The Current and Future Uses of Biotechnology in Animal Agriculture

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Abstract. Biotechnologies have been an integral part of improvements in animal genetics, nutrition and health over the past century. Many biotechnologies have become fundamental components of efficient livestock production systems. The genetic improvements that have been enabled by biotechnologies have dramatically decreased the environmental footprint of animal protein production in many parts of the world, and continued innovation is required to address the projected increase in demand for animal products in the future. Breeding programs increasingly utilize a combination of advanced reproductive technologies and genomic tools to accelerate the rate of genetic gain by manipulating components of the breeder's equation. The use of these biotechnologies and breeding methods has met with little public opposition. In contrast, the use of modern biotechnologies, defined as those that employ the use of *in vitro* nucleic acid techniques, have been highly controversial, especially those involving the use of genetic engineering. This modern biotechnology distinction is somewhat arbitrary as there are a number of biotechnologies that involve the use of *in vitro* processes, and many result in genetic modifications that are indistinguishable from the naturally-occurring variation that is the driver of both traditional breeding programs and evolution. A number of useful traits including disease resistance and animal welfare traits have been successfully introduced into various livestock species using both genetic engineering and gene editing techniques. Ultimately these techniques complement the genetic improvement that can be accomplished using traditional selection techniques and, if judged acceptable, offer an opportunity to synergistically accelerate genetic improvement in food animal species.

Key words: Biotechnology, gene editing, genetic engineering

Los Usos Actuales y Futuros de la Biotecnología en la Agricultura Pecuaria

Resumen. Las biotecnologías han sido una parte integral de las mejoras en la genética animal, nutrición y sanidad a lo largo del siglo pasado. Muchas biotecnologías se han vuelto componentes fundamentales de los sistemas de producción pecuaria eficientes. Las mejoras genéticas que han sido habilitadas por las biotecnologías han disminuido dramáticamente la huella ambiental de la producción de proteína animal en muchas partes del mundo, e innovaciones continuas son necesarias para cumplir con el aumento proyectado en la demanda para los productos animales en el futuro. Los programas de reproducción cada vez más usan una combinación de tecnologías reproductivas avanzadas y las herramientas genómicas para acelerar la tasa de ganancia genética al manipular los componentes de la ecuación del reproductor. El uso de estas biotecnologías y métodos de crianza se ha encontrado con poca oposición pública. En contraste, el uso de tecnologías modernas, definidas como aquellas que usan técnicas de ácidos nucleicos *in vitro* ha sido altamente controversial, especialmente aquellas que involucran el uso de ingeniería genética. Esta distinción de biotecnología moderna es algo arbitraria ya que hay un número de biotecnologías que involucran el uso de procesos *in vitro*, y muchos resultan en modificaciones genéticas que no se pueden distinguir de la variación que ocurre naturalmente y que es el impulsor de programas de crianza y la evolución. Un número de características útiles incluyendo la resistencia a enfermedades y las características de bienestar animal han sido introducidas con éxito en varias especies de animales pecuarios usando la ingeniería genética y técnicas de edición genética. Por último, estas técnicas complementan las mejoras genéticas que pueden lograrse usando técnicas de selección tradicional y si es aceptable, ofrecen una oportunidad para acelerar el mejoramiento genético con sinergia en especies animales.

Palabras clave: Biotecnología, edición de genes, ingeniería genética.

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Introducción

Biotechnology is defined in the Cartagena protocol as "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use." From this definition it is clear that some applications of biotechnology have been used in animal agriculture for many years. Biotechnologies have directly benefitted the three core scientific disciplines of animal science - genetics, nutrition, and health, as summarized in Table 1 (FAO 2010).

Table 1. Biotechnologies used in animal production (adapted from FAO 2010).

Genetics/breeding	Nutrition	Health
Artificial insemination	Single cell proteins	Molecular diagnostics
Progesterone monitoring	Probiotics and Prebiotics	DNA vaccines
Estrus synchronization	Recombinant somatotropins	Marker vaccines
In vitro fertilization and embryo transfer	Solid state fermentation of lignocellulosics	Virus-vectored vaccines
Molecular markers; Marker-assisted and genomic selection	Feed additives: Amino acids, enzymes & probiotics	Sterile insect technique (SIT)
Cryopreservation	Ionophores	Bioinformatics
Semen and embryo sexing	Molecular gut microbiology	
Cloning	Silage additives (enzymes and microbial inoculants)	
Genetic Engineering/Transgenesis	Recombinant metabolic modifiers	
Genome Editing		

To impact the rate of genetic change (ΔG) in a breeding program, biotechnologies must impact some component of the breeder's equation:

$$\Delta G = \frac{[(\text{Accuracy of Selection}) \times (\text{Selection Intensity}) \times (\text{Genetic Standard Deviation})]}{\text{Generation Interval}}$$

Artificial insemination (AI) is a biotechnology that greatly increases the selection intensity by enabling the high use of genetically superior sires. AI plays a major role in design of breeding programs and dissemination of advanced genetics. AI technology was introduced into the dairy industry and commercialized in the United States during the late 1930s to early 1940s (Foote 1999). Today, approximately 80% of all dairy cows in the US are bred using AI.

To put the extensive use of AI in the US dairy industry in perspective, a single US bull named Elevation, born in 1965, had over 80,000 daughters, 2.3 million granddaughters, and 6.5 million great-granddaughters (VanRaden 2007). Such extensive use of this single exceptional bull clearly accelerated the rate of genetic gain, but also has the potential to reduce the genetic diversity of the dairy cattle population. About half of the 369% increase in milk production efficiency (Figure 1) is attributable to genetic improvement enabled by AI; the remainder is due to improved management and nutrition.

Although AI is used routinely in animal breeding, it was initially viewed with skepticism. There was a fear that AI would lead to abnormalities, and influential cattle breeders were originally opposed to the concept as they believed it would destroy their bull market (Foote 2002). When independent, university research demonstrated that the AI could be used to provide superior bulls, control venereal disease, and produce healthy calves, subsequent industry adoption was swift.

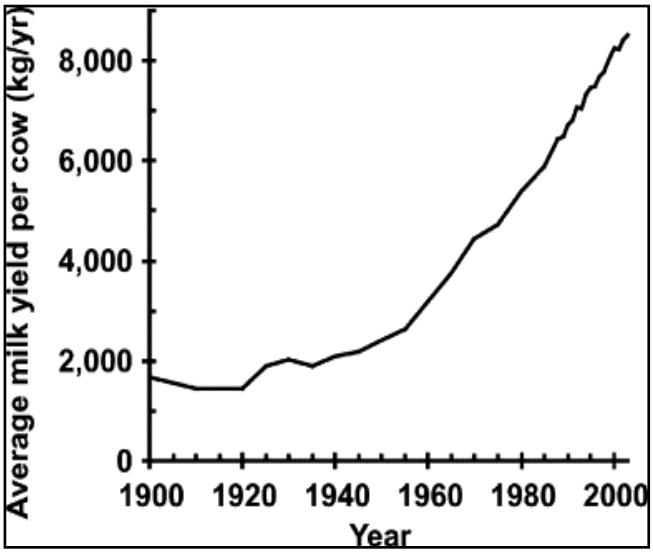

Figure 1. Milk production per cow in the United States over the past 100 years (VandeHaar and St-Pierre 2006).

It has been observed that despite intense selection on specific traits (e.g. 8-week body weight in broilers or milk yield in dairy cattle), the selection response per generation for these traits shows no sign of decreasing. Mean milk yield in the U.S. has increased at a rate of 1% per generation for decades. It is likely that this sustained response is fueled by new mutations that arise each generation (Hill and Kirkpatrick 2010).

From an environmental perspective, genetic improvement over the past 50 years has also resulted in reductions in greenhouse gas (GHG) emissions and global warming potential per ton of animal product (Table 2; Jones *et al*. 2008). Capper *et al.* (2009) reported that although the carbon footprint per individual cow increased when comparing 1944 to 2007 due to increases in the milk production per cow, the carbon footprint per unit of milk in 2007 was 63% lower than in 1944. Progress in decreasing the environmental footprint per unit of ruminant meat production has been noticeably less than in other animal-source proteins.

Industries that have less vertical integration (e.g. beef and sheep) have generally made slower genetic progress. Animal breeding in these industries tends to be driven by breed associations, and because the traits differ among industry sectors (e.g. breeder, farmer, feeder, processor); it is difficult to develop a single, industry-wide breeding objective that is economically rational for all sectors. This leads to an important concept in animal breeding, the role of the decision maker (Olesen *et al.* 2000). In the absence of vertical integration, breeding goals will be developed based on the producers' financial interests. The producer is the one investing in breeding stock and in a competitive market their decision will be based on the ways they perceive that animals contribute to farm profit.

Table 2. 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 (Jones *et al*. 2008).

Livestock Industry	CH_4	NH_3	N_20	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

Recent developments in animal breeding combine several biotechnologies together to impact multiple components of the breeder's equation (Figure 2). Advanced reproductive technologies that reduce the generation interval and increase the intensity of selection are being combined with genomic selection to increase the accuracy of selection in cell lines that are then cloned and transferred using embryo transfer to produce high genetic merit calves. This approach results in a substantial reduction in the generation interval by producing animals with the desired genetics in a one year timeframe.

A subset of biotechnologies are termed modern biotechnologies, and are defined in the Cartagena protocol as the application of a) *in vitro* nucleic acid techniques, including recombinant deoxyribonucleic acid (DNA) and direct injection of nucleic acid into cells or organelles, or b) fusion of cells beyond the taxonomic family, that overcome natural physiological reproductive or recombination barriers and that are not techniques used in traditional breeding and selection. This distinction between traditional and modern breeding methods is somewhat arbitrary as many of the techniques (Figure 2) involve the use of *in vitro*

techniques, and some of the recent breeding methods exactly mimic the natural processes of mutation and the end result is indistinguishable from naturally-occurring variation.

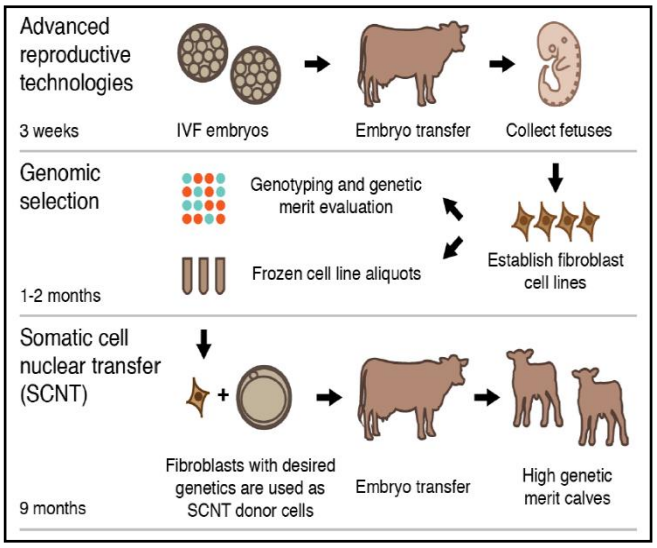

Figure 2. Production of high genetic merit calves using a range of biotechnologies. Image taken from Kasinathan *et al*. (2015).

Some have argued that if genetic modification has "for at least part of the procedure, been handled outside the organism by people" then the resulting organism is a genetically modified organism or GMO (Cotter *et al*. 2015). Using this definition, many animal breeding techniques that are currently a routine part of genetic improvement programs shown in Figure 2 (embryo transfer, ovum pick up, embryo transfer, artificial insemination, *in vitro* fertilization, somatic cell nuclear transfer cloning) would result in a GMO.

Genetic Engineering

Genetic engineering (GE) is a process in which scientists use recombinant DNA (rDNA) technology to introduce desirable traits into an organism. Because the genetic code for all organisms is made up of the same four nucleotide building blocks, this means that a gene encodes the same protein whether it is made in an animal, a plant or a microbe. Recombinant DNA refers to DNA fragments from two or more different sources that have been joined together in a laboratory. The resultant rDNA construct is usually designed to express a protein, or proteins, that are encoded by the gene(s) included in the construct. GE involves producing and introducing the rDNA construct into an organism so that new or changed traits can be given to that organism. A GE animal is an animal that carries a known sequence of rDNA in its cells, and passes that DNA on to its offspring. GE animals are sometimes referred to as living modified organisms, transgenic, GMOs or bioengineered animals. GE animals were first produced in the late 1970s. Forty years later, transgenic animals have been produced in many species, including those traditionally consumed as food, although most have not moved from the laboratory to commercialization (Table 3).

Genetically Engineered Animals for Agriculture

GE fits in as a component of the breeder's equation in that it introduces useful genetic variation into breeding programs. The real power of this technology is in bringing in genetic variation not available in the target species, especially for traits like disease resistance. DNA from viral and bacterial species pathogens can conceptually be used to permanently genetically-immunize species against microbial pathogens. A number of different GE animals have been produced by researchers globally, and those specifically targeting traits of agricultural importance (Table 3). To date, only one application has been approved for food purposes, the fast-growing AquAdvantage® Atlantic salmon. This fish was approved in 2015 for commercialization under specific production conditions by the US Food and Drug Administration after a prolonged regulatory evaluation. As of March 2016, its future was still uncertain due to the introduction of a legislative bill to require a third-party review of the FDA's decision to pronounce AquAdvantage® salmon safe for human consumption.

Many of the goals listed in Table 3 are common traits included in the breeding objectives of livestock genetic improvement programs. Breeders could conceptually use GE alongside conventional breeding methods to facilitate genetic improvement. To date, the expense of the regulatory process has precluded the commercialization of GE animals for food purposes. There have been some GE animals approved for biomedical pharmaceutical production including goats, rabbits and chickens and also some trials using GE insects for pest control applications.

Table 3. Examples of transgenic animals for agricultural applications. The only product to obtain regulatory approval is the AquAdvantage® fast-growing salmon (bold). Adapted from Lievens *et al.* 2015.

Species	Transgene	Origin	Effect/Goal
Cattle	Lysozyme	Human	Milk composition
	PrP	Knockout	Animal health
	α−,κ-Casein	Bovine	Milk composition
	Omega-3	Nematode	Milk composition
	Lysostaphin	Bacterial	Mastitis resistance
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
	Monosaturated fatty acid	Rat-Bovine	Mastitis resistance
	Lactoferrin	Human	Prophylactic treatment
	Human beta-defensin 3	Human	Milk composition
Pig	Phytase	*E. coli*-Mouse	Feed uptake
	Growth hormone	Human-Porcine	Growth rate
	cSKI	Chicken	Muscle development
	Lysozyme	Human	Piglet survival
	Unsat. fat. acid	Spinach	Meat composition
	Omega-3	Nematode	Meat composition
	α-lactalbumin	Bovine	Piglet survival
	Mx1	Murine	influenza resistance
Salmon	**Growth hormone**	**Piscine**	**Growth rate**
	Lysozyme	Piscine	Animal health
	wflAFP-6	Piscine	Cold tolerance
Sheep	IGF-1	Ovine	Wool growth
	CsK	Bacterial	Wool growth
	Visna resistance	Viral	Disease resistance
	PrP	Knockout	Animal health
Silkworm	eGFP, DsRed, or mKO	Cnidarian	Silk color
	A2S814	Arachnid	Silk strength
Trout	Follistatin	Piscine	Muscle development

Gene Editing

Gene editing is a technique that employs site-directed nucleases (SDN) to precisely edit or change the genetic code. As the name gene editing suggests, these technologies enable researchers to add, delete, or replace letters in the genetic code. In the same way that spell check identifies and corrects single letter errors in a word or grammar errors in a sentence, gene editing can be used to identify and change the letters that make up the genetic code (i.e. DNA) within an individual.

Gene editing has many potential applications. For example, it can be used to correct diseases and

disorders that have a genetic basis. It could also be used to change a less desirable form of a gene (called an allele) to a more desirable allele without the need to introgress (repeatedly backcross) or bring in that allele through outcrossing with an animal that carries the desirable allele. Therefore, gene editing is really more like precision breeding where breeders can introduce the specific sequences that they would like to select for using gene editing technologies.

Gene editing is different from traditional genetic engineering. Continuing with the analogy of a word processor, genetic engineering enables a gene sequence of foreign DNA to be cut and pasted from one species to another; typically the location where the new DNA sequence inserts into the genome is random. Gene editing can add, delete, or replace a series of letters in the genetic code at a very precise location in the genome.

The basic idea behind gene editing is that molecular scissors called site-directed nucleases (SDN) are used to cut DNA at a specific location in the genome based on recognition of the specific, unique target DNA sequence. The cut site is then repaired using the DNA repair mechanisms of the cell. These repairs can be directed to introduce, delete, or replace a series of letters in the genetic code. This essentially enables the introduction of known, desired alleles based on what is understood about naturally-occurring genetic variation in the target species.

Without the addition of template DNA, the double stranded breaks created at a precise location in the genome by the nucleases are repaired by the cell's natural DNA repair mechanism (non-homologous end joining (NHEJ), and this typically results in single nucleotide changes, deletions or small (1-2 nucleotide) insertions at the DNA cut site, sometimes called SDN-1. In this case, although the location of the cut site is very precise, the exact change that occurs when the DNA is repaired is random and so a number of different outcomes representing minor sequence changes are possible (Figure 3).

Supplied with a nucleic acid template, however, the double stranded breaks initiated by the nucleases are repaired via the cell's homologous recombination (HR) repair pathway whereby the template dictates the sequence resulting from the repair, allowing the introduction of the DNA sequence dictated by the template into the host genome. Such changes might range from nucleotide-specific changes, to large (whole gene) insertions or substitutions depending upon the template. The end result of this maybe a targeted SNP edit (e.g. the nucleotide A at a given location in the genome is deliberately replaced by T), the replacement of one naturally occurring allele with another naturally occurring allele at target genetic gene locus within a species, or the introduction of a novel DNA sequence as encoded by the template at the target location in the genome, sometimes called SDN-3. There are many potential uses of this technology ranging from human medicine to plant and animal breeding.

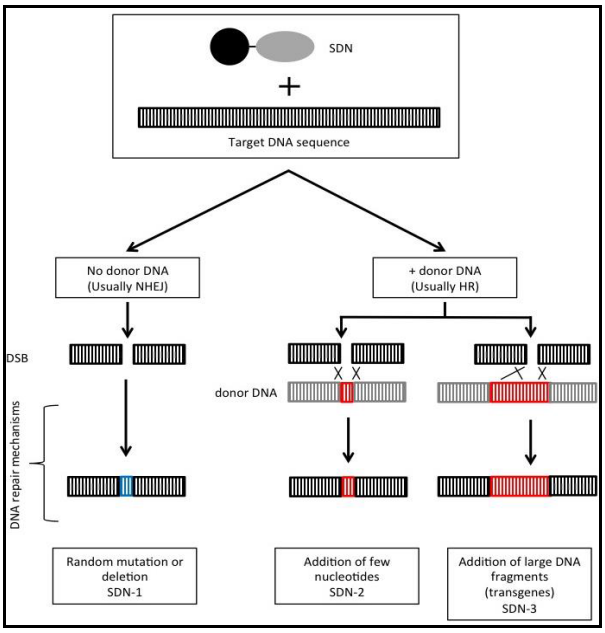

Figure 3. Schematic representation of intended modification by site-directed nuclease (SDN) types. Double-stranded break repair can occur via non-homologous end-joining (NHEJ; SDN-1) or homologous recombination (HR) when a donor DNA is present (SDN-2; -3). Figure obtained from http://genok.no/wp-content/uploads/2015/06/250615_Emerging_technologies_final.pdf.

How Might Gene Editing be Used in Animal Breeding?

In the last 5 years, genome editing technologies (zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regulatory interspersed short palindromic repeats

(CRISPRs) associated system) have been used to mediate the generation of more than 300 edited pigs, cattle, sheep and goats (Tan *et al*. 2016). Table 4 lists some of those that were directly targeted to agricultural applications including product yield, animal health and welfare.

Gene editing has been used to produce genetically hornless Holstein dairy cattle by replacing the Holstein horned allele with the naturally-occurring Angus polled allele at the gene that is responsible for horn development (Tan *et al.* 2013), and to generate pigs with edits in the haplotype of a gene that may confer resilience to African Swine Fever Virus (Lillico *et al.* 2016). Another group of gene edited pigs are protected from porcine respiratory and reproductive syndrome (PRRS) virus, a particularly devastating disease of the global pork industry (Whitworth *et al.* 2016) Gene editing has also been used to introduce changes in the *myostatin* gene in sheep, cattle (Proudfoot *et al*. 2015), and goats (Ni *et al.* 2014). As the Latin origin of the word *myostatin* (muscle/stop) suggests, turning off this gene results in muscle growth. Naturally-occurring mutations in this gene have historically been selected by conventional animal breeders and are the genetic basis for the "double muscled" phenotype that is seen in cattle breeds like the Belgian Blue, and the bully phenotype in whippet dogs.

Gene editing effectively mimics the natural processes that form the basis of selective breeding programs, and for that matter, natural selection. Breeders work with the genetic variation that exists within a species, and that genetic variation ultimately arises from naturally-occurring mutations. Although the word "mutation" sounds negative, it simply refers to variations in DNA sequences. These variations, or mutations, are responsible for virtually all genetic differences that exist between individuals, such as having blue eyes instead of brown.

Although different mammals have many of the same genes, some people do not appreciate that the genetic code that makes up those genes differs among animals of different breeds, and even among animals within the same breed. In fact, with the exception of identical twins, there are literally millions of DNA sequence variations between two individuals of any species. For example, an enormous number of genetic variants have accumulated within cattle since the advent of domestication and selective breeding due to the naturally-occurring processes that lead to a small number of mutations each generation. In one recent analysis of whole-genome sequence data from 234 taurine cattle representing three breeds, more than 28 million variants were observed, including insertions, deletions and single nucleotide variants (Boussaha *et al.* 2015). A small fraction of these mutations are those that have been selected by breeders; most of them are silent and have no impact on traits of importance to breeding programs. Occasionally, such mutations result in a genetic condition such as red or black coat color or an undesirable disease condition such as dwarfism.

Table 4. Examples of successful gene edited agricultural applications in food animal species.

Species	Target	Publication	Effect/Goal
Cattle	Polled	Tan *et al.* 2013	No horns
	Myostatin	Proudfoot *et al.* 2015	Increased muscle growth
Chicken	Ovalbumin	Park *et al.* 2014	Elimination of ovalbumin in egg
Goat	Myostatin Prion protein Beta-lactoglobulin	Ni *et al.* 2014	Increased muscle growth Elimination of prion protein Elimination of milk allergen
Pig	CD163	Whitworth *et al.* 2015	PRRSV Resistance
	RELA	Lillico *et al.* 2016	African Swine Fever Resistance
Sheep	Myostatin	Proudfoot *et al.* 2015	Increased muscle growth

How Might Gene Editing Intersect with Conventional Breeding?

Data coming out of some of the large-scale genomic and sequencing projects are revealing situations in which the sequence of one naturally-occurring allele results in superior performance than observed when animals inherit an alternative allele of that gene. It is envisioned that it might be possible to edit an animal's genome to the superior allele, and to do that at several genomic locations, or for several different genes. The advantage of gene editing over conventional selection to move these naturally-occurring alleles from one animal to another is that favorable alleles rarely all occur in one single individual and editing offers the opportunity to increase the frequency of desirable alleles in an individual or a breed more rapidly than could occur through conventional breeding.

One could potentially envision editing several alleles for different traits – such as disease resistance, polled and to correct a known genetic defect – all while using conventional selection methods to keep making genetic progress towards a selection objective. One study found that combining gene editing with genomic selection could improve the response to selection four fold after 20 generations (Jenko *et al.* 2015).

It should be remembered that complex traits are typically impacted by many different genes. It is not likely that all of the genes impacting such traits are known, nor is it typically evident which might be the desirable molecular edits for these genes (i.e. what is the sequence of the desirable allele). It is likely that editing will be focused on large effect loci and known targets to correct genetic defects or decrease disease susceptibility, and conventional selection will continue to make progress in selecting for all of the many small effect loci that impact the complex traits that contribute to the breeding objective.

Gene editing offers an approach to translate the thousands of SNP markers discovered through livestock sequencing projects, the information obtained from numerous genome wide association studies, and the discovery of causative SNPs (Quantitative Trait Nucleotides; QTNs) into useful genetic variation for use in animal breeding programs (Hickey 2013).

Will Gene Editing be Regulated?

At the current time, it is unclear whether gene editing will be formally regulated as is the case with animals that have been produced using genetic engineering. Animal breeding *per se* is not regulated by the federal government, although it is illegal to sell an unsafe food product regardless of the breeding method that was used to produce it. Gene editing does not necessarily introduce any foreign genetic DNA or transgenic sequences into the genome, and many of the changes produced would not be distinguishable from naturally-occurring alleles and variation. As such, many applications will not fit the classical definition of genetic engineering. For example, many edits are likely to edit alleles of a given gene using a template nucleic acid dictated by the sequence of a naturally-occurring allele from the same species (e.g. the hornless Holstein example described earlier used template sequence based on the polled allele from Angus). As such, there will be no novel DNA sequence present in the genome of the edited animal, and likewise no novel phenotype associated with that sequence. It is not evident what unique risks might be associated with an animal that is carrying such an allele given the exact same sequence and resulting phenotype that would be observed in the breed from which the allele sequence was derived.

It is possible that nucleases might introduce double stranded breaks at locations other than the target locus, and thereby introduce alterations elsewhere in the genome. Such off-target events are analogous to spontaneous mutations and can be minimized by careful design of the gene editing reagents.

Governments and regulators globally are currently deliberating about how or if gene-edited animals should be regulated. It is likely that gene editing will be considered on a case-by-case basis depending upon the novelty of the edited DNA sequence and the resulting attributes or phenotype displayed by the animal. Although gene editing is a very versatile tool, many applications will likely result in animals carrying desirable alleles with sequences that originated in other breeds or individuals from within that species. As such, this process is directly analogous with conventional breeding. There is a need to ensure that the extent of regulatory oversight is proportional to the

unique risks, if any, associated with the novel phenotypes. This question is of course important from the point of view of technology development, innovation and international trade.

Conclusion

Biotechnology is a broad term that encompasses many technologies that are used in animal agriculture. Emerging biotechnologies offer great potential, especially in the area of animal breeding. While regulation to ensure the safety of new technologies is necessary, in a world facing burgeoning demands on animal agriculture from population and economic growth, unaccountable delay of safe, effective technologies is a luxury that food security can ill afford.

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