The future of genetic alteration in food animal production

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Abstract

For centuries, animal breeders have intentionally selected the parents of the next generation based on their contended 'ideal' animal. The dramatic variation evidenced by the appearance and productivity of different breeds demonstrates the power of selective breeding. There are 4 variables that can be altered in breeding programs: 1) the accuracy of selection, 2) selection intensity, 3) the amount of genetic variation that is available among the selection candidates, and 4) the average age of the parents when their offspring are born. Any approach or technology that can improve 1 of these 4 components of the so-called "breeder's equation" can accelerate the rate of genetic gain. Animal breeders have routinely used both assisted reproductive technologies (e.g. artificial insemination) and advanced breeding methods (e.g. genomic selection) concurrently to accelerate genetic gains. Molecular methods to introduce useful genetic variation such as genetic engineering (GE) have met with regulatory obstacles and delay, and activist opposition. In 2017 the FDA issued a draft guidance proposing the regulation of all "intentional" genomic alterations as new animal drugs. There is a real possibility that this will preclude the development of beneficial GE and gene edited applications to the detriment of global food security and agricultural sustainability.

Key words: genome editing, genetic engineering, genomic alteration, genetically modified organism (GMO), animal breeding

Résumé

Depuis des siècles, les éleveurs d'animaux ont intentionnellement sélectionné les parents de la génération subséquente sur la base de leur vision de l'animal idéal. L'existence de variation dramatique dans l'apparence et la productivité des différentes races démontre la puissance de l'élevage sélectif. Quatre variables peuvent être modifiées dans un programme d'élevage : 1) la fiabilité de la sélection, 2) l'intensité de la sélection, 3) le degré de variation génétique présent parmi les candidats à la sélection, et 4) l'âge moyen des parents au moment de la naissance des jeunes. Toute approche ou technologie qui permet d'améliorer l'une des quatre variables de l'équation de l'éleveur accélèrera le taux de gain génétique. Les éleveurs d'animaux ont souvent eu recours conjointement aux technologies de reproduction assistée (e.g. l'insémination artificielle) et à des méthodes d'élevage de pointe (e.g. sélection génomique) pour accroître le gain génétique. Les méthodes moléculaires qui introduisent de la variation génétique utile tel que le génie génétique ont fait face à des obstacles réglementaires, à des délais et de l'opposition militante. En 2017, la FDA a fourni une ébauche de ligne directrice envisageant la règlementation de toute modification génomique intentionnelle comme une nouvelle drogue. Il est bien possible que cela empêche le développement de percées en génie génétique et d'applications reliées à la manipulation des gènes au détriment de la sécurité alimentaire globale et de la durabilité de l'agriculture.

Introduction

Animal breeders have been genetically altering farm animals for centuries. At first, genetic improvement programs simply involved selecting those animals with the desired appearance or characteristics to be the parents of the next generation. This aspect of animal breeding has not changed; what has changed over time is how breeders identify the desired animals and the addition of assisted reproductive technologies to enable the amplified use of both genetically superior and younger animals with high genetic merit to be parents of the next generation. All of this contributes to an accelerated rate of genetic gain towards the stated or implicit breeding objective.

A breeding objective (BO) defines the 'ideal' animal towards which the breeding program aspires. The BO can be thought of as the overall goal of the breeding program. The role of the animal breeder is to maximize the response to selection. This is defined as the difference between the average performance of the offspring of the selected parents as compared to the average performance of the whole of the parental generation before selection. The rate of genetic change (ΔG) in animal breeding programs is directly proportional to 3 factors 1) the accuracy of selection (how well breeders can identify the best animals), 2) selection intensity (the proportion of animals that are used as the parents of the next generation), 3) the amount of genetic variation that is available in the selection candidates, and is inversely proportional to 4) the generation interval (the average age of the parents when their offspring are born). Any approach or technology that can improve 1 of these 4 components of the so-called "breeder's equation" can accelerate the rate of genetic progress towards the BO.

Breeding Objectives

Breeding objectives traditionally focused on production traits such as milk yield, growth rate, and meat yield. Key social goals such as food safety, food quality, environmental protection, and animal welfare were often not overtly included in historical BO. Irrespective, it must be recognized that from an environmental perspective, food animal genetic improvement over the past 50 years has resulted in dramatic reductions in greenhouse gas (GHG) emissions and global warming potential per unit of animal product due in part to the dilution of maintenance energy (i.e. more of the total feed consumed by productive animals goes towards making animal product, and less towards meeting maintenance energy requirements for survival). Perhaps this is nowhere more evident than in poultry breeding (Table 1).

The body weight of broiler (meat) chickens at 8-weeks of age increased from 1.79 lb to 6.92 lb (0.81 kg to 3.14 kg) between 1957 and 2001, and approximately 80% of this 4-fold increase was due to genetic selection.[22] Increased productivity clearly benefits the economics of production. Animals that can be grown to market weight at a younger age use proportionally less of their total feed intake on maintenance energy. In 1960, the average time needed to produce a broiler chicken in the United States was 72 days. By 1995, this was reduced to 48 days, including an increase in average slaughter weight of 0.88 lb (0.4 kg) as is dramatically illustrated in Figure 1. Concurrently, the feed conversion ratio (lb feed/lb gain) was reduced by 15%. Conventional selection has clearly resulted in dramatic reductions of the inputs required to produce a pound of chicken.

Early breeding programs tended to focus solely on production traits. Growth traits often have a negative genetic correlation with other traits such as reproduction and health. This means that selecting for improved growth can negatively impact reproduction and health. Therefore selection programs need to appropriately weight all of the different traits that are important in the BO. Hazel developed selection index methodology for optimized multiple trait selection.[24] Multiple-trait selection indexes can be developed to optimize profit given a specific BO, with different traits being assigned

Table 1. Proportional changes (%) in greenhouse gas (GHG) emissions and global warming potential (GWP_{100}) per unit of animal product achieved as a result of 20 years (1988-2007) of genetic improvement as calculated by Department for Environment, Food, and Rural Affairs. (Jones 2008)

	CH4	NH3	N_2O	GWP_{100}
Chickens – layers	-30	-36	-29	-25
Chickens – broilers	-20	10	-23	-23
Pigs	-17	-18	-14	-15
Cattle – dairy	-25	-17	-30	-16
Cattle – beef	0	0	0	0
Sheep	-1	0	0	-1

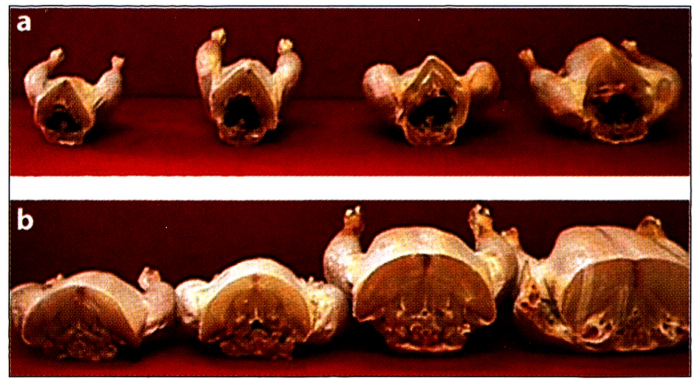

Figure 1. Contemporary comparison of a) 1957 control and b) 2001 selected broiler carcasses slaughtered at different ages (from left; 43, 57, 71, and 85 days.). Photo by G.A. Havenstein. Permission for use of Figure 1 granted from the Annual Review of Ecology Evolution and Systematics, Volume 41:1-19 © 2010 by Annual Reviews www.annualreviews.org.

an economic weight based upon their contribution to profit. This results in an economic index ranking of an animal which is equivalent to the term fitness in wild populations, with the highest ranked individual being the "fittest", or most profitable, according to the BO and therefore a desirable parent for a given production system.

With classical index selection, the BO determines the targeted direction of genetic change for the traits, weighted by their respective market values (MV). This MV is the economic value per unit increment in the trait (e.g. $/lb, $/egg). The breeding goal (H) or aggregate genotype can be represented in the following equation:

$$H = MV_1\ EBV_1 + MV_2\ EBV_2 + \ldots + MV_n\ EBV_n;$$

where EBV_i is the additive genetic value of trait i, and MV_i is the market value (also known as economic value) of trait i, defined by the change in profit of a unit change in the trait i.[24]

Selection for animal health and welfare

It has been argued that genetic improvement of animals has been achieved without adequately considering important animal health and welfare components of sustainability. This was true in early selection programs. As can be seen in the evolution of the selection index for dairy cattle in the United States (Table 2), now called Net Merit ($NM), selection was initially focused only on milk and protein. Over time the index has evolved and selection for cows with lower somatic cell counts (SCC), an indicator trait of mastitis, has been in the index since 1994. Likewise traits associated with improved udder and feed and leg conformation were included in the index in 2000. Today it can be seen that the economic weighting on milk is actually -1, and more than 50% of the weighting is on "functional traits" associated with changes that have taken place in the past decade to include functional traits such as longevity, reproduction and health, rather than

Table 2. History of the main changes in USDA economic selection indexes for dairy cattle and relative emphasis placed on the different traits included in the index.

Traits included in US dairy cattle selection index	USDA genetic-economic index (and year introduced)								
	PD$ (1971)	MFP$ (1976)	CY$ (1984)	NM$ (1994)	NM$ (2000)	NM$ (2003)	NM$ (2006)	NM$ (2010)	NM$ (2014)
Milk	52	27	−2	6	5	0	0	0	**−1**
Fat	48	46	45	25	21	22	23	19	**22**
Protein		27	53	43	36	33	23	16	**20**
Productive life				20	14	11	17	22	**19**
Somatic cell score				−6	−9	−9	−9	−10	**−7**
Udder composite					7	7	6	7	**8**
Feet/legs composite					4	4	3	4	**3**
Body size composite					−4	−3	−4	−6	**−5**
Daughter pregnancy rate						7	9	11	**7**
Cow conception rate									2
Heifer conception rate									1
Calving ability							6	5	**5**

the sole focus on production traits that was seen in the early selection indexes.

Similarly, early broiler breeding programs did not include selection emphasis on feet and leg conformation, resulting in welfare concerns associated with gait. The genetic correlation between body weight and incidence of leg disorders in broilers is positive, so appropriate multi-trait selection indexes have been developed to permit a genetic improvement in leg health concurrently with continued, though more modest, improvement in growth rate. This trait should arguably have been included in BO from the beginning as it ultimately affects the profitability of an enterprise. Where health and welfare traits have declined as a result of selection, it is generally due to their absence in the BO. There are several reasons why they have not been included in BO including low heritability (the proportion of observed variation that can be attributed to inherited genetic factors in contrast to environmental ones), lower MV than production traits, difficulty to obtain suitable selection criteria or indicator traits, and/or negative genetic correlations with production traits.

Including functional traits in the BO reduces the selection pressure (and hence slow genetic progress) in production traits. Production traits typically possess both high heritability and high MV, and so low heritability, low MV functional traits need to be given an inflated emphasis in the selection index to achieve the same rate of genetic progress, or at least to minimize their decline. Animal breeders are always negotiating trade-offs among competing goals, especially when it comes to breeding for sustainability.[15] Typically it has been difficult to obtain records on functional traits, and the old adage that "you can't manage what you do not measure" is particularly true for animal breeders. This raises an important point as it relates to breeding for animal health and welfare. Animal breeding programs that involve complex traits such as robustness, animal well-being, or disease resistance in the selection objectives require well-defined phenotypes (records) upon which to base selection decisions. Identifying a phenotype that can be observed with high repeatability (test-retest reliability) and which can be used as selection criteria to quantify complex functional traits in the BO can be difficult, and ideal traits may be very expensive or impractical to measure. In that regard, an important advance will be development of objective, quantifiable measures of welfare which could be used as selection criteria for breeding decisions.[26]

These types of measures are now being collected in broiler breeding programs. For example, 1 poultry breeding company, Cobb,[a] records 56 individual observations on each pedigree selection candidate in their broiler breeding program, and more than half of the measurements associated with some aspect of health and fitness. These include records on bone and skeletal health as determined by noninvasive surveys of bird joints by x-ray scanners, "anatomical deficiencies such as valgus, varus, rotated tibia, straightness of toes, footpad dermatitis, *Staphylococcus* species infection, and red hocks".[28] Birds with defects are removed from populations. Later, families with an increased incidence of any of these problems are identified and removed from the program. Each pedigree candidate's gait is measured for motor ability, and cardiovascular measurements are collected via blood oximeter machines and physical examination for skin color

which could indicate compromised oxygen circulation and thus is used to identify individuals and families susceptible to ascities and sudden death syndrome. As a result, although current broiler breeding programs are improving the efficiency of meat production in the broiler industry by 2 to 3% per year, livability (survival expectancy) of broilers is also improving 0.22% per year, and condemnation rates have decreased 0.7% per year. In recent years, genetic selection has also had a major impact on decreasing the incidence of skeletal disorders in broiler chickens.[50]

It is important to understand that animal welfare concerns are not solely associated with the BO of agricultural breeding programs, or even sophisticated breeding methods like genomic selection or genetic engineering. For example, every 1 of the 50 most popular pedigreed dog breeds has at least one aspect of its physical conformation that predisposes it to a genetic disorder resulting from conventional selection programs.[1] The authors of this study on genetic ailments in dogs cautioned that "the association of some of these conditions with official breed standards...make conformational extremes an area which needs to be addressed to safeguard the welfare of pedigreed dogs in the future."

Maximizing the Response to Selection

Any method or technology that can impact 1 of the 4 components of the breeder's equations to increase the rate of genetic gain towards a given BO will be of potential value to animal breeders. Increasing the intensity of selection can be achieved using a variety of approaches such as artificial insemination to maximize the use of superior breeding stock. The accuracy of selection can be increased though progeny testing programs, or by using information from genetic markers and genomic selection. Breeders can increase the amount of genetic variability that exists in the prospective parental population by bringing in new traits or breeds. The generation interval can be decreased by selecting animals at a younger age or through the use of assisted reproductive technologies. Figure 2 illustrates the ultimate goal of different breeding programs, and it is likely that different breeding

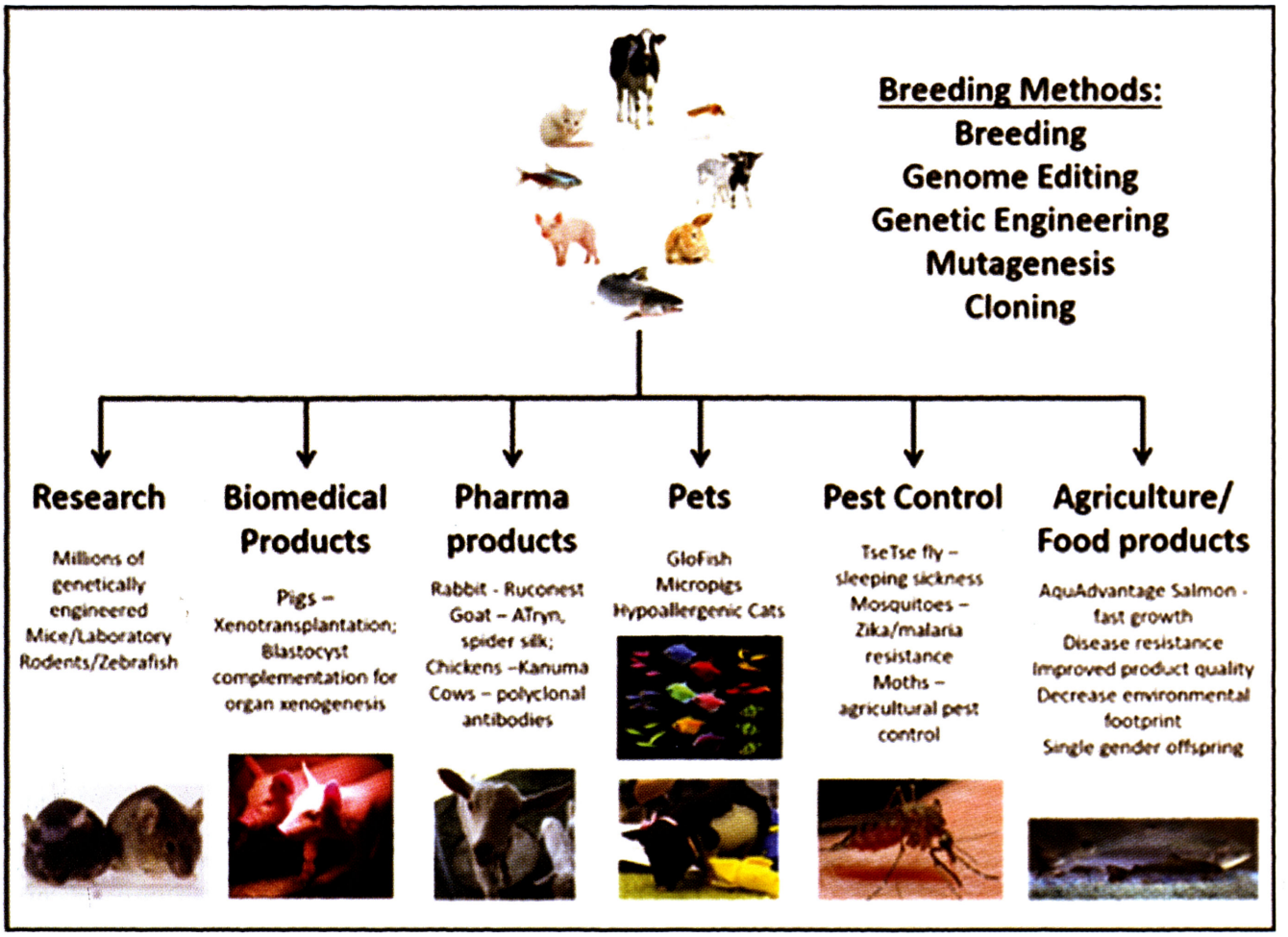

Figure 2. The 6 main animal use categories for which animal breeding programs exist. Different breeding methods will be employed depending upon the breeding objective being targeted.

methods will be used to select for the very different breeding objectives that are associated with these 6 overarching animal use categories.

Modern Biotechnologies

One approach to increase the amount of genetic variation that is available in the selection candidates is to introduce new variability using genetic engineering (GE) and genome editing. Genetic engineering refers to the process of introducing recombinant DNA (rDNA) into the genome of an animal such that the rDNA modification is stably transmitted to their offspring in a Mendelian fashion. Traditional animal breeding methods are typically used for the propagation of the rDNA or transgene once the founder animal has been produced. The use of GE is most appealing when the allele substitution effect is very large, resulting in a profound change in phenotype that would be difficult, if not impossible, to achieve using traditional breeding approaches (e.g. expressing a protein that confers a trait like resistance to a specific disease) if the trait is not found in that species.

Genetic engineering has been used to produce animals for all 6 of the animal use categories outlined in Figure 2. There are literally millions of genetically modified laboratory animals that have been developed for research purposes.[52,53] There are catalogs of GE mice and zebrafish varieties available to researchers. Other groups are working on biomedical products such as pigs that do not express the major antigenic proteins that result in organ rejection when xenotransplanted into human patients,[38] and pigs with a tissue-lineage specific protein knockout to allow for the xenogeneic organ production from a human patient's own cells using blastocyst complementation.[36]

There are also GE animals that have been developed to produce transgenic proteins in blood, urine, semen, salivary gland, egg white or milk that can be collected, purified and used as pharmaceutical, or biopharming products[3] These include 3 approved human pharmaceutical products: goats producing the anticoagulant ATryn® (human antithrombin-III),[12,29] rabbits producing Ruconest™ (Rhucin® outside the EU) for the management of hereditary angioedema,[57] and chickens producing Kanuma™ (sebelipase alfa) in their eggs for long-term enzyme replacement therapy in patients with lyposomal acid lipase deficiency.[45] Cows that produce polyclonal human antibodies,[33,35] and a number of GE animals that produce monoclonal human antibodies have also been developed. The monoclonal antibody market is the fastest growing segment of the pharmaceutical industry. Goats that made spider silk (BioSteel™) in their milk were developed by the now-defunct Genzyme.[b] In response to instructions from the Ministry of Defense, goats were developed that made butyrylcholinesterase,[c] a highly active enzyme that efficiently protects against organophosphate poisons[7] but unfortunately, expression of butyrylcholinesterase significantly reduced their productivity.[2]

In addition to pharmaceutical applications, other non-food GE animals have also been developed. These GE pets include fluorescent aquarium fish (Glofish)[17] that are available in all 50 US states and micropigs that have been developed in China.[9] There are also a number of GE insects being developed for disease control, and some work is being done to use GE and gene drives to control unwanted feral populations such as mosquitoes[46] and the diamond back moth.[21] There are also less well-documented proposals to engineer white-footed mice to be immune to the bacteria that cause Lyme and other tick-borne diseases, eliminate mosquitoes in Hawaii to save an endangered endemic bird, the honeycreeper, from avian malaria,[20] efforts to produce a "daughterless house mouse" on the Farallon Islands with field tests aimed for 2020,[23] and proposals to exterminate mice on New Zealand.[51] Ironically, many of these proposals that are focused on unwanted invasive species are also in areas with some of the most vocal opposition to the use of genetic engineering in agricultural production systems.

Genetically Engineered Food Animals

The first GE food animals were developed in 1985. Since that time, public sector researchers throughout the world have developed a number of GE animal applications. Not surprisingly, breeders tended to focus on trying to develop GE solutions for traits that are important to the BO. As such, the focus tends to be on production and functional traits such as longevity, reproduction, and health. Additionally, some applications have targeted food quality traits such as milk composition and reducing the environmental impact of animals through improved feed efficiency and reduced phosphorus excretion.

Despite the promise of GE food animals to address some breeding goals including animal health (e.g. mastitis resistance[58]), environmental protection (75% decrease in phosphates in the feces of transgenic pigs[16]), and improved animal welfare (e.g. piglet survival[62]), only a single GE animal, the fast-growing AquAdvantage Atlantic salmon, has been approved for food purposes. The founder of this GE fish line was generated in 1989,[11] and the product underwent a lengthy and unpredictable regulatory evaluation.[56]

Although it was approved in December, 2015 by the United States Food and Drug Administration (FDA), its future remains uncertain. Commercial sale of the fish for food is currently blocked by a pending federal bill introduced to the United States House of Representatives on March 4, 2016 by lawmakers from the state of Alaska. The budget rider requires the FDA to develop mandatory labeling of the product before fillets can be imported into the US, and an additional review of "the study of genetically modified salmon's impact on wild salmon stocks carried out by the FDA" by an independent scientific organization". Although the fate of the salmon remains uncertain in the United States, Health Canada, which has a regulatory evaluation process triggered by the novelty of the

product rather than a breeding method used to produce the product, gave the final approval for the AquAdvantage salmon to be produced, sold, and consumed in Canada in 2016.

Genome editing and animal breeding

In recent years, new tools have been introduced that allow for more targeted genetic changes. Genome or gene editing refers to the use of site-directed nucleases (SDN) to precisely introduce a double stranded break (DSB) at a predetermined location in the genome. The cell can repair that DSB break in 1 of 2 ways: homologous recombination (HR) using a nucleic acid template that includes the sequences homologous to either side of the double-strand break, or non-homologous end joining (NHEJ)-mediated repair can produce variable-length insertion and deletion mutations at the site of the DSB. The outcomes of these repair processes result in precision gene edits or random mutations called indels (for insertions/deletions), respectively (Figure 3).

Genome editing technologies enable breeders to efficiently turn off a gene through NHEJ or introduce specific allelic variants,[4,30] and conceptually entire genes or transgenes[31,32,65] as dictated by the HR template nucleic acid sequence, that they would like in their target population using SDN. Genome editing provides an alternative approach to bring in desired genetic variation rather than employing "artificial" selection, cross breeding, and/or genetic engineering using random integration. The outcome of gene editing can range from the addition of useful genetic variation from an entirely different species (i.e. transgenesis), to targeted gene editing of the endogenous genome. The latter enables precise changes to be made at a specific location in the genome (e.g. creating a gene knock-out) without any other changes to the genome of an animal (i.e., without selection markers, or even the genome-wide changes caused by crossbreeding). This approach can also be used to replace a target allele present in 1 population or breed with a preferred allele with known effect from another population in the same (intraspecies allele substitution), or different (interspecies allele substitution), species.

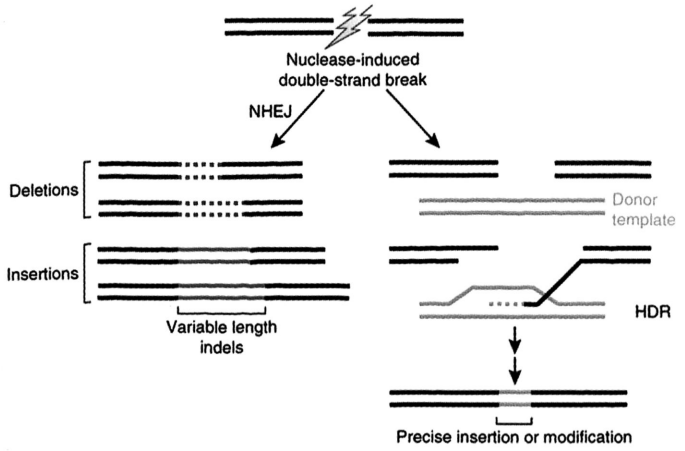

Figure 3. Nuclease induced double-strand breaks (DSBs) can be repaired by NHEJ or HDR pathways. Imprecise NHEJ-mediated repair can produce variable-length insertion and deletion mutations at the site of the DSB. HDR-mediated repair can introduce precise point mutations or insertions from a single-stranded or double-stranded DNA donor template. Reproduced with permission from Sander and Joung, 2014.

Gene editing has many potential applications for all 6 of the animal use categories outlined in Figure 2. It will undoubtedly be used in research, perhaps even more widely than GE animals were used given its ability to make precise deletions and allele substitutions. It will facilitate the development of knockouts in large animals where previous efforts were frustrated by the lack of embryonic stem cells for homologous gene targeting. This will necessitate the employment of somatic cell nuclear cloning to produce animals from targeted knockouts that have been achieved in cell culture, although it should be noted that cloning is already being used in the elite seedstock sector of conventional selection programs. Gene editing could also be used to repair the multitude of genetic defects associated with inbreeding in pedigreed pet populations, or to prevent diseases that have a genetic susceptibility by altering the allele to a resistant form. Perhaps the most powerful application could be its use to substitute a less desirable allele of a gene to a more desirable allele without the need to outcross with an animal that happens to carry the desirable allele, but is genetically inferior in terms of its polygenic inheritance toward the targeted BO.

Both GE and genome editing are breeding methods that have the potential to help animal breeders achieve genetic progress towards the BO. Some goals of food animal breeding programs (e.g. disease resistance) would seem to align with multiple sustainability metrics, such as improving animal health and 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% of turnover in developing countries and 17% in the developed world.[44] Improving animal health using genetics has the added benefit of reducing the need for veterinary interventions and the use of antibiotics and other medicinal treatments to treat sick animals. Efforts are underway to generate trypanosome resistance in cattle which is a major problem for beef and dairy population in East Africa.[64] These methods could also provide a humane method for sex selection in dairy and egg industries, where females provide the animal product (i.e. milk and eggs). Gene supplementation that feminizes male embryos[47] or eliminates the production of male sperm in sires[25] is technically feasible; the latter approach has the desirable outcome that the animals that are produced are not themselves GE.[14] This change to sex-biased or sex-specific production of offspring would have the additional advantage of increasing overall efficiency of the production system.[26] There are also efforts underway to target animal welfare concerns directly, such as the intraspecies allele substitution of the polled loci to genetically

dehorn dairy cattle breeds. Whether breeders will be able to use these tools in their animal breeding programs is very much dependent on the national and international regulatory governance that is put in place for these breeding tools.

Regulation

Regulatory systems provide 1 way for society to find a balance among the potential benefits, risks, and concerns associated with new technologies. The United States "Coordinated Framework for the Regulation of Biotechnology," promulgated in the 1980s, is technically agnostic towards the technology or process under review. According to the Office of Science and Technology Policy (OSTP), "Exercise of oversight in the scope of discretion afforded by statute should be based on the risk posed by the introduction and should not turn on the fact that an organism has been modified by a particular process or technique ... (O)versight will be exercised only where the risk posed by the introduction is unreasonable, that is, when the value of the reduction in risk obtained by additional oversight is greater than the cost thereby imposed".[13] This suggests that the US only exercises regulatory authority over organisms — plant or animal — based on the risks they pose. This is irrespective of the breeding technique used to produce them, and used only when the risk posed is unreasonable, which is clarified to mean the cost of regulatory oversight is not greater than the reduction in risk obtained by that oversight.

In practice, this is not actually what happens. The trigger for the US Food and Drug Administration (FDA) regulation of GE animals based on the 2009 #187 Guidance for Industry entitled, "Regulation of Genetically Engineered Animals" was those animals modified by recombinant DNA (rDNA) techniques, including the entire lineage of animals that contain the modification. All GE animals are captured under these provisions, regardless of their intended use. Thus, although the regulatory evaluation is based on the product (the characteristics and novel phenotype of the GE animal), the method used to produce the genetic change (i.e. rDNA versus other breeding methods) that results in the product is the trigger for regulatory oversight.

In January 2017, the FDA revised the #187 Guidance for Industry to one entitled, "Regulation of Intentionally Altered Genomic DNA in Animals".[54] This 2017 guidance for producers and developers of genetically improved animals and their products defines all intentional DNA alterations in animals as "drugs". No longer is it the presence of an rDNA construct in the genome of a GE animal that is considered to be a drug, as was the case when the guidance was written for GE animals in 2009, but rather it is proposed that the presence of ANY "intentionally altered genomic DNA" in the animal should trigger a new animal drug application.

The guidance states that "intentionally altered genomic DNA may result from random or targeted DNA sequence changes including nucleotide insertions, substitutions, or deletions"; however, it clarifies selective breeding or other assisted reproductive technologies, including random mutagenesis followed by phenotypic selection, are not included as triggers. This suggests that if a breeder intended for a change to happen such as the myostatin knockout Nellore cattle produced at Texas A&M using gene editing,[41] then the alteration would be regulated. However, if random mutations happened in nature or due to mutagenesis breeding – such as all of the other myostatin mutations that are present in cattle breeds like the double-muscled Belgian Blue, then those alterations would not trigger new animal drug regulatory oversight.

The new draft guidance then goes on to state "a specific DNA alteration is an article that meets the definition of a new animal drug at each site in the genome where the alteration (insertion, substitution or deletion) occurs. The specific alteration sequence and the site at which the alteration is located can affect both the health of the animals in the lineage and the level and control of expression of the altered sequence, which influences its effectiveness in that lineage. Therefore, in general, each specific genomic alteration is considered to be a separate new animal drug subject to new animal drug approval requirements." What this effectively means is that FDA is interpreting every intentionally-induced SNP or alteration to be a separate new animal drug, but not the exact same SNP(s) and alterations resulting from de novo mutations.

Such a precautionary approach based on the fact the genomic alterations were introduced intentionally, rather than randomly, seems to have little to do with the risk of the resulting product. If regulatory oversight should be exercised only when the risk posed by the introduction of a new variety is unreasonable as stated by the OSTP, there does not seem to be clear rationale for regulating varieties exhibiting the exact same genetic trait and DNA sequence produced using classical breeding techniques differently from those exhibiting the same trait produced using molecular techniques. Process-based regulatory oversight would seem to be justified if there is something inherently risky about the process that results in unreasonable risks in the resulting product.

For example, the polled (hornless) Holstein dairy cow was produced by an intraspecies allele substitution and carries the exact same DNA sequence at the polled locus as exists naturally in other cattle breeds (e.g., Angus). It is therefore unclear why gene edited polled animals should be subjected to regulatory review when an animal with exactly the same genotype and phenotype produced using crossbreeding and gene introgression would be subject to none.[6] Likewise, it is difficult to envision how the food safety and environmental risks posed by the polled trait in the Holstein breed are different to those posed by the polled trait in the Angus breed.

The proposed draft guidance "Regulation of Intentionally Altered Genomic DNA in Animals" as currently written is neither risk-triggered nor product-driven. It has the potential to overregulate products that have proven track records of safety (e.g. polled cattle) based on the breeding methods used to obtain those products. The costs associated with regulatory

Table 3. Examples of genetically engineered (GE) food animals that have been produced for agricultural applications. Reproduced with permission from Van Eenennaam, 2017.[55]

Species	Transgene	Origin	Trait/Goal
Cattle	Lysozyme, Lactoferrin	Human	Milk composition; animal health; mastitis resistance
	Prion protein (PrP) shRNA	Knockout	Animal health
	α-,κ-Casein	Bovine	Milk composition
	Omega-3 (Fat-1)	Nematode	Milk composition
	β-Casein miRNA	Cattle	Milk composition
	Lysostaphin	Bacterial	Mastitis resistance
	SP110	Murine	Bovine tuberculosis resistance
	Myostatin shRNA	Knockout	Increased muscle yield
Chicken	alv6 envelope glycoprotein	Viral	Disease resistance
	short hairpin RNA	Viral	Disease resistance
	LacZ	Bacterial	Animal health
Carp	Growth hormone	Piscine	Growth rate
	Lactorferrin	Human	Disease resistance
Catfish	Cercopin B	Insect	Disease resistance
Goat	Lysozyme	Human-bovine	Animal health
	Stearoyl-CoA desaturase	Rat-bovine	Mastitis resistance
	Lactoferrin	Human	Prophylactic treatment
	Human beta-defensin 3	Human	Milk composition
	Myostatin shRNA	Knockout	Increased muscle yield
	Prion protein (PrP) shRNA	Knockout	Animal health
Pig	Phytase	*E. coli*-mouse	Feed uptake; decreased phosphorus in manure
	Growth hormone, growth hormone releasing factor, insulin-like growth factor-1	Human-porcine	Growth rate
	cSKI	Chicken	Muscle development
	Lysozyme	Human	Piglet survival
	Unsat. fat. acid (FAD2)	Spinach	Meat composition
	Omega-3 (Fat-1)	Nematode	Meat composition
	α-lactalbumin	Bovine	Piglet survival
	Mx, Iga, mouse monoclonal antibody (mAb)	Murine	Disease Influenza resistance
Salmon	Growth hormone	Piscine	Growth rate
	Lysozyme	Piscine	Animal health
	wflAFP-6	Piscine	Cold tolerance
Sheep	Growth hormone, growth hormone releasing factor,	Ovine	Growth rate
	IGF-1, wool intermediate filament keratin, CsK	Ovine, Bacterial	Wool growth
	Visna resistance	Viral	Disease resistance
	Omega-3 (Fat-1)	Nematode	Meat composition
	Prion protein (PrP)	Knockout	Animal health
	Mouse monoclonal antibody	Murine	Disease influenza resistance
Trout	Follistatin	Piscine	Muscle development

compliance will potentially preclude public sector scientists and small companies from being able to use techniques that intentionally alter the genomic DNA of animals, irrespective of the risk posed by such products. And, as has been the case with GE animals, the proposed regulatory approach does not allow consideration of the potential benefits associated with the newly-developed varieties. Consideration of the benefits associated with the novel phenotype associated with the animals carrying intentionally altered DNA would represent a shift away from the current precautionary risk-assessment process that focuses only on potential and frequently hypothetical risks, to one that addresses the probability of whether the potential benefits outweigh any attendant potential risks.

Ideally, the regulatory evaluation of animals carrying intentional genomic alterations would be triggered by unreasonable unique risks associated with the novel trait(s) in that species in relation to known risks associated with existing varieties, rather than the breeding method used to obtain the alteration. And the trigger for a comprehensive food safety evaluation of products derived from animals

Table 4. Examples of successful gene edited agricultural applications in food animal species. KO = knock out or inactivation of gene function. Reproduced and modified with permission from Van Eenennaam, 2017.

Species	Target	Publication(s)	Trait/Goal
Cattle	Intraspecies *POLLED* allele substitution	36,41	No horns
	Myostatin KO	42,43	Increased muscle yield
	Beta-lactoglobulin KO	44	Elimination of milk allergen
	Lysostaphin transgene	38	Disease resistance
	Lysozyme transgene	39	Disease resistance
	SP110 transgene	40	Resistance to tuberculosis
Chicken	*Ovalbumin KO*	45,46	Elimination of ovalbumin in egg
	Immunoglobulin heavy chain locus	47	Germline gene editing
Goat	Beta-lactoglobulin KO	48	Elimination of milk allergen
	FGF5	49	Hair length for cashmere production
	Myostatin	48,49	Increased muscle growth
	Prion protein KO	48	Elimination of prion protein
Pig	CD163 KO	50	PRRS virus resistance
	RELA interspecies allele substitution	37, 51	African Swine Fever resistance
	Myostatin KO	52-54	Increased muscle yield
	vWF	55	Improved bleeding efficiency
Sheep	ASIP	56	Black/white coat color
	BCO2	56	Disease resistance
	Myostatin KO	43,56-58	Increased muscle yield

carrying intentional genomic alterations should be based on the likelihood that the intentional genetic alteration will result in the presence of a completely new protein in the food supply, changes in the macronutrient composition of animals products, increase in the level of a natural toxicant, or presence of a novel allergen.

A comprehensive food safety analysis of animals carrying SNP mutations or intraspecies allele substitutions, such as the polled and myostatin alleles that have been safely consumed for decades, would seem to run counter to the OSTP's stated intent that regulatory oversight should not turn on the fact that an organism has been modified by a particular process or technique, and that it will only be exercised when the risk posed by the introduction is unreasonable, that is, when the value of the reduction in risk obtained by additional oversight is greater than the cost thereby imposed. There is no science-based rationale for regulating animals exhibiting a genetic trait produced using classical breeding techniques differently from those exhibiting the same trait produced using molecular techniques.

Conclusion

Access to modern biotechnological innovations in US animal breeding programs is currently uncertain. Despite the demonstrated and sustained impact of genetic improvement programs on reducing the environmental footprint of animal protein production and improving functional traits such as longevity, reproduction, and health, there are serious questions as to whether animal breeders will be able to use modern molecular methods to achieve these goals. The proposed draft guidance for mandatory premarket regulatory evaluation of animals carrying intentionally altered genomic DNA, irrespective of product novelty or risk, has the potential to disincentivize the development of beneficial GE and gene edited applications. Regulatory evaluations should focus on the risks and benefits posed by any novel traits in animals carrying intentionally altered genomic DNA sequences, irrespective of which breeding method was used to introduce those traits. While animal breeders will continue to make progress uisng whatever technologies and methods are legally available to them, it makes little sense to impede their access to safe breeding methods in the absence of a demonstrated risk. It is the innovation equivalent of tying breeders' hands behind their backs. Slowing down progress in animal breeding programs comes with a very high opportunity cost given the projected global increase in demand for milk, meat, and eggs.

Endnotes

[a]Cobb-Vantress Inc., Siloam Springs, AR
[b]Genzyme, Framingham, MA
[c]Protexia™, PharmAthene Inc. (USA)

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