, Yaskowiak ES , King MJ, Goddard SV. Gene transfer: potential to enhance the genome of Atlantic salmon for aquaculture. Australian Journal of Experimental Agriculture 2004;44(11) 1095-1100

Table 3. Animal welfare will be improved for genetically engineered animals

Trait: Improving disease resistance

Type of Animal: Cattle, chickens, fish, mollusks, pigs

- Resistance to disease: Bovine spongiform encephthalopathy, Avian influenza, Brucellosis, Mastitis, K88-positive *E. Coli,* Parasitic organisms, Viral or bacterial pathogens, Genetic diseases
- Self-immunization: Raising antibody titers
- Natural resistance: Cloning

Enhancing Milk

Advances in recombinant DNA technology have provided the opportunity either to improve the composition of milk or to produce entirely novel proteins in milk. These changes may add value to, as well as increase, the potential uses of milk.
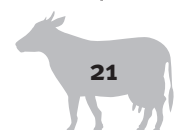
The improvement of livestock growth or survivability through the modification of milk composition requires production of genetically engineered animals that: 1) produce a greater quantity of milk; 2) produce milk of higher nutrient content; or 3) produce milk that contains a beneficial "nutriceutical" protein. The major nutrients in milk are protein, fat and lactose. By elevating any of these components, we can improve growth and health of the developing offspring that consumer the enhanced milk. In many production species such as cattle, sheep and goats, the nutrients available to the young may not be limiting. However, milk production in the sow limits piglet growth and therefore pig production.[67] Methods that increase the growth of piglets during suckling result in increased weaning weights,[68] decreased time to reach market weight, and thus decreased feed requirements.

Cattle, sheep and goats used for meat production may also benefit from improved milk yield or composition. In tropical climates, *Bos indicus* cattle breeds do not produce copious quantities of milk. Increases in milk yield of as little as 2–4 liters per day may have a profound affect on weaning weights in cattle such as the Nelore breed in Brazil. Similar comparisons can be made with improving weaning weights in meat type breeds like the Texel sheep and Boer goat. This application of genetic engineering could lead to improved growth and survival of offspring.

A second mechanism by which changing milk composition may improve animal growth is the addition or supplementation of beneficial naturally occurring hormones, growth factors or bioactive factors to the milk through the use of genetic engineering. It has been suggested that bioactive substances in milk possess important functions in the neonate with regard to regulation of growth, development and maturation of the gut, immune system and

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[67] Hartmann PE, McCauley I, Gooneratne AD, Whitely JL. Inadequacies of sow lactation: survival of the fittest. Symp Zool Soc Lond 1984;51: 301-326

[68] Noble MS, Rodriguez-Zas S, Bleck GT, Cook JS, Hurley WL, Wheeler MB. Lactational performance of first parity transgenic gilts expressing bovine ±-lactalbumin in their milk. J Anim Sci 2002;80: 1090-1096

The overall result of genetic engineering to modify milk will be the creation of more uses of milk and milk products in both agriculture and medicine.

endocrine organs.[69] Transgenic alteration of milk composition has the potential to enhance the production of certain proteins and/or growth factors that are deficient in milk.[70] The increased expression of a number of these proteins in milk may improve growth, development, health and survivability of the developing offspring. Some of these factors are insulin-like growth factor 1 (IGF-I), epidermal growth factor (EGF), transforming growth factor beta (TGF-b) and lactoferrin.[60, 71]

Other properties of milk that bear consideration for modifications are those that affect human and animal health. It has been shown that specific antibodies can be produced in genetically engineered animals.[72] It should be possible to produce antibodies in the mammary gland that are capable of preventing mastitis in cattle, sheep and goats and MMA (mastitis-metritis-agalactia) in pigs, and/or antibodies that aid in the prevention of domestic animal or human diseases.[59] Another example is to increase proteins that have physiological roles within the mammary gland itself such as lysozyme,[73] lysostaphin[74] or other anti-microbial peptides.

It is important to consider the use of transgenics to increase specific components, which are already present in milk for manufacturing purposes. An example might be to increase one of the casein components in milk. This could increase the value of milk in manufacturing processes such as production of cheese or yogurt. One might also alter the physical properties of a protein such as β-casein or κ-casein.[75] By increasing the glycosylation of β-casein,[76] one could increase its solubility in milk, which would reduce the time required for rennet coagulation and whey expulsion. This would produce firmer curds that are valuable in cheese making. Changes in other physical properties could result in dairy foods with improved characteristics, such as better tasting low fat cheese.[77] It should also be possible to increase the concentration of milk components while maintaining a constant volume. This could lead to greater product yield (i.e. more protein, fat or carbohydrate from a liter of milk). This would also aid in manufacturing processes while also decreasing transportation costs for the more concentrated products in fluid milk. The end result would be more saleable product for the dairy producer, and a reduced environmental footprint.

The overall result of genetic engineering to modify milk will be the creation of more uses of milk and milk products in both agriculture[59] and medicine.[78] This is truly a *"value-added"*

[69] Grosvenor CE, Picciano MF, Baumrucker CR. Hormones and growth factors in milk. Endocrinol Rev. 1993;14 [6], 710-728.

[70] Wall RJ, Pursel VG, Shamay A, McKnight RA, Pittius CW, Henninhausen L. High-level synthesis of a heterologous milk protein in the mammary glands of transgenic swine. Proc Natl Acad Sci USA 1991;88: 1696-1700.

[71] Konakci et.al. 2005. Alpha-gal on bioprostheses: xenograft immune response in cardiac surgery. Eur. J. of Clin. Invest. 35, p17-33; Malcarney et.al. 2005. Early inflammatory reactions after rotator cuff repair with porcine small intestine submucosal implant: a report of 4 cases. Am. J. of Sports Med. 33, p907.

[72] Storb U. Transgenic mice with immunoglobin genes. Annu Rev Immunol 1987;5: 151-174.

[73] Maga EA, Anderson GB, Murray JD. The effect of mammary gland expression of human lysozyme on the properties of milk from transgenic mice. J Dairy Sci 1995;78: 2645-2652.

[74] Donovan DM, Kerr DE, Wall RJ. Engineering disease resistant cattle. Transgenic Res 2005;14: 563-567.

[75] Brophy B, Smolenski G, Wheeler T, Wells D, L'huillier P, Laible G. Cloned transgenic cattle produce milk with higher levels of β-casein and κ-casein. Nature 2003;21: 157-162.

[76] Choi BK, Bleck GT, Wheeler MB, Jiminez-Flores R. Genetic modification of bovine-casein and its expression in the milk of transgenic mice. J Agric Food Chem 1996;44: 953-960.

[77] Bleck GT, Jiminez-Flores R, Wheeler MB. Production of genetically engineered animals with altered milk as a tool to modify milk composition, increase animal growth and improve reproductive performance. In: Greppi GF, Enne G [ed], Animal Production & Biotechnology. Elsevier, Amsterdam, 1995; 1-19.

opportunity for animal agriculture by increasing the concentrations of existing proteins or producing entirely new proteins in milk.

Enhancing Growth Rates and Carcass Composition

The production of genetically engineered livestock has been instrumental in providing new insights into the mechanisms of gene action governing growth.[79] Using transgenic technology, it is possible to manipulate growth factors, growth factor receptors and growth modulators. Transgenic sheep and pigs have been used to examine postnatal growth of mammals. Growth hormone (GH) and IGF genes have been incorporated and expressed at various levels in genetically engineered animals.[80] Transgenic livestock as well as salmon and catfish have been produced which contain an exogenous GH gene. This type of work enabled the study of chronic expression of these hormones on growth in mammals and fish. Results from one study have shown that an increase in porcine-produced GH as a result of a transgene leads to enhanced growth and feed efficiency in pigs.[81] In fish, dramatic increases have been shown in growth rate of transgenic Atlantic salmon using the gene promoter and growth hormone gene derived from fish species.[82] These researchers also indicate that fish used in aquaculture would be made sterile, thus minimizing the ecological impact due to accidental escape of fish that might be raised in ocean pens. Introduction of salmonid GH constructs has resulted in a 5–11-fold increase in weight after one year of growth.[83] This demonstrates that increased growth rate and ultimately increased rate of protein production can be achieved via genetic engineering. In addition, the production of these growth-enhanced salmon will have vast positive environmental benefits. Cutting in half the time required to raise salmon means supply can be increased without proportionately increasing the use of coastal waters. In addition, land-based systems become economically viable and competitive with ocean-pen systems further reducing environmental impact. The apparent increase in food conversion rates means that fewer natural resources are required to produce the fish, thus enhancing sustainability.

The production of genetically engineered livestock has been instrumental in providing new insights into the mechanisms of gene action governing growth of the animal.

[78] Van Berkel PHC, Welling, MM, Geerts M, van Veen HA, Ravensbergen B, Salajeddine M, Pauwels EKJ, Pieper F, Nuijens JH, Nibbering PH. Large-scale production of recombinant human lactoferin in the milk of transgenic cows. Nature 2002;20: 484-487.

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The use of genetic engineering to improve feed efficiency and/or appetite could profoundly impact livestock production and deliver significant benefits to producers, processors, and consumers.

The Rendement Napole (RN) or Acid-Meat gene has been implicated in lower processing yields in lines of Hampshire and Hampshire crossbred pigs. "Knocking-out" the RN gene may provide a method to alter, post-mortem pH, and, thereby, increase meat tenderness. Other specific loci, which may affect growth patterns, are the ryanodine receptor, the *myo-D,*[84] GH releasing factor, high affinity IGF binding proteins (IGFBP-1 to IGFBP-6), the sheep callipyge[85] and the myostatin (growth/differentiation factor-8, *GDF-8*) genes.[86] Based on a recent report on the mouse, the myostatin gene is an exceptionally intriguing potential locus for "knocking-out" in meat producing species. [76] The loss of the myostatin protein results in an increase in lean muscle mass. Certainly, there are numerous potential genes related to growth, including growth factors, receptors or modulators which have not yet been used, but may be of practical importance in producing genetically engineered livestock with increased growth rates and/or feed efficiencies.

Altering the fat or cholesterol composition of the carcass is another valuable benefit that can be delivered via genetic engineering. By changing the metabolism or uptake of cholesterol and/or fatty acids, the content of fat and cholesterol of meats, eggs and cheeses could be lowered. There is also the possibility of introducing beneficial fats such as the omega-3 fatty acids from fish or other animals into our livestock.[87] Receptors such as the low-density lipoprotein (LDL) receptor gene and hormones like leptin are also potential targets that would decrease fat and cholesterol in animal products.

The use of genetic engineering to improve feed efficiency and/or appetite could profoundly impact livestock production and deliver significant benefits to producers, processors, and consumers. Increased uptake of nutrients in the digestive tract, by alteration of the enzyme profiles in the gut, could increase feed efficiency. The ability to introduce enzymes such as phytase or xylanase into the gut of species where they are not normally present, such as swine or poultry, is particularly attractive. The introduction of phytase would increase the bioavailability of phosphorus from phytic acid in corn and soy products. One group has reported the production of transgenic pigs expressing salivary phytase as early as seven days of age.[88] The salivary phytase provided essentially complete digestion of the dietary phytate phosphorus in addition to reducing phosphorus output in waste by up to 75 percent. Furthermore, transgenic pigs required almost no inorganic phosphorus supplementation to the diet to achieve normal growth. The use of phytase transgenic pigs in commercial pork production could result in significantly decreased environmental phosphorus pollution from livestock operations.

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Optimal Animal Welfare through Improved Disease Resistance

The impact of genetic engineering on animal welfare is compelling. Genetic engineering of agricultural animals has the potential to improve disease resistance by introducing specific genes into livestock. Identification of single genes in the major histocompatibility complex (MHC), which influence the immune response, was instrumental in the recognition of the genetic basis of disease resistance/susceptibility.[89] The application of transgenic technology to specific aspects of the immune system should provide opportunities to genetically engineer livestock that are healthier and have superior disease resistance.

It has only been realized recently that there are many aspects of disease resistance or susceptibility in livestock that are genetically determined.[90] One specific example where transgenesis has been applied to disease resistance in livestock is the attempt to produce cattle resistant to mastitis. Mastitis is an infectious disease of the mammary gland that causes decreased milk production and lost productivity. Treatment and prevention of mastitis is costly and labor intensive. Lysostaphin is an antimicrobial peptide that protects mammary glands against *Staphylococcus aureus* infection by killing the bacteria in a dose-dependent manner.[64] Transgenic dairy cows that secrete lysostaphin into their milk have been produced to address the mastitis issue.

The application of nuclear transfer technology, or cloning, will enable the augmentation of beneficial alleles and/or the removal (via gene "knock-out") of undesirable alleles associated with disease resistance or susceptibility. An example is "knocking-out" the intestinal receptor for the K88 antigen. The absence of this antigen has been shown to confer resistance to infection of K88-positive *E. coli*.[91] Potential areas of investigation include resistance to: 1) parasitic organisms such as trypanosomes and nematodes, 2) viral or bacterial pathogens such as bovine leukemia virus, pseudorabies virus, foot and mouth virus, clostridium and streptococcus, and 3) genetic diseases such as deficiency of uridine monophosphate synthase (DUMPS), mule foot and bovine leukocyte adhesion deficiency (BLAD).

The opportunity to produce animals that could self-immunize against pathogens is an exciting application of genetic engineering. Transgenes could be designed to produce antigens resulting in immunization of the genetically engineered animal to particular diseases. Transgenes will be designed that could be turned on by administering, for example, zinc in feed, or a specific antibiotic to produce antigens that could raise protective antibody titers.

Transgenic dairy cows that secrete lysostaphin into their milk have been produced to reduce mastitis infections of the mammary gland.

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[89] Benacerraf B, McDevitt HO. Histocompatibility linked immune response genes. A new class of genes that controls the formation of species immune response has been identified. Science 1972;175: 273-279.

[90] Lewin HA. Disease resistance and immune response genes in cattle: strategies for their detection and evidence of their existence. J. Dairy Sci 1989;72: 1334-1348.

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The manipulation of reproductive processes using transgenic methodologies is only beginning, and it should be a very rich area for research and livestock improvement in the future.

Using the genetics from naturally resistant animals in cloning applications will produce animals resistant to a variety of diseases including BSE, and scrapie. An example of this kind of application is the production of transgenic mice expressing either the human or bovine prion protein. Each of these mouse strains was inoculated with the prions that cause bovine spongiform encephalopathy (BSE) or with a variant of Creutzfeldt-Jakob disease (vCJD). BSE was transmitted to the mice containing the bovine prion protein but was not transmitted to transgenic mice containing the human prion protein.[92] However, all three transgenic mouse lines containing the human prion protein showed transmission of the disease when inoculated with vCJD. Recently, cattle have been produced lacking the prion protein.[93] Analysis of these animals to determine whether they are in fact resistant to BSE is ongoing, but this is a major step toward developing cattle that do not develop "mad-cow" disease. Another example of this potential application is the production of fetuses that are resistant to brucellosis,[94] a highly contagious bacterial disease of cattle that can be transmitted from cattle to humans and causes high fever and muscular pain. This is only a partial list of organisms or genetic diseases that, when targeted for improvement via transgenic methodologies will increase production efficiency and enhance animal welfare.

Improving Reproductive Performance and Fecundity

Several genes have recently been identified which may profoundly affect reproductive performance. These include the estrogen receptor (ESR) and the Boroola fecundity (FECB) genes. It has been shown that a specific form of the ESR gene is associated with 1.4 more pigs born per litter than is typical in lines of pigs that do not contain this specific ESR gene type.[95] Introduction of a mutated or polymorphic ESR gene could increase litter size in a number of diverse breeds of pigs. A single major gene for fecundity, the FECB gene, which allows for increased ovulation rate, has been identified in Merino sheep.[96] Each copy of the gene has been shown to increase ovulation rate by approximately 1.5 ova per cycle. Production of transgenic sheep containing the appropriate FECB allele could increase fecundity in a number of diverse breeds. Identification of additional genes involved in fecundity from hyperprolific breeds/strains of swine (Meishan); sheep (Finnish Landrace) and cattle (high twinning) will provide additional opportunities to improve reproductive performance. The manipulation of reproductive processes using transgenic methodologies is only beginning, and it should be a very rich area for research and livestock improvement in the future.

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Table 4. Genetically engineered animals will enhance public health through healthier, high quality, and abundant food

Trait: Enhancing milk for use by animals

Type of Animal: Pigs

- Natural proteins fortified: α Lactalbumin, Insulin-like growth factor-1, Epidermal growth factor, Transforming growth factor-β, Lactoferrin, Antibodies to mastitis, Lysozyme, Lysostaphin

Trait: Enhancing milk for direct use by humans

Type of Animal: Cattle, sheep

- Natural components fortified: β-casein, κ-casein, Protein, Fat, Lactose

Trait: Enhancing growth rates and carcass composition

Type of Animal: Cattle, crustaceans, fish, pigs, sheep

- Increasing growth factors, hormones: Growth hormone, Insulin-like growth factors
- Tenderness of meat: Knock-out of acid-meat gene
- Increased muscle protein synthesis or growth rate: Ryanodine receptor, Myo-D, Growth hormone releasing factor, Insulin-like growth factor binding protein-1 to Insulin-like growth factor binding protein-6, Sheep callipyge gene, Myostatin gene
- Altered fat or cholesterol in meat: Omega-3 fatty acids, Low-density lipoproteins, Leptin hormone

Trait: Enhancement of reproductive performance

Type of Animal: Pigs, sheep

- Genes that increase fecundity: Estrogen receptor, Boroola fecundity genes

Trait: Enhancement of hair and fiber

Type of Animal: Sheep

- Wool: Quality, Length, Fineness, Crimp
- Fiber: Elasticity, Strength

Improving Hair and Fiber

The control of the quality, color, yield and ease of harvest of hair, wool and fiber for fabric and yarn production has been an area of focus for genetic engineering in livestock. The manipulation of the quality, length, fineness and crimp of the wool and hair fiber from sheep and goats has been examined using transgenic methods.[97] Transgenic methods will also allow improvements to fiber elasticity and strength.[98] In the future transgenic manipulation of wool will focus on the surface of the fibers. Decreasing the surface interactions between fibers could decrease shrinkage of garments made from such fibers.[94]

A novel approach to produce useful fiber has been recently accomplished using the milk of transgenic goats.[99] Spiders that produce orb-webs synthesize as many as seven different types of silk used in making these webs. Each of these silks has specific mechanical properties that make them distinct from other synthetic and natural fibers.[95] One of the most durable varieties is dragline silk. This material can be elongated up to 35 percent and has tensile properties close to those of the synthetic fiber Kevlar®. This silk has a greater capacity to absorb energy before snapping than steel. The protein monomers that assemble to produce these spider silk fibers have been produced in the milk of transgenic goats. The numerous potential applications of these fibers include medical devices, suture, ballistic protection, aircraft, automotive composites and clothing to name a few.

Table 5. More abundant, high-value industrial proteins may be produced by genetically engineered animals

Trait: Tensile properties for biodefense or medical uses

Type of Animal: Goats

- Natural proteins: Spider silk

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Powell BC, Walker SK, Bawden CS, Sivaprasad AV, Rogers GE. Transgenic sheep and wool growth: possibilities and current status. Reprod Fertil Dev 1994; 6 [5]: 615-623.

[98] Bawden CS, Powell, BC Walker, SK, Rogers GE. Expression of a wool intermediate filament keratin transgene in sheep fiber alters structure. Transgenic Res 1998;7: 273-287.

Bawden CS, Dunn SM, McLaughlan CJ, Nesci A, Powell BC, Walker SK, Rogers GE.Transgenesis with ovine keratin genes: expression in the sheep wool follicle for fibres with new properties. Transgenic Res 1999; 8: 474 abstr.

[99] Karatzas CN, Zhou JF, Huang Y, Duguay F, Chretien N, Bhatia B, Bilodeau A, Keyston R, Tao T, Keefer CL, Wang B, Baldassare H, Lazaris A. Production of recombinant spider silk [BiosteelTM] in the milk of genetically engineered animals. Transgenic Res 1999;8: 476-477.

Regulatory Landscape and Challenges

The technology involved in production of genetically engineered animals holds great promise of benefits through both biomedicine and agriculture. This scientific promise compels us to pursue a regulatory pathway for enabling these new technologies. While the first practical medical applications of genetic engineering of animals in the United States—through development of new drugs, biologics, and xenotransplants—are still a few years from market due to continued research, the agricultural applications are already upon us.

Enabling Both Agricultural and Biomedical Applications of Genetic Engineering

Genetically engineered animals in agriculture are poised to deliver benefits at many stages, to producers, processors, the environment and to individual consumers. Improvements in food production efficiency become more urgently needed in the face of projected increases in demand driven by population growth and prosperity.[100] Aside from increasing production efficiency, the examples of livestock able to resist specific diseases, and thus improving animal welfare, decreases the use of antibiotics in the food supply, clearly a consumer benefit as well as producing more healthful products such as meat high in omega-3 fatty acids. The increased food safety aspects of eliminating BSE or certain bacteria in milk production and dairy products clearly benefit consumers.

One of the most promising areas of research and development involves the farm animals bred to deliver environmental benefits, such as the Enviro-Pig™. Because of its unique attributes, it excretes feces that contain up to 75 percent less phosphorus than non-transgenic pigs fed the same conventional diet.[101] As a result, 33 percent less land would be required to absorb the manure from these pigs as fertilizer. If this were combined with animal diets adjusted to decrease crude protein, even less land would be required.[102] In addition, notwithstanding the impact on the land, there will also be a direct positive impact on human health as the negative impact on environmental quality is reduced.

The transgenic pig and salmon embody the leading edge of various types of genetically engineered animals that will reduce the environmental footprint of animal agriculture through enhanced metabolic capabilities. Consumer surveys suggest that genetic engineering directed to issues involving environmental sustainability and food safety receive meaningful support.[103] Likewise, similar to environmentally-friendly agriculture, another more immediate and obvious application of genetic engineering is the development of animals that have improved food production qualities, creating efficiencies, cost savings, and qualitative improvements
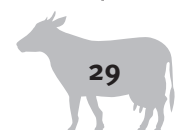
Genetically engineered animals in agriculture are poised to deliver benefits at many stages, to producers, processors, the environment and to individual consumers.

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[100] United Nations Food & Agriculture Organization, 2002. World Agriculture: Towards 2015/2030 at http://www.fao.org/docrep/004/y3557e/y3557e00.HTM.

[101] Golovan SP, Meidinger RG, Ajakaiye A, Cottrill M, Wiederkehr MZ, Barney DJ, Plante C, Pollard JW, Fan MZ, Hayes MA, Laursen J, Hjorth JP, Hacker RR, Phillips JP, Forsberg CW. Pigs expressing salivary phytase produce low-phosphorus manure. Nature Biotechnol 2001;19: 741-745.

[102] If transgenic phytase pigs were raised in place of conventional pigs, the land area required for spreading would be reduced by 33% before manure N would be applied in excess. It is generally recognized that for each 1% decrease in protein in the diet, there is an 8 to 10% reduction in manure (Lenis, N. P., and A. W. Jongbloed. 1999. New technologies in low pollution swine diets: Diet manipulation and use of synthetic amino acids, phytase and phase feeding for reduction of nitrogen and phosphorus excretion and ammonia emission. Asian Aus. J. Anim. Sci. 12:305-327

[103] Santerre, C. R., and K. L. Machtmes. 2002. The impact of consumer food biotechnology training on knowledge and attitude. J. Am. Coll. Nutr. 21(Suppl. 3):174-177.

Science-based regulation of genetically engineered animals and their products ensures safety of the products and public confidence.

in food production that can enable farmers worldwide to extend food supplies while using fewer natural resources. Any genetically engineered animal that grows more efficiently also provides a substantial positive environmental impact. For example, the genetically engineered salmon, AquAdvantage™ salmon, that is bred to grow to a mature size more quickly, increases the efficiency of food production while providing a huge environmental benefit.[104][105][106][107][108][109] The positive environmental impact will be significant in aquaculture that uses genetic engineering.

These agricultural applications remain the most immediate opportunity, but they are limited by the absence of a clear regulatory framework for enabling their availability. In that regard, they share a common obstacle with the perhaps even more compelling biomedical applications of genetic engineering, which also remain hobbled by the same obstacles. Despite the public health and consumer benefits that stand to be realized, genetically engineered animals remain undeveloped. A rigorous regulatory process is in place under the current law for evaluating chemically derived medical products. But for the medical products derived from genetic engineering of agricultural animals, only one product has come to market worldwide, and that was approved by the European Commission. Many more are likely.

Why Regulate?

Science-based regulation of genetically engineered animals and their products ensures safety of the products and public confidence. The federal government set the precedent for reasonable oversight of biotechnology through the development of its genetically engineered plant regulatory framework. To implement a genetically engineered animal framework that is deemed to be any less than what is in place for plants invites sharp criticism and probably places the future commercial viability of genetically engineered animals in jeopardy. Furthermore, any new technology can create doubt and mistrust in some sectors. To forestall that doubt and to, in part, ensure consumer acceptance, reasonable regulation based upon an internationally recognized approval process will lead to more efficient commercialization of genetically engineered animals, processes and products. Another significant factor that emphasizes the need for decisions on federal government regulation is to enable continued growth and leadership of U.S. animal biotechnology research and development.

The fact remains that enabling this science requires robust and comprehensive coordination for regulation that bridges the divide between food and biomedical products. In addition, federal government study of the regulation of genetic engineering of plants and microbes has

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[104] Alestrøm P (1995) Genetic engineering in aquaculture. In 'Sustainable fish farming'. (Eds H Reinertsen, H Haaland) (AA Balkema: Rotterdam)

[105] Berkowitz DB, Kryspin-Sorensen I (1994) Transgenic fish: safe to eat? Biol. Technology (Elmsford, N.Y.) 12, 247-252.

[106] Devlin RH (1997) Transgenic salmonids. In 'Transgenic animals, generation and use'. (Ed. LM Houdebine) pp. 105-117. (Harwood Academic Publishers)

[107] Devlin RH, Donaldson EM (1992) Containment of genetically altered fish with emphasis on salmonids. In 'Transgenic fish'. (Eds CL Hew, GL Fletcher) pp. 229-265. (World Scientific)

[108] Fletcher GL, Alderson R, Chin-Dixon EA, Shears MA, Goddard SV, Hew CL (1999a) Transgenic fish for sustainable aquaculture. In 'Proceedings of the 2nd international symposium on sustainable aquaculture'. (Eds N Svennevig, H Reinertsen, M New) pp. 193-201. (AA Balkema: Rotterdam)

[109] Fletcher GL, Davies PL (1991) Transgenic fish for aquaculture. In 'Genetic engineering, principles and methods'. (Ed. JK Setlow) pp. 331-370. (Plenum Press: New York)

eclipsed animals for over two decades. Despite the Office of Science and Technology Policy's (OSTP) 1986 intensive study and publication of the coordinated framework for policy and regulation of agricultural biotechnology, which outlined agency responsibilities for regulation of genetically engineered plants, microbes and animals,[110] and in-depth case reviews of the regulation of various genetically engineered animals by the Council on Environmental Quality[111] (CEQ), the policy environment has not yet moved forward. The OSTP analysis focused on the statutory authorities of FDA and the U.S. Department of Agriculture (USDA). Published in 2001, the CEQ case studies for both transgenic growth-enhanced salmon and transgenic goats producing a human drug indicate that the animals are subject to FDA oversight according to the Food Drug and Cosmetic Act (FDCA) because they are considered to contain a "new animal drug" as defined in the law.

The FDA's Center for Veterinary Medicine claimed jurisdiction over genetically engineered animals several years ago[112], defined a regulatory pathway, and invited parties from industry and academia to apply for an Investigational New Animal Drug (INAD), but there has been no publication of guidance documents or regulations on the process. Although several applications have been submitted to FDA over the past at least eight years, no genetically engineered animals have gone beyond the INAD stage nor received approval for an animal-made pharmaceutical. A significant development is that the Codex Alimentarius Commission is expected to approve an international standard for food safety risk assessment for genetically engineered animals in July 2008 that is consistent with the new animal drug process.[113] The USDA has evaluated their authorities and role in regulation of genetically engineered animals and is coordinating with the FDA.

Without a clear understanding of the regulatory framework, the development of genetically engineered animals for biomedical products development can remain prohibitively expensive and inefficient. This is because, not only are the animals themselves expensive to maintain once they have grown past their useful life cycle as animals that produce recombinant drugs, but also because the process of making genetically engineered animals for drug production also can sometimes produce large numbers of surrogate dams or non-genetically engineered offspring that have no drug producing qualities. These animals are nonetheless difficult and expensive to continue to maintain, and therefore are ideally suited for placement in the food supply chain. In fact, some observations suggest that using genetically engineered animals to develop drugs is only cost efficient when these surrogate dams and (or) non-genetically engineered offspring can be safely harvested for human consumption.

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[110] Office of Science & Technology Policy (OSTP), Executive Office of the President, 1986 (June 26). Coordinated Framework for the Regulation of Biotechnology. 51 FR 23302.

[111] OSTP-Council on Environmental Quality, 2001. 'CEQ and OSTP Assessment: Case Studies of Environmental Regulations for Biotechnology.' http://www.ostp.gov/html/ceq_ostp_study1.pdf.

[112] National Research Council. 2002. Animal Biotechnology: Science-based Concerns. Washington, DC. National Academy Press.

[113] Codex Alimentarius Commission. 2008. Joint FAO/WHO Food Standards Programme, 31st Session, Geneva, Switzerland, 30 June - 5 July 2008, Report of the Seventh Session of the Codex Ad Hoc Intergovernmental Task Force on Foods Derived from Biotechnology. al31_42e[1].pdf at www.codexalimentarius.net

From a regulatory standpoint, there were basically two options for FDA regulation of animals produced by genetic engineering, both of which are based in statutory authority, and seek appropriate science-based regulatory review.

Regulatory Options

It is clear that existing laws governing the oversight of food and drugs were not crafted with the challenges in mind of ensuring the safety of food, feed and drugs derived from genetically engineered animals. The challenges are not, however, novel or unique, and OSTP and others have concluded that there is ample statutory authority to regulate genetically engineered plants, microbes and animals. The existing coordinated framework has served the purpose of enabling the development and commercialization of genetically engineered plants for well over 15 years; indeed there has not been one human health nor safety issue attributed to the planting, production and consumption of over 70 approved genetically engineered plants, and global acreage continues to rise by double digits every year, as it did for 2007.[114] In an ideal environment, Congress could legislate separately on the issue of genetically engineered animals and develop a statute narrowly tailored to the appropriate regulation of these products. Such a solution, however, is unrealistic and impractical. Moreover, statutory authority already exists.

From a regulatory standpoint, there were basically two options for FDA regulation of animals produced by genetic engineering, both of which are based in statutory authority, and seek appropriate science-based regulatory review. One approach shares oversight of the genetically engineered animal among the various reviews of products; the other begins with oversight of the genetically engineered animal, and both are based on the FDCA. The approaches for regulation of genetically engineered animals are generally called the "foods" approach, and the "new animal drug" (NAD) approach, respectively. The FDA's Center for Veterinary Medicine has accepted applications under NAD over the past 10 years, and industry applicants support this approach. However, it's worth outlining the considerations of the "foods" approach in part to help explain the reasons FDA is currently accepting applications under the NAD approach.

Under the "foods" approach, food proteins produced by genetically engineered animals would be regulated using the food additive provision of the FDCA and (or) the process known as "generally recognized as safe" (GRAS) notification. Therapeutic proteins produced by genetically engineered animals would be regulated as drugs or biologics. FDA would regulate food and animal feed derived from animals produced by genetic engineering as foods and animal feeds under the FDCA. The GRAS notification process is not mandatory, and it results in a letter from the FDA indicating the agency has "no further questions" for all intents and purposes clearing the way for the company to market the product. Only one biotechnology-derived product, the kanamycin-resistant marker gene, has been finalized as safe by FDA final rule using the food additive provision since 1994. This gene was found in the transgenic Flavor-Savor™ tomato, which was commercially available for several years until retired by the new parent company after a takeover.

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[114] ISAAA 2007. Global Status of Commercialized Biotech/GM Crops: 2007. ISAAA Briefs 37-2007 at http://www.isaaa.org/resources/publications/briefs/37/default.html.

Under the NAD approach, FDA would regulate animals and substances created in genetically engineered animals according to the FDCA provisions applying to new animal drugs. The statutory definition of a NAD is anything that affects the structure or function of an animal. Based on that definition, the transgene would be the NAD. Animals produced by genetic engineering and from which foods and animal feeds are derived would be regulated by FDA under the new animal drug provisions of the FDCA. This review would include evaluation of the genetic construct or the transgene in the animal, including its efficacy.

Comparing Regulatory Approaches

The two approaches differ primarily in their approach to oversight of the animals and of food derived from such animals. Under the "foods" approach, FDA could regulate the animals when used in production of therapeutic products or when put to other uses (including research), if it is reasonable to expect the animal could enter the food supply. FDA could also regulate food derived from such animals. Under the NAD approach, by contrast, FDA would have the option to regulate all the animals, or alternatively could elect to regulate some defined subset of such animals through regulatory discretion. Both approaches rely on established procedures for notifying or seeking approval from FDA. The "foods" approach would likely also entail the development of a voluntary consultation process, analogous to the Agency's process for new genetically engineered plant varieties. The NAD approach would require FDA to adapt procedures originally designed for new animal drugs to all animals produced by genetic engineering, including those not intended to produce therapies.

The "foods" approach would have any substance created in an animal, such as a protein, that is intended for human consumption regulated by FDA for safety according to the food additive provisions of the FDCA. The food additive provisions would require pre-market review, except that substances deemed as GRAS would be subject to a voluntary biotechnology consultation process. The food additive petition provision is not applicable to all transgenes in all genetically engineered animals. The food additive provision does not offer a formal "approval" per se, nor does it confer market exclusivity. This is one reason it has not received support among industry participants as a template for regulation of genetically engineered animals.

The NAD approach is a mandatory process that provides an "approval." Many industry participants believe this imprimatur is necessary for successful commercialization and appropriate to the technology and products. They believe that this rigorous, science based approval process will improve consumer acceptance because of the mandatory framework for approval. The biotechnology industry has been on record for several years as supporting the new animal drug approach. In regards to the use of scarce resources at the FDA, the new animal drug approach consolidates regulatory review and oversight for the animal's health, human health and the environment, affording an efficient process with regard to use of expertise and other resources. Industry's expectation is that the process will avoid duplicative and burdensome process steps to a science-based, seamless and smooth path toward approvals.

The New Animal Drug approach is a mandatory process that provides an "approval." Many industry participants believe this imprimatur is necessary for successful commercialization and is appropriate to the technology and products.

The considerations have been thoroughly studied and debated among scientists, regulators, industry and academia, and consensus has formed around having genetically engineered animals regulated according to the New Animal Drug framework.

Regarding the public, consumer advocacy groups argue that a voluntary regulatory approach does not provide consumers with a comparable level of confidence to that from the mandatory new animal drug review and approval process. On the other hand, the primary concern about the new animal drug approach, heard from some consumer groups is that it is a "black box" (i.e. confidential) and thus public transparency is achieved only upon approval of the product.

The considerations have been thoroughly studied and debated among scientists, regulators, industry and academia, and consensus seems to be forming around having genetically engineered animals regulated as animal drugs[115] on the principle that the insertion of the gene as part of the process of making a genetically engineered animal, as well as its residue in subsequent generations of animals, meets the definition of an animal drug intended to alter the structure or function of the animal itself. To some who avoid the statutory definition but instead place emphasis on the term "drug", it has appeared to be an awkward application of the definition. Scientists and product developers believe the "foods" approach is too restrictive and does not account for even a fraction of the transgenes being developed nor the benefits yet to be realized. The latter includes those being developed to confer traits in genetically engineered animals for non-food applications such as a fluorescent aquarium fish,[116] a disease resistant horse, or a hypoallergenic cat. As a result of these various views, a policy compromise has been difficult to achieve.

FDA has tailored its existing statutory authority to support regulations that satisfy the theoretical and practical concerns of critics while providing transparent and reasonable boundaries on how genetically engineered animals can be safely developed for human consumption. Draft FDA guidance for a regulatory framework based on the NAD framework may be forthcoming and will provide an opportunity for the public to comment.

Conclusion

On a political level, the creation of a comprehensive regulatory framework for these products has been stymied by continued policy debate. Meanwhile, consumer critics cite a myriad of criticisms of the technology, including the "yuck" factor, used to refer to some people's reflexive disaffection for the results of innovative technologies applied to food production. The criticisms offered generally fail under analysis, in the end leaving opponents standing on little more than emotion. However, the industry must continue to educate stakeholders and consumers to achieve acceptance of the technology.

Significant opportunity costs will be levied if a specific regulatory pathway is not defined soon. While the agricultural application of this science is compelling, the medical applications are groundbreaking, and the needs for both public health and food security are urgent.

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[115] Pew Initiative on Food and Biotechnology, 2006. Public Sentiment About Genetically Modified Food at http://www.pewtrusts.org/uploadedFiles/wwwpewtrustsorg/Public_Opinion/Food_and_Biotechnology/2006summary.pdf.

[116] Gong Z, Wan H, Tay TL, Wang H, Chen M, Yan T. (2003) Development of transgenic fish for ornamental and bioreactor by strong expression of fluorescent proteins in the skeletal muscle. Biochemical and Biophysical Research Communications 308: 58-63.
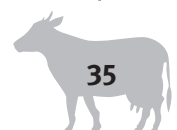
Genetically engineered animals promise not only safer, lower-cost proteins and drugs that could increase access and enable essential changes in medical practice but also fundamentally better medical products that can provide substantial improvements over today's medicines. The drugs that genetically engineered animals can produce – blood components, replacement proteins, antibodies, and xenotransplants – remain among the most expensive drugs to produce in the world. Genetically engineered animals can deliver substantial improvements in terms of cost, safety and availability of urgently needed drugs and treatments, bringing substantial public health benefits. Likewise genetically engineered animals can also sustainably and in an environmentally friendly and pro-welfare friendly manner, meet the growing global demand for high quality and safe animal food products.

The human health benefits will not be realized if we do not resolve the regulatory framework for governing how these animals can also provide food and agricultural benefits. This fact cannot be wished away by those who would embrace the human health aspects of genetically engineered animals while treating the food and agricultural aspects as something to be resisted or prohibited. The boundaries that govern the science of genetic engineering of animals do not allow for these easy dichotomies in policy. Until we resolve how we are going to deal with the food capabilities of this science, the medical possibilities will remain largely undeveloped and many of their attendant opportunities will go unrealized. The solution is simple if we follow the lead of the science based regulatory agency that has the scientific and regulatory expertise in animal biotechnology. The FDA has worked closely with the industry and academia on the diverse applications of the technology for over ten years, and it has mapped the road forward with a rigorous science-based framework.

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