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Animal Biotechnologies and Agricultural Sustainability

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Animal production systems can be broadly classified into three categories: grazing, mixed crop-livestock, and intensive. Originally, all livestock production was grassland based and fell into the grazing category. Grassland systems effectively convert human-inedible materials to high-quality human food. Where climatic, soil, and disease conditions permitted, grassland-based systems developed into mixed crop-livestock systems. On a global basis, mixed crop-livestock systems involve the largest number of animals, generate the most total production, and serve the largest number of people (Seré and Steinfeld 1996). Intensive animal agriculture systems developed more recently, mostly in the vicinity of urban centers when urbanization and income exceeded certain levels. Intensive livestock production is the fastest growing category as a result of several factors, including declining real prices for feed grains, and improved feed conversion ratios (unit feed per unit animal product), animal health, and reproductive rates (Naylor et al. 2005). That animals of most species produce more product per animal in less time when fed nutrient-dense grain diets is one of the factors favoring the growth of intensive systems.

Large-scale intensive operations, in which animals are raised in confinement, now account for three-quarters of the global poultry supply, 40 percent of the pork supply, and more than two-thirds of all eggs (Bruinsma 2003). Intensive livestock production systems have dramatically reduced the amount of land needed to produce a unit of animal product, such as a gallon of milk or a pound of meat. For example, over the last century, advances in the genetics, nutrition, and management of U.S. dairy cows have resulted in more than a fourfold increase in milk production per cow, and a threefold improvement in productive efficiency (milk output per feed resource input; VandeHaar and St-Pierre 2006). However, the environmental and ethical sustainability of these intensive production systems is coming increasingly under scrutiny. Large-scale animal operations concentrate environmental pollutants and result in ecological disturbances, and consumers in some countries are increasingly concerned about the health and well-being of animals raised in concentrated animal production systems.

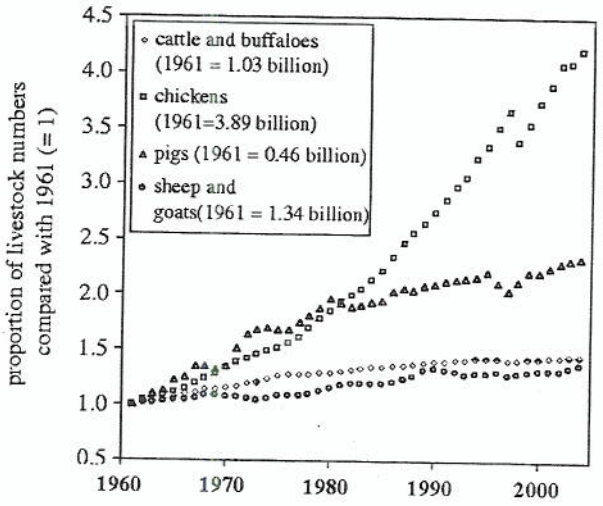

Figure 6.1. Proportional Increase in World Head of Livestock 1961–2004. *Source:* Pretty (2008).

These factors have led to opposition to the very existence of animal agriculture by some, whereas others question the production of meat in a world in which millions of people are starving. However, animal agriculture is an integral component of global food production systems. Animal products provide one-sixth of human food energy and more than one-third of the protein on a global basis (Bradford 1999). In less developed countries, much of this energy and protein has traditionally been derived from extensive, mixed-production systems in which livestock convert human-inedible materials (forages and byproducts) into high-quality human food. Animal agriculture also serves other functions, including the provision of draft power and transportation, nutrient recycling, wealth accumulation, and rangeland management functions, which are important to the efficiency and sustainability of food production systems. Evidence also exists to support the conclusion that the inclusion of foods of animal origin in the diets of young children with currently low levels of these foods leads to marked improvement in both physical and mental development (Allen et al. 1992; Grillenberger et al. 2007, 2006; Neumann et al. 2007).

Since the early 1960s, livestock production has grown rapidly, with a worldwide fourfold increase in the number of chickens, a twofold increase in the number of pigs, and a 40 to 50 percent increase in the numbers of cattle, sheep, and goats (Figure 6.1). Meat demand is expected to rise rapidly with continued economic growth, which will have important ramifications for world agricultural production systems. This rapidly growing demand for livestock products has been coined the "Livestock Revolution," after the better known "Green Revolution." To put this in perspective, from the beginning of the 1970s to the mid-1990s, consumption of meat and milk in developing countries increased by 70 and 105 Tg, respectively. The market value of that increase totaled approximately $155 billion (real 1990 dollars), which was more

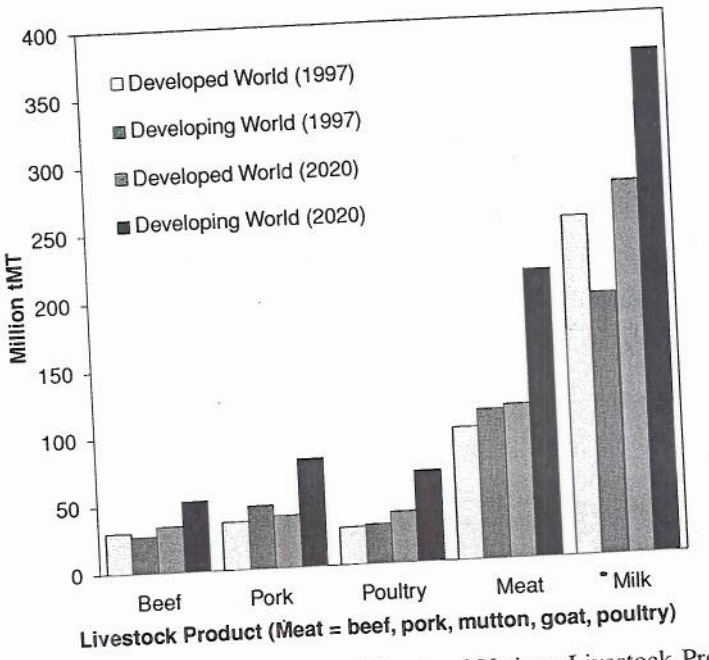

Figure 6.2. Projected Food Consumption Trends of Various Livestock Products to the Year 2020. *Source:* Based on data derived from Delgado (2003).

than *twice* the market value of increased wheat, rice, and maize consumption resulting from the Green Revolution (Delgado 2003). The Livestock Revolution is primarily being driven by demand: Lower income people everywhere are eating more animal products as their incomes rise. It is estimated that by 2020 developing countries will consume 107 Tg more meat and 177 Tg more milk than they did in 1996/1998, dwarfing developed-country increases of 19 Tg for meat and 32 Tg for milk (Figure 6.2). The growing demand for animal products in developing countries cannot be ignored and shows no evidence of diminishing.

Currently 40 percent of the land on earth is used for food production (Nonhebel 2005). It is estimated that more than 60 percent of the arable land is used for the production of animal feeds. In industrialized countries, 73 percent of cereals are fed to animals, whereas in developing countries some 37 percent are (Pretty 2008). Projections made by FAO show an approximate global doubling of the demand for animal food products in the period 2000–20 due to population growth and increases in consumption in developing countries, with poultry, meat, and egg consumption rising markedly more quickly than beef and pork (Bruinsma 2003). Table 6.1 shows the projected increase in the use of cereals as feed through 2020 needed to meet this increased demand for animal products.

On a global scale, doubling the land required for feed production is not possible simply because the quantity of good agricultural soils is insufficient. High-quality arable

Table 6.1. *Past and projected trends in use of cereal as animal feed to the year 2020*

Region	Annual growth rates (percent per year)			Per capita cereal use as feed (kg)	
	Cereal production 1982–93	Cereal use as feed 1982–93	Projected cereal use as feed 1993–2020	1993	2020
China	2.0	5.8	3.2	62	120
India	3.2	3.5	3.0	4	6
Other East Asia	−2.0	6.7	2.5	115	183
Other South Asia	2.1	1.5	2.9	7	8
Southeast Asia	2.4	8.6	2.9	32	49
Latin America	0.7	2.5	1.9	118	137
West Asia/North Africa	3.9	1.8	2.1	92	94
Sub-Saharan Africa	4.1	5.3	2.3	4	4
United States	0.0	1.0	0.9	603	622
Developing World	2.3	4.3	2.6	45	62
Developed World	0.2	−0.1	0.7	346	386
WORLD	1.3	0.9	1.4	115	120

Source: Bradford (1999).

land is becoming scarcer because of ongoing industrialization, urbanization, infrastructural development, and desertification. Given this fact, this chapter focuses on ways that biotechnologies may help animal agriculture meet the growing demand for animal products more sustainably – by balancing environmental, social, and economic goals. We recognize that there are often tradeoffs among these sustainability goals; therefore it is impossible to present a single animal production system or biotechnology that will satisfy all aspects of sustainability concurrently. We outline biotechnologies that may assist animal agriculture to become more efficient, decrease its impact on the environment, and improve animal well-being. Although some biotechnological approaches are prohibited by agricultural production systems that are purported to be sustainable, we consider that any biotechnology that works to improve efficiency or animal well-being and does not deleteriously affect the environment is likely to have some sustainability benefits.

What Is Animal Biotechnology?

Biotechnology can be defined as the application of science to living organisms. From this definition it is clear that a broad range of strategies for the genetic improvement of livestock, including widely used practices such as selective breeding, artificial insemination, and embryo transfer, qualify as animal biotechnologies. More recently,

the term has become associated with the controversial technologies of GE and cloning. For the purposes of this chapter we discuss the subset of biotechnologies that focus on materials and methods related to the genetic manipulations of RNA and DNA and on genomic approaches to improve the sustainability of animal agriculture. However, we do recognize that traditional animal breeding and husbandry practices have resulted in major improvements in the efficiency of animal agriculture. For example, the average time to produce a broiler chicken in the United States was reduced from 72 days in 1960 to 48 days in 1995, and the slaughter weight rose from 1.8 to 2.2 kg. Concurrently, feed conversion ratios (kg feed/kg gain) were reduced by 15 percent. These remarkable improvements in production efficiency have dramatically decreased the inputs required to produce a unit of output, although it might be argued that the processes were employed without adequately considering environmental, social, and animal welfare goals. Broiler improvements highlight the point that environmental and animal well-being concerns associated with efficiency gains are not the sole purview of modern DNA-based biotechnologies. They are equally associated with the use of conventional breeding methods for the genetic improvement of livestock.

Animal breeders are always trying to maximize the response to selection. This is defined as the difference in the mean phenotypic value between the offspring of the selected parents as compared to that of the whole of the parental generation before selection. The genetic gain (ΔG) in animal breeding programs can be calculated as

$$\Delta G = \frac{i^* r^* \sigma_A}{L}$$

where i is the intensity of selection, r is the accuracy of selection, σ_A is the additive genetic standard deviation in the potential parent population, and L is the generation interval. If biotechnologies affect any of the variables in this equation, they can influence the genetic gain per generation. For example, increasing the intensity of selection (i.e., the proportion of animals in the parental population that are actually selected to produce offspring) can be achieved using a variety of approaches such as artificial insemination or cloning to maximize the use of superior breeding stock. The accuracy of selection can be increased through progeny testing programs or by using information from genetic markers. Breeders can increase the amount of genetic variability that exists in the prospective parental population (e.g., by increasing the number of breeds of potential selection candidates, or by using either GE to bring in new traits, or by increasing the additive genetic variability of existing traits). Likewise, they can also decrease the generation interval by selecting animals at a younger age or through the use of assisted reproductive technologies. Any biotechnology that can affect one of these four factors influencing genetic gain will be of potential value to animal breeders.

Genetic Engineering

The first GE livestock were generated more than 25 years ago using pronuclear microinjection techniques (Hammer et al. 1985). Since then a modest number of GE animals have been developed, and many more are envisioned for agricultural applications (Table 6.2). GE animals carry a segment of recombinant deoxyribonucleic acid (rDNA) – the transgene – that is stably transmitted to their offspring in a Mendelian fashion. The efficiency of pronuclear microinjection is low; usually only 3 to 5 percent of the animals born carry the rDNA, and not all lines express the transgene. An encouraging recent development is the use of viral vectors, particularly those based on lentiviruses, which results in much higher rates of germ-line positive GE animals (Golding et al. 2006). Additionally, nuclear transfer techniques (cloning) have been used to produce GE animals with precise genetic changes; for example, animals with targeted disruption of endogenous genes including cattle lacking the prion protein responsible for bovine spongiform encephalopathy (BSE) or "mad cow disease" (Kuroiwa et al. 2004; Richt et al. 2007). Despite these advances, the techniques for generating transgenic livestock and poultry remain somewhat inefficient and expensive, and at the current time no food products derived from GE livestock have reached the marketplace.

There are a number of GE livestock applications – both extant and proposed – that could be envisaged to align with agricultural sustainability goals (Table 6.2). These applications include GE animals with improved product quality, reduced environmental impact, and enhanced disease resistance. Additionally, there are GE examples where animals have improved productivity, including increased milk production and growth rate, improved feed utilization, enhanced reproductive performance, and/or increased prolificacy.

Potential Benefits

Breeding: Conventional breeding programs are limited to naturally occurring genetic variation in the parent population. GE offers a way to increase the genetic variability available for selection (i.e., the additive genetic variance term from Equation 1). It is likely to be most useful in developing novel genetic traits (e.g., providing mammals the ability to endogenously synthesize n-3 fatty acids) or genetic variation that did not exist in a specific population, breed, or even species. Transgenic laboratory animals have become increasingly important for biological and biomedical research, and the scientific literature associated with these applications is vast and growing. For example, there was a tenfold increase in the number of GE animals used in research in the United Kingdom between 1995–2005 (Lane 2005). Transgenic livestock are also increasingly being produced specifically as biomedical research models (Forsberg 2005; Petters et al. 1997). The public is mostly supportive of such applications, and

Table 6.2. *Extant and envisioned genetically engineered livestock applications for agriculture*

EXTANT APPLICATIONS	Species	Gene	Approach	Reference
PRODUCTIVITY				
Enhanced growth rate	Various fish species	Growth hormone	Transgene expression	Aerni (2004); Bessey et al. (2004); Cook et al. (2000); Martinez et al. (2000); Nam et al. (2001); Rahman et al. (1998)
Enhanced milk production	Swine	α-lactalbumin	Transgene expression	Marshall et al. (2006); Wheeler, Bleck, and Donovan (2001)
Enhanced growth rate	Swine	Growth hormone	Transgene expression	Pursel et al. (1989); Pursel et al. (1997)
Enhanced growth rate	Swine	Insulin-like growth factor (IGF1)	Transgene expression	Pursel et al. (2004)
DISEASE RESISTANCE				
BSE resistance	Cattle, goats, and sheep	Prion	Knockout	Denning et al. (2001); Richt et al. (2007); Yu et al. (2006)
Mastitis resistance	Cattle	Lysostaphin	Transgene expression	Wall et al. (2005)
Mastitis resistance	Cattle	Lactoferrin	Transgene expression	van Berkel et al. (2002)
BSE resistance	Goat	Prion	RNAi transgene	Golding et al. (2006)
Visna virus resistance	Sheep	Visna virus envelope gene	Transgene expression	Clements et al. (1994)
Mastitis resistance	Goats	Lysozyme	Transgene expression	Maga, Cullor, et al. (2006); Maga, Shoemaker, et al. (2006)
GCH virus resistance	Grass Carp	Lactoferrin	Transgene expression	Zhong et al. (2002)
Bacterial resistance	Channel Catfish	Cecropin B gene	Transgene expression	Dunham et al. (2002)
Prevent spread of avian influenza	Chicken	Decoy molecule	Transgene expression	Lyall et al. (2011)

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ENVIRONMENTAL				
Decreased P in manure	Swine	Phytase	Transgene expression	Golovan et al. (2001)
PRODUCT QUALITY				
Increased ω-3 fatty acids in meat	Swine	n-3 fatty acid desaturase	Clone/transgene expression	Lai et al. (2006)
Increase cheese yield from milk	Cattle	β-casein, κ-casein	Clone/transgene expression	Brophy et al. (2003)
Increased mono-unsaturates in milk	Goat	Rat stearoyl-CoA desaturase	Transgene expression	Reh et al. (2004)

ENVISIONED APPLICATIONS	Species	Target	Proposed approach	Background information
Increased lean-muscle growth	Cattle	Myostatin	RNAi /knockout	McPherron and Lee (1997)
Increased postnatal growth	Various	Socs2	RNAi /knockout	Horvat and Medrano (2001)
Enhanced mammary gland development	Various	Socs1	RNAi /knockout	Lindeman et al. (2001)
Suppressing infectious pathogens	Various	RNA viruses (e.g., foot and mouth, fowl plague, swine fever)	RNAi	Clark and Whitelaw (2003); Whitelaw and Sang (2005)
Coronavirus resistance	Swine	Aminopeptidase N	RNAi /knockout	Schwegmann-Wessels et al. (2002)
Avian flu resistance	Poultry	Avian influenza	RNAi	Sang (1994); Tompkins et al. (2004)
Low-lactose milk	Cattle	Lactase	Transgene expression	Jost et al. (1999)
Low-lactose milk	Cattle	α-lactalbumin	RNAi /knockout	Stacey et al. (1995)
Increased ovulation rate	Sheep	GDF9, BMP15, ALK6/BMPR1B	RNAi /knockout	Melo et al. (2007)
High omega-3 fatty acid milk	Cattle	n-3 and n-6 fatty acid desaturase	Transgene expression	Morimoto et al. (2005)
Resistance to brucellosis	Cattle	NRAMP1	Transgene expression	Barthel et al. (2001)
Decreased P in manure	Poultry	MINPP	Transgene expression	Cho et al. (2005)
Decreased P in manure	Poultry	Phytase	Transgene expression	Guenther et al. (2005)

scientists pursuing such research are generally viewed as contributing toward societal good. Interestingly, the production and use of this vast number of transgenic animals for research purposes, estimated in Mak (2008) to be 10–50 million animals annually in the United States, have received little attention or comment from either the activist or the scientific community.

Sustainability: Whether GE livestock fit in with sustainability goals is greatly dependent on which goal and production system one is considering. However, some GE livestock applications (e.g., disease resistance) would seem to align with almost any definition of sustainability and clearly with the goal of improving animal well-being. Infectious diseases have major negative effects on poultry and livestock production, both in terms of economics and animal welfare. The costs of disease are estimated to be 35 to 50 percent of turnover in developing countries and 17 percent in the developed world. Improving animal health using GE has an added benefit in that it reduces the need for veterinary interventions and the use of antibiotics and other medicinal treatments. However, on ideological grounds, some may determine that disease-resistant GE animals have no place in sustainable production systems. For example, the standards of the U.S. Department of Agriculture (USDA) National Organic Program (NOP) specifically prohibit the use of GE. Such regulatory decisions should consider the fact that the combined employment of both disease-resistant livestock and improved animal management practices to minimize disease incidence is compatible with multiple sustainability goals. For example, the 2001 foot and mouth outbreak in the United Kingdom resulted in the slaughter and incineration of 4,078,000 animals (McConnell and Stark 2002). Clark and Whitelaw (2003) speculated that raising GE foot and mouth disease–resistant livestock might align more with sustainability goals than the mass slaughter and adverse environmental consequences that were associated with this disease outbreak in the United Kingdom.

Similarly, the use of more productive GE animals – animals that produce more units of output, such as gallons of milk or pounds of meat, with the same or fewer inputs – should be given due consideration in the context of sustainability. Consider the example of the AquaAdvantage™ salmon, the first GE food animal to go through the U.S. regulatory approval process. Since the mid-1980s, the yield of food fish from capture fisheries has been static at about 60 Tg per year. The growth of the fish supply since that time has largely come from aquaculture. Fletcher et al. (2004) calculated that an extra 52 Tg of aquaculture production will be needed by 2025 if the current rate of fish consumption is to be maintained. Atlantic salmon remains the most important farmed food fish in global trade. Salmon is a carnivorous fish, and aquaculturalists have been working to improve feed conversion rates and efficiencies through selective breeding and inclusion of plant-based protein (soy, rapeseed oil, and corn gluten) in feed formulations. As a consequence, feed input per fish has decreased to 44 percent of 1972 levels; likewise, current diets contain approximately half the content of fishmeal that they did at that time (Aerni 2004).

The AquaAdvantage salmon is an Atlantic salmon carrying a Chinook salmon growth hormone gene controlled by an antifreeze protein promoter from a third species, the ocean pout. The mature weight of these fish remains the same as other farmed salmon, but their growth rate is increased by 400 to 600 percent, with a concomitant 25 percent decrease in feed input, decreased waste per unit of product, and a shortened time to market (Cook et al. 2000; Du et al. 1992). Unlike other food animal species in which selective breeding programs have been ongoing for decades, many fish farmers are still reliant on brood fish collected from the wild. GE may offer one component of an approach to sustainably increase the efficiency of aquacultural production to meet the needs of the 21st century.

Concerns

Social acceptance and sustainability goals: After publication of a paper detailing the generation of a transgenic pig able to endogenously produce omega-3 fatty acids (Lai et al. 2006), a letter to the editor in that same journal criticized the research, stating that "the use of transgenic technology for this application represents the worst kind of research waste" and that "the animal biotech industry needs to confine its work to projects necessary for the achievement of important health, safety, or medical goals" (Fiester 2006). This criticism came despite the fact that an overwhelming number of studies document both the health benefits of increased omega-3 fatty acids in the diet (Connor 2000; Simopoulos 2004) and the fragility of the current supply of long-chain omega-3 fatty acids (Pauly et al. 2003). Additionally, traditional animal breeders are actively pursuing quantitative trait loci (QTL) associated with fatty acid composition in swine meat (Clop et al. 2003; Nii et al. 2006), and both academic and commercial plant metabolic engineering groups have been vigorously pursuing the land-based production of long-chain omega-3 fatty acids in plants (Domergue et al. 2005; Robert 2006; Robert et al. 2005; Wu et al. 2005). It is an interesting conundrum that agricultural research using GE animals to achieve a goal is considered a "research waste," a verboten mechanism to achieve the same goal that other researchers and companies consider to be an important one and that the scientific community is actively pursuing using a variety of non-GE approaches.

Other examples of transgenic livestock that have been developed for agricultural applications have likewise been subject to wide-ranging criticism from a variety of sources including activists, the popular media, and scientific colleagues. Transgenic animals with disease resistance attributes (Maga, Shoemaker, et al. 2006; Richt et al. 2007; Wall et al. 2005) and potential environmental benefit have been critiqued not for their phenotypes, but rather for the production systems that led to the problems that they attempt to address. Take the example of the GE "Enviropig." It produces dramatically lower manure phosphorus levels because it produces the enzyme phytase in its saliva and is therefore able to metabolize dietary phytate (Golovan et al. 2001).

Given the large increase that is expected in both pig and poultry production in the developing world over the next 20 years, decreasing the phosphorus levels in the manure of these monogastric species would likely have a huge worldwide environmental benefit (CAST 2006). However, using GE to reduce the levels of this important pollutant in swine manure has been subject to the criticism that this kind of approach encourages "non-sustainable, un-ecological approaches to livestock management," according to E. Ann Clark (Philipkoski 2001, para. 13). In that article, critics argue that if farmers really want to be environmentally friendly, they should let pigs graze on greens instead of feeding them grain. However, outdoor pig farming can exacerbate nutrient leaching into the soil and groundwater (Williams et al. 2000). This example highlights the fact that sustainability goals are sometimes in conflict, and managing a system for optimal environmental stewardship may clash with animal welfare objectives (Siegford, Powers, and Grimes-Casey 2008). Evaluation of the sustainability of animal agricultural systems therefore depends on the sustainability goal(s) under consideration.

To illustrate this point, an interesting case study from Sweden examined three scenarios for pig production based on different sustainability goals (Stern et al. 2005). The first focused on animal welfare and the natural behavior of the animals. The second focused on environmental goals and the efficient use of natural resources. The third focused on product quality and safety. The cost per pound of pork and land use was highest for the animal welfare scenario and similar for the other two scenarios. Not surprisingly, the environmental scenario had the lowest environmental impact using the life-cycle assessment (LCA) methodology. Stern et al. (2005, 402) summarized that "each scenario fulfilled different aspects of sustainability, but there were goal conflicts because no scenario fulfilled all sustainability goals." The authors also wrote that the evaluation and ranking of sustainability goals are mainly a political question. Leaving sustainability goal evaluation to the political process potentially exposes the process to subjective interpretation and political pressure from special interest groups. It would seem preferable to allow science to objectively evaluate the sustainability implications of different agricultural systems. This evaluation method would give the scientific community an opportunity to develop measures of product and system-level performance to assess and compare the ability of different systems to sustainably meet the needs of both animal and human populations.

Science-based concerns: The main science-based concerns associated with the use of GE food animals relate to food safety, the health and well-being of the animal, and the environment. A report by the National Academy of Sciences considered the ability of GE organisms, particularly fish and insects, to escape confinement and become feral to be the greatest concern facing the animal biotechnology industry (NRC 2002). Models have predicted that under certain circumstances, the interbreeding of GE fish with increased fitness attributes (e.g., younger age at sexual maturity or

increased mating success) could have serious ecological consequences for native fish populations (Muir and Howard 1999, 2001, 2002).

The actual environmental risk posed by each species/transgene combination will depend on a number of factors, including the containment strategy(s), species mobility, ability to become feral, net fitness of the transgenic animal, genotype by environmental interactions, and the stability of the receiving community. Likewise, food safety concerns related to transgenic animals will be similarly case-specific depending on the attributes of the recombinant protein being expressed and whether it is intended to be a pharmaceutical, industrial, or food protein.

Regulatory concerns: There has been public discussion about whether the U.S. Coordinated Framework for the Regulation of Biotechnology, first published in the *Federal Register* on December 31, 1984, will be able to adequately address the safety and commercialization issues associated with the introduction of GE animals into the food supply. In January 2009, the U.S. Food and Drug Administration (FDA) issued a final guidance for industry on the regulation of GE animals (CVM of FDA 2009). The guidance explains the process by which FDA regulates GE animals and provides a set of recommendations to producers of GE animals to help them meet their obligations and responsibilities under the law. That document clarifies that the Center for Veterinary Medicine of the FDA plans to regulate GE animals under the new animal drug provisions of the Federal Food Drug and Cosmetics Act (FFDCA). The FFDCA requires that each new animal drug be approved through a new animal drug application (NADA) based on a demonstration that it is safe and effective for its intended use. The rationale behind regulating GE animals using the new animal drug approach is based on the fact that the rDNA construct in a GE animal is intended to affect the structure or function of the body of the GE animals. Under this interpretation, the rDNA construct meets the FFDCA definition of a drug. Use of a new animal drug is unsafe unless the FDA has approved a NADA based on a demonstration that it is safe and effective for its intended use. All transgenic animals are subject to these premarket approval requirements. The new animal drug regulatory approach focuses on three questions: (1) Is the new animal drug safe for the animal?; (2) is the new animal drug effective?; and (3) if the drug is for a food-producing animal, is the resulting food safe to eat? The FDA new animal drug approval process does not consider ethical and social concerns; regulatory approvals are based solely on safety and effectiveness.

Ethical concerns: These concerns include fundamental objections to the manipulation and use of animals, objections to specific modifications, and concerns about the consequences of genetic modifications, but many of these issues are not unique to GE livestock. Additionally, the current inefficiency of transgenic techniques results in the production of many more animals than would be necessary under higher success rates. To date, unknown consumer acceptance and uncertainties in the regulatory timeline

have effectively halted commercial investment in the development of GE livestock for agricultural applications in the United States. There has been little public discussion of the consequences of not using this significant technology on animal well-being and especially for the development of disease-resistant animals (Murray and Maga 2009).

Cloning

A clone is an organism that is descended from and genetically identical to a single common ancestor. Cloning involves making genetically identical copies of an animal using asexual reproduction. Animals can be cloned by two different methods: mechanical embryo splitting or nuclear transfer. Embryo splitting involves bisecting the multicellular embryo at an early stage of development to generate clones or "twins." A 32-cell embryo, for example, might be bisected into two 16-cell twins. This type of cloning occurs naturally (human identical twins result from this process, but fraternal twins do not); it can also be performed in a laboratory, where it has been successfully used to produce clones from a number of different animal species. This technique was first used in agriculture to replicate valuable dairy breeding animals in the 1980s. The Holstein Association USA registered its first embryo split clone in 1982, and more than 2,300 had been registered by October 2002 (Norman and Walsh 2004). This method has a practical limitation in cattle (Johnson et al. 1995) and sheep (Willadsen 1981), in that a maximum of four clones can be produced from each embryo.

Cloning can also be accomplished by nuclear transfer, in which the genetic material from the nucleus of one cell is placed into a recipient egg. A recipient egg is an unfertilized egg that has had its own genetic material removed by enucleation. To begin the developmental process, the donor nucleus must be fused with the recipient egg through the administration of a brief electrical pulse or a chemical fusion process, after which the embryo starts to divide as if it had been fertilized. In the case of mammals, the embryo is then placed into a surrogate mother, where it will develop until birth and will be delivered as any newborn. Mammals were first cloned via nuclear transfer during the early 1980s, almost 30 years after the initial successful experiments with frogs (Briggs and King 1952). Numerous mammalian clones followed – including mice, rats, rabbits, pigs, goats, sheep (Willadsen 1986), cattle (Robl et al. 1987), and even two rhesus monkeys named Neti and Detto (Meng et al. 1997) – all as a result of nuclear transfer. The Holstein Association USA registered its first embryo nuclear transfer clone in 1989, and approximately 1,200–1,500 cows and bulls were produced by embryonic cell nuclear transfer in North America in the 1980s and 1990s (Yang et al. 2007). Because all of these clones were produced from the transfer of nuclei derived from early (8- to 32-cell) embryos, a theoretical maximum of only 32 clones could be produced from each individual embryo.

In 1996, the famous cloned sheep, Dolly, was born. Dolly was the first animal to be cloned via nuclear transfer from a cultured somatic cell derived from an adult (Wilmut et al. 1997). This process, known as somatic cell nuclear transfer (SCNT) cloning, opened the way for cloning to be performed on a potentially unlimited number of cells from adult animals. It allowed cloning technology to be extended to make copies of elite breeding animals with well-established breeding superiority based on their own performance records and those of their offspring. A diverse range of species have now been successfully cloned from adult tissues using SCNT, including cattle (Kato et al. 1998), mice (Wakayama et al. 1998), pigs (Polejaeva et al. 2000), cats (Shin et al. 2002), rabbits (Chesne et al. 2002), horses (Galli et al. 2003), goats (Keefer et al. 2001), dogs (B. C. Lee et al. 2005), rats (Zhou et al. 2003), and zebra fish (K. Y. Lee et al. 2002). In October 2007, there were approximately 500–600 SCNT livestock clones in the United States (B. Glenn, pers. comm.).[1] Very few of these clones of valuable breeding stock will enter the human food supply themselves; instead, food products like milk and meat will likely be derived from the sexually produced offspring of these SCNT clones.

Potential Benefits

Clones may provide a genetic insurance policy in cases of extremely valuable animals or by producing several identical genetically superior sires in production environments where artificial insemination (AI) is not a feasible option. This practice effectively provides a source of "proven" bulls with superior breeding values relative to the bulls that might otherwise have been used (i.e., increasing both the intensity and accuracy of selection terms from Equation 1). Clones could conceptually also be used to reproduce a genotype that is particularly well suited to a given environment. The advantage of this approach is that a genotype that is proven to do especially well in a particular location could be maintained indefinitely, without the genetic shuffle that normally occurs every generation with conventional reproduction. This genetic shuffle effectively decreases the accuracy term from Equation 1, until that young bull has been subsequently proven to carry superior genes though progeny testing.

Although cloning of elite breeding stock as a genetic insurance policy may provide a limited market for clones, the most significant impact of cloning will likely result from methods to make targeted genetic modifications to cells before using them for SCNT cloning (Forsberg 2005). Cloning enhances the efficiency of GE by offering the opportunity to produce 100 percent transgenic offspring from cell lines that are known to contain the transgene. This has already enabled the generation of animals

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with agricultural potential, including cattle without the prion protein responsible for bovine BSE (Richt et al. 2007), pigs able to endogenously produce meat with omega-3 fatty acids (Prather 2006), and dairy cows that express elevated levels of milk proteins in their milk (Brophy et al. 2003). The ability to make targeted changes in cell culture and its subsequent cloning opens the way for the previously impracticable targeted deletion of undesirable traits and for the more efficient addition of desirable traits using GE techniques.

Concerns

The proportion of adult somatic cell nuclei that successfully develop into live offspring after transfer into an enucleated egg is very low (Tsunoda and Kato 2002). High rates of pregnancy loss have been observed after transfer of the eggs containing the adult cell nuclei into recipient animals (Hill et al. 2000). On average, only 9 percent of transferred embryos result in calves, with efficiencies ranging from 0 to 45 percent depending on the type of somatic tissue from which the transferred nucleus was derived (Beyhan et al. 2007). The problems associated with the cloning process are not unique to SCNT cloning, and all have been observed in animals derived via other commonly used assisted reproductive technologies (e.g., embryo transfer, in vitro fertilization) and even natural mating (Rudenko, Matheson, and Sundlof 2007). However, the frequency of these problems tends to be higher in SCNT clones.

Various abnormalities, such as "large offspring syndrome" (in which cloned lambs and calves are often large at birth), placental abnormalities, edema, and perinatal deaths, have been observed in cloned animals with frequencies that are at least partially dependent on the type of somatic tissue from which the transferred nucleus was derived. On average, 42 percent of cloned calves die between delivery and 150 days of life (Panarace et al. 2007). Although cloning poses no risks that are unique or distinct from those encountered in modern agricultural practices, the frequency of the risks is increased in cattle during the early stages of the life cycle. However, some adult cloned cows have been observed to have normal breeding and calving rates, and cloned bulls produce high-quality semen and have normal fertility when used for artificial insemination and natural mating. To date, there has been no evidence of clone-associated abnormalities being passed on to their offspring following sexual reproduction. This suggests that abnormalities seen in clones are not heritable and appear to be corrected during gametogenesis (the formation of eggs and sperm).

Studies examining the composition of food products derived from clones have found that they have the same composition as milk or meat from conventionally produced animals (Heyman et al. 2007; Laible et al. 2007; Norman and Walsh 2004; Takahashi and Ito 2004; Tian et al. 2005; Tomé, Dubarry, and Fromentin 2004; Walker

et al. 2007; Walsh et al. 2003; Yamaguchi, Ito, and Takahashi 2007; Yang et al. 2007). Milk and meat from clones produced by embryo splitting and nuclear transfer of embryonic cells have been entering the human food supply for more than 20 years with no evidence of problems. Nevertheless, in 2001 the Center for Veterinary Medicine at the FDA determined that it should complete a comprehensive risk assessment to identify hazards and characterize food consumption risks that may result from SCNT animal clones (Rudenko and Matheson 2007), and it asked companies not to introduce these cloned animals, their progeny, or their food products (e.g., milk or meat) into the human or animal food supply. Because there was no fundamental reason to suspect that clones would produce novel toxins or allergens, the main underlying food safety concern was whether the SCNT cloning process resulted in subtle changes in the composition of animal food products (Rudenko et al. 2004).

On January 15, 2008, the FDA published its final 968-page risk assessment on animal cloning, which examined all existing data relevant to the health of clones and their progeny or to food consumption risks resulting from their edible products. This report, which summarized all available data on clones and their progeny, concluded that meat and milk products from cloned cattle, swine, and goats, and the offspring of clones of any species traditionally consumed as food, were as safe to eat as those of conventionally bred animals (CVM of FDA 2008). This conclusion opened the door for animal products from SCNT clones and their offspring to enter the food supply.

Currently, the cost of obtaining cloned animals (~$15,000/head for cattle) is prohibitive for commercial cattle producers. However, if costs were to decrease so that cloning became more widely adopted, some have expressed concerns regarding its potential to decrease genetic diversity and render livestock populations vulnerable to a catastrophic disease outbreak or be singularly ill suited to changes that may occur in the environment. Although such criticisms could be equally relevant to many forms of assisted reproductive technology (e.g., artificial insemination, embryo transfer), for any single genotype to prove superior in all economically relevant traits across all production systems and environments is unlikely. Even if clones were to become widely used, producers in different regions would likely select different clonal lines from a range of breeds based on their decision as to which genotype best matched their region and production environment.

Genomic Selection

Traditional genetic improvement of livestock relies on developing accurate genetic merit predictions or "breeding values" for animals based on their performance and that of their ancestors and offspring. Selection of animals with the best breeding values for production traits has been very effective in improving the efficiency of livestock production. However, selection has not been as successful for traits that are difficult

to measure such as disease resistance or traits that are not available until late in an animal's life, such as fertility or longevity.

The cow, chicken, and pig genomes have all recently been sequenced, and this has led to the discovery of many thousands of naturally occurring DNA sequence variations between individuals in the form of single nucleotide polymorphisms (SNPs). Researchers are now working to determine which variations are associated with desirable characteristics, such as disease resistance. It is hoped that using information on variation in DNA sequence between animals will help improve the accuracy of breeding values; that is, it will give breeders more confidence they are selecting the best animals. Additionally, because DNA is available from birth, it may be possible to predict the genetic potential of animals at a very young age and then keep only the very best animals for breeding purposes. This may pave the way for producers to select animals to become parents of the next generation based on breeding values calculated from DNA marker data alone, a process called "whole genome selection."

Whole genome selection involves the simultaneous use of a large number of markers (e.g., the 50,000 SNP bovine panel) to predict the genetic merit of genotyped animals for many different traits. This approach relies on a two-step analysis involving "training data" to estimate molecular breeding values (MBVs) of SNP haplotypes (Meuwissen, Hayes, and Goddard 2001) or alleles (Solberg et al. 2008). An overall measure of the genetic merit for genotyped individuals outside the training dataset can then be obtained by genotyping that animal and adding up the genetic merit of each of the chromosome fragments inherited. This process allows prediction of MBV at an early age, thereby removing many limitations of current phenotype-based breeding programs and providing a clear time advantage in developing genetic estimates for sex-limited traits or for traits that are not available until late in an animal's life, such as fertility or longevity. Genomic selection has the potential to affect the generation interval (age at sire selection), and both the intensity and accuracy of selection components of Equation 1.

Genomic technologies may also offer new opportunities to develop management systems to optimize the production environment based on an animal's DNA genotype. For example, the genotype of some beef and dairy cattle may be better suited to grass-based production systems. It may also be possible to select animals that are able to grow to a certain size using less feed or that are more resistant to certain diseases. These technologies have the potential to achieve sustainability goals, including the production of safer and more nutritious food with less environmental impact and improved animal welfare due to lower disease incidence.

Potential Benefits

If additive genetic merit can be precisely predicted from MBV (i.e., increase the accuracy of selection term in Equation 1), the design of breeding programs will

rapidly evolve and rates of genetic improvement will increase. A major goal of the genomics programs in livestock and poultry is the identification of natural resistance genes or genes that enhance immune response (Müller and Brem 1998). Genomic selection offers a non-GE approach to improve selection for disease resistance.

It is also thought that the use of MBV may help decrease rates of inbreeding per generation because selection using this approach increases the emphasis on the Mendelian sampling of genes an individual receives from its parents, as distinct from emphasizing the parent average used by traditional selection methods (Daetwyler et al. 2007). Basically, genome-wide prediction increases the accuracy of breeding values through revealing which specific chromosome fragments an animal received from its parents, rather than estimating the value of an "average" offspring derived from those two parents. The latter approach favors the co-selection of siblings from elite parents in breeding programs, with a resultant emphasis on closely related individuals.

Concerns

Whole genome selection is an unproven technology. Although preliminary data coming from the dairy industry look promising (VanRaden et al. 2009), it is not known how well it will work in livestock industries with a wider diversity of breeds and less extensive phenotype and data collection resources. Additionally, simulations have shown that the process of selection itself based on MBVs rapidly reduces the accuracy of MBV, following selection on MBV (Muir 2007). Although genomic selection may initially encounter less public opposition because it uses naturally occurring genetic variation, some applications aimed at reducing generation interval, and hence increasing genetic gain per unit of time in Equation 1, may elicit public discomfort. For example, genomic selection enables an approach to decrease generation interval by harvesting immature oocytes from in utero calves (Georges and Massey 1991). Others have even proposed schemes in which breeding is essentially done in the laboratory, and genome scans allow for an estimation of the MBV of cells derived from in vitro meiosis events (Haley and Visscher 1998). Such animal breeding scenarios are largely hypothetical, but analogous manipulations in the world of plant breeding have certainly met with more success and both less regulation and public opposition than those engendered by the prospect of GE animals.

Functional Genomics

Genes usually influence the phenotype through regulatory networks. Functional genomics concentrates on mechanisms that regulate gene transcription and translation in these networks. Gene regulation can be modulated by environmental inputs as well as by DNA sequence variation. Whereas structural genomics is based on

the discovery of alternative DNA sequences, functional genomics is based on the measurement of the abundance of mRNA associated with transcribed genes. The relative abundance of mRNA produced by each gene can be quantified in many ways, but for assessing large number of genes, the microarray is the current technology of choice. Gene expression profiles can be related to environmental perturbations and/or stage of development. Structural and functional genomics can be combined to find chromosomal locations or QTL associated with trait differences. Jansen and Nap (2001) defined the combination of these two fields as "genetical genomics." With this approach, DNA variation that controls gene expression, called expression quantitative trait loci (eQTL), can be mapped to a specific chromosomal location. One of the important outcomes of genetical genomics is the identification of eQTL that map to either the same (*cis*-acting loci) or different (*trans*-acting loci) genomic locations as the gene expressing the transcript being quantified (Pomp, Nehrenberg, and Estroda-Smith 2008). These data can then be combined to infer causal relationships among eQTL, *cis*- and *trans*- gene expression, and phenotypic traits (Sieberts and Schadt 2007).

Potential Benefits

Animal breeding programs that involve complex traits such as robustness, animal well-being, or disease resistance require a well-defined phenotype on which to base selection. For complex traits, identifying a selection criterion that has high repeatability and quantifies the breeding objective can be difficult, and ideal traits may be very expensive or impractical to measure. Functional genomics has the potential to provide biomarkers, which can be used to define complex traits such as behavior, stress, or disease in unique, quantifiable ways (Kadarmideen, von Rohr, and Janss 2006).

Most reported QTL in animals have large confidence intervals that possibly harbor hundreds of genes, making the determination of which is the causative gene difficult or unattainable (Kadarmideen et al. 2006). Genetical genomics may offer a potential solution to this problem. One of the first examples of the successful use of this approach was given by Liu et al. (2001), who mapped QTL in an F2 cross to find genes responsible for Marek's disease resistance in chickens. A concurrent microarray study was conducted on the founder lines for the cross to find genes that were differentially expressed after infection. Fifteen of these genes were subsequently mapped onto the chicken genome, and two of them mapped to a QTL region for Marek's disease resistance.

Genetical genomics can also generate substantial additional insight into the function and interrelation of gene products and gene action, which can then be used to unravel networks of gene regulation (de Koning, Carlborg, and Haley 2005; Jansen and

Nap 2001). These insights have importance to animal breeding because no selection program in commercial operations is based on a single trait (Emmerson 2003; Groen 2003). Pleiotropic effects due to common genes in pathways can result in either favorable or unfavorable correlations among traits. Understanding how genes interact in pathways on multiple traits through functional genomics can lead to the discovery of QTL that have either neutral or favorable pleiotropic effect, thus overcoming an unfavorable correlation.

One of the problems with applying molecular genetics to animal breeding stems from using linked markers, rather than tracking the actual DNA variant that causes the phenotypic difference. Linkage breaks down over time due to recombination, and markers may be in different phases in different families. Finding the causative genetic variants would greatly facilitate such breeding programs. *Cis*-eQTL are highly heritable and easier to identify because genetic control is generally highly robust. When a *cis*-eQTL localizes to the confidence interval of a phenotypic QTL, it becomes a relevant positional candidate (Pomp et al. 2008) and can be selected for without concern for linkage.

However, inclusion of molecular information in breeding programs requires an understanding of how genes interact, that is, do genes work independently or in certain combinations with others. It is increasingly apparent that genes interact extensively and that epistasis is common (Carlborg et al. 2006). Thus, using QTL in breeding programs requires understanding interacting networks of genes to determine the proper combinations for optimal response. The experimental basis for understanding heritable traits has largely involved studying biological systems one gene at a time. Yet the genome consists of tens of thousands of genes compounded by intricate interactions between genes (i.e., epistasis), and between genes and the environment (Pomp et al. 2008). All epistatic QTL would, by definition, be detected as a *trans*-eQTL in a microarray study (Kadarmideen et al. 2006). Aylor and Zeng (2008) proposed a framework for estimating and interpreting epistasis from expression data that combines quantitative genetics approaches with classical genetics associating genes with pathway regulation. With these approaches it may be possible to directly use epistatic variation in breeding programs.

Additionally, variation in gene expression between animals or different lines following disease challenge could unravel the genetics underlying immune response (de Koning et al. 2005), as well as characterize those parts of the molecular networks that help drive disease progression. Sieberts and Schadt (2007) concluded that the integration of gene expression and genotypic data will be critical to understanding how genetic and environmental perturbations lead to disease. A systematic approach is therefore needed to dissect the genetic basis for diseases and understand how genes interact with one another, and with environmental factors, to determine disease phenotypes (Zhu et al. 2007).

A final potential benefit of functional genomics was proposed by Haley and de Koning (2006) who suggested that certain genes and networks could be explored in tissue culture, thereby moving the focus of experimental studies from whole animals to in vitro systems. They concluded that functional genomics may offer "an animal-friendly means of tackling welfare and other problems and hence enhancing sustainable livestock agriculture in a world where the demand for animal protein is expected to increase substantially over the next decade" (Haley and de Koning 2006, 12).

Concerns

Concerns associated with functional genomics are generally related to cost, false positives, a lack of power, choice of tissue, and optimal time to collect samples. Compared to the experimental designs commonly encountered in QTL detection, eQTL experiments to date have been very small and therefore have had inadequate statistical power (de Koning et al. 2005). Multiple testing is a major problem in eQTL experiments. In eQTL mapping, this testing occurs on two levels. First, multiple correlated tests are carried out during the genome scan for eQTL. Second, eQTL analyses are performed for thousands of potentially highly correlated gene expression levels (de Koning et al. 2005). Another concern is the choice of tissue and the developmental stage at which to profile gene expression. This decision is critical, but proper choice requires a priori understanding of which tissue(s) is associated with regulation of the phenotype. A researcher's assumptions could be incorrect, multiple tissues may require examination, or several time points may be necessary (Doerge 2002).

Other Biotechnologies

RNAi

RNAi is a sequence-specific method to selectively knock down endogenous gene expression. It works by introducing transgenic homologous double-stranded gene constructs that enable the stable expression of small interfering RNA (siRNAs) that constitutively suppress target gene expression (Martin and Caplen 2007). Transgenic goats carrying lentivectors that express siRNAs against the prion protein have been reported (Golding et al. 2006). Similarly, knockdown of porcine endogenous retrovirus (PERV) expression was recently reported in transgenic pigs (Dieckhoff et al. 2008). In that study, pig fibroblasts were transfected using a lentiviral vector expressing a corresponding short hairpin RNA (shRNA), and transgenic pigs were produced by SCNT cloning. All seven of the piglets that were born had integrated the transgene. Expression of the shRNA was found in all tissues investigated, and PERV expression

was significantly inhibited when compared with wildtype control animals. These recent developments suggest that this approach may be a highly efficient method to generate GE animals with targeted gene knockouts in the future, including GE animals that can knockdown infections caused by important contagious RNA viruses such as foot and mouth disease, classic swine fever, and fowl plague (Clark and Whitelaw 2003).

Modification of Rumen Microorganisms

Although not "animal" biotechnology per se, genetic manipulation of rumen microorganisms has enormous potential to reduce the environmental footprint of ruminant livestock agriculture, as well as enhance product quality (Edwards et al. 2008).

Recombinant Bovine Somatotropin

Perhaps no other animal biotechnology has stimulated more vigorous public debate than the use of recombinant bovine somatotropin (rBST) derived from GE bacteria. This protein, which results in increased milk production when administered to lactating cows, is widely used in the U.S. dairy industry. Administering the protein rBST does not modify the DNA of the cow, nor does the cow become GE. The use of rBST can increase milk production by as much as 30 percent in well-managed herds. Currently banned in Europe, the administration of rBST to dairy cows was approved by the U.S. FDA in 1993 after extensive testing by numerous medical associations and scientific societies revealed no health or safety concerns for consumers (Bauman 1999). Since then, there have been a number of negative campaigns targeting this product, which have resulted in the development of a value-added market for rBST-free milk. At least one paper has examined the environmental impact of rBST use in dairy production (Capper et al. 2008). Not surprisingly, the use of rBST not only markedly improved the efficiency of milk production but also mitigated the environmental impacts associated with the production of a gallon of milk (decreased eutrophication, acidification, greenhouse gas emissions, and fossil fuel use). This example emphasizes the need to weigh decisions to restrict producer access to high-yield technologies or genetic resources that improve productive efficiency against the potential negative impact such decisions may have on achieving environmental sustainability goals. Pretty (2008, 451) captured this idea succinctly when he wrote, "The idea of agricultural sustainability, though, does not mean ruling out any technologies or practices on ideological grounds. If a technology works to improve productivity for farmers and does not cause undue harm to the environment, then it is likely to have some sustainability benefits."

Conclusion

The demand for meat and dairy products is expected to rise rapidly with economic growth in the developing world. It is likely that this growth will increasingly occur in intensive systems where animals are fed with cereals and oils, rather than by using forage and byproducts that cannot be consumed by humans. Intensive systems have some sustainability benefits in that they minimize the resources required to produce a unit of animal product, but often have high external costs on the environment and may give rise to some animal health and well-being concerns. When the external costs of an agricultural system are high and can be reduced by the adoption of new practices and technologies, adoption of intensive systems is a move toward sustainability. A variety of animal biotechnologies offer sustainability benefits. Some are technologies that help animal breeders select the best genotypes to minimize the environmental footprint of animal agriculture, whereas others offer clear animal health and well-being benefits. These biotechnologies may allow intensive animal agricultural systems to proceed in a more sustainable direction. Given the projected demand for animal products in the future, serious consideration must be given to all technologies that can move animal agriculture toward production systems that integrate a sustainable balance of environmental, animal well-being, social, and economic goals.

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