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PROCEEDINGS PAPER

Application of genome editing in farm animals: cattle

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Abstract Milk and meat from cattle and buffaloes contribute 45% of the global animal protein supply, followed by chickens (31%), and pigs (20%). In 2016, the global cattle population of 1.0 billion head produced 6.5 billion tons of cows' milk, and 66 million tons of beef. In the past century, cattle breeding programs have greatly increased the yield per animal with a resultant decrease in the emissions intensity per unit of milk or beef, but this has not been true in all regions. Genome editing research in cattle to date has focused on disease resistance (e.g. tuberculosis), production (e.g. myostatin knockout; production of all-male offspring), elimination of allergens (e.g. beta-lactoglobulin knockout) and welfare (e.g. polled or hornlessness) traits. Modeling has revealed how the use of genome editing to introduce beneficial alleles into cattle breeds could maintain or even accelerate the rate of genetic gain accomplished by conventional breeding programs, and is a superior approach to the lengthy process of introgressing those same alleles from distant breeds. Genome editing could be used to precisely introduce useful alleles (e.g. heat tolerance, disease resistance) and haplotypes into native locally-adapted cattle breeds, thereby helping to improve their productivity. As with earlier genetic engineering approaches, whether breeders will be able to employ genome editing in cattle genetic improvement programs will very much depend upon global decisions around the regulatory framework and governance of genome editing for food animals.

Introduction

Animal products, namely milk, meat and eggs, provide approximately 13% of the energy and 28% of the protein consumed globally. In developed countries, these numbers increase to 20% and 48%, respectively (FAO 2009). Milk and meat from cattle and buffaloes contribute 45% of the global animal protein supply, followed by chickens (31%), and pigs (20%) (Mottet et al. 2017). Despite impressive advances in animal protein production over the past 50 years, projections suggest demand for pork could increase by up to 43% and demand for beef by as much as 66% to feed the predicted global population of 9 billion by 2050

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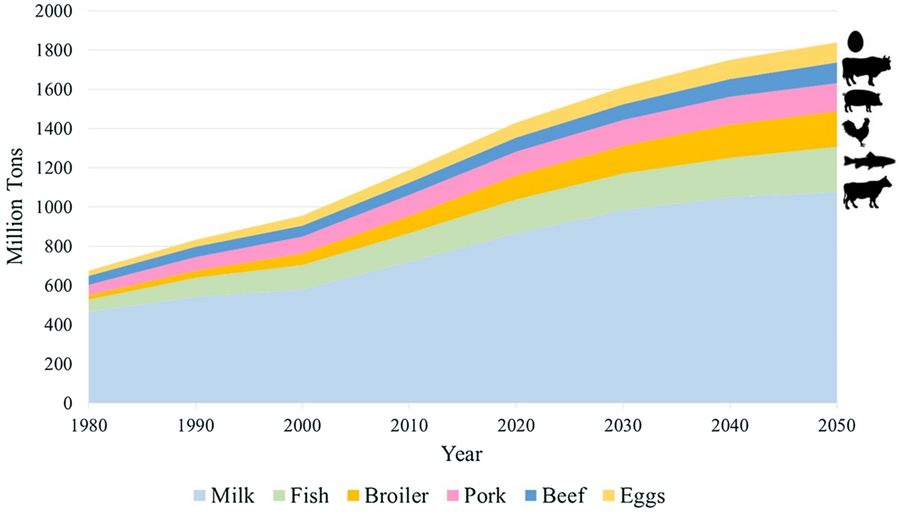
Fig. 1 Egg, beef, pork, chicken, fish and milk production since 1980 and projected to 2050 (FAO 2018; Alexandratos and Bruinsma 2012)



(Fig. 1). The greatest increase is expected for poultry products, with demand for poultry meat increasing by as much as 121% and eggs by 65% (Mottet and Tempio 2017).

In 2016, the global cattle population of 1.0 billion head, including 270 million dairy cows, produced 6.5 billion tons of cows' milk and 66 million tons of beef (FAO 2018). In the past century, cattle breeding programs have greatly increased the yield per animal with a resultant decrease in the emissions intensity per unit of milk or beef, but this has not been true in all regions (Capper and Bauman 2013). Many countries with the lowest production per cow are also those with the most cows (Fig. 2), and therefore the greenhouse gas emissions per unit of milk are highest in these countries (FAO 2010). A similar trend can be seen for beef cattle (Fig. 3), and the selection for improvement in beef yield that has been occurring in the United States since 1980 is evident as total beef production has been rising despite a falling cattle inventory. It is likely that future growth in meat and dairy production will be accomplished through larger herds and higher output per animal (Britt et al. 2018), with global meat production expected to expand by almost 40 million tons (Mt) and world milk production by 178 Mt by 2026 (OECD/FAO 2012). In order to meet increased demands, it will be necessary to accelerate the rate of genetic gain in global breeding programs for both dairy and beef cattle.

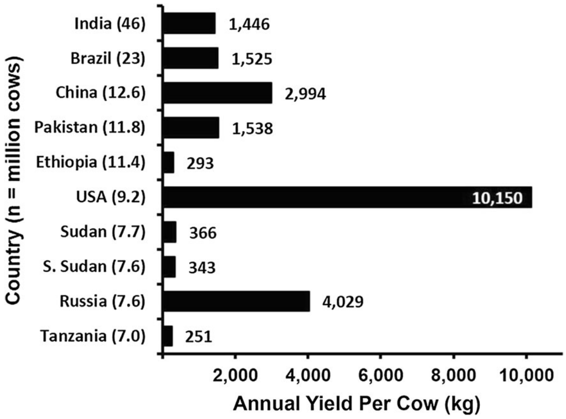


Fig. 2 Number of cows (n, millions) and average annual yield (kg) per cow for the 10 countries with the greatest number of milk cows in 2014. These countries comprise 150 million milk cows, about 46% of the world's inventory (Britt et al. 2018)

The United States is the world's largest producer of beef in part because of selection for higher yielding carcasses since the 1980s. Figure 4 show that despite a falling cattle inventory, total beef production has been rising due to the increased beef yield per carcass.

In order to achieve such progress, producers breed animals that contribute to their breeding objective, or overall goal of the breeding program, which is traditionally focused on production traits such as milk or meat yield or growth rate. Animal breeders work to maximize the response to selection towards their breeding objective. The rate of genetic gain depends on the four components of the breeders' equation:

$$Genetic\ change\ per\ year = \frac{Reliability \times Intensity \times \sqrt{Genetic\ Variation}}{Generation\ Interval}$$

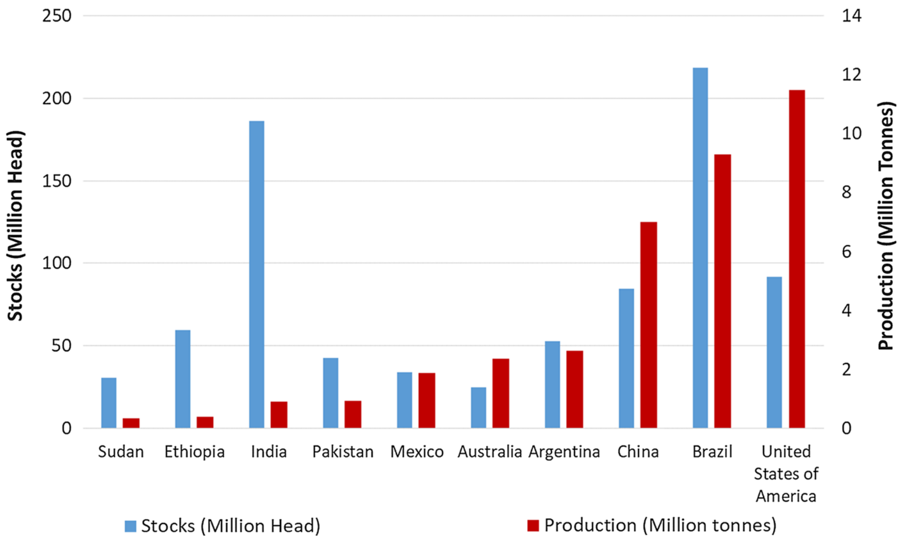


Fig. 3 2016 global beef production: cattle numbers (Million Head; blue, left) versus beef production (Million Tonnes; red, right). (FAO 2018)

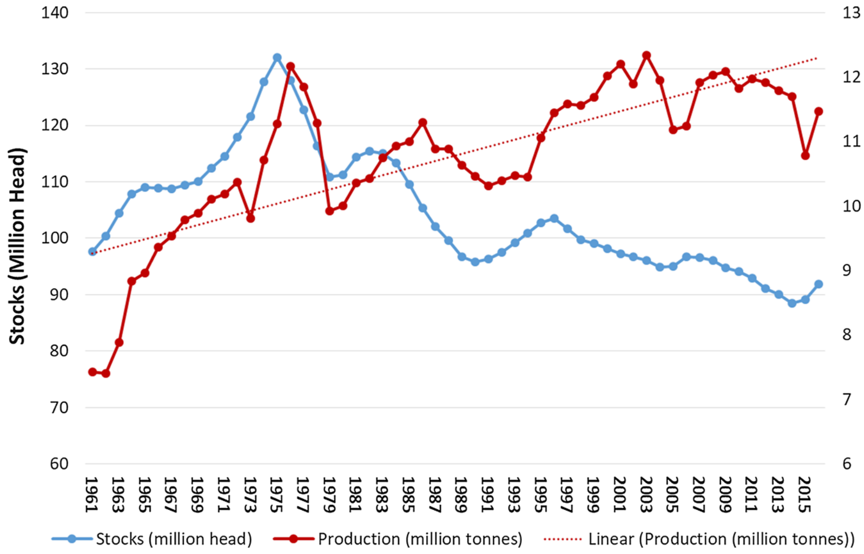


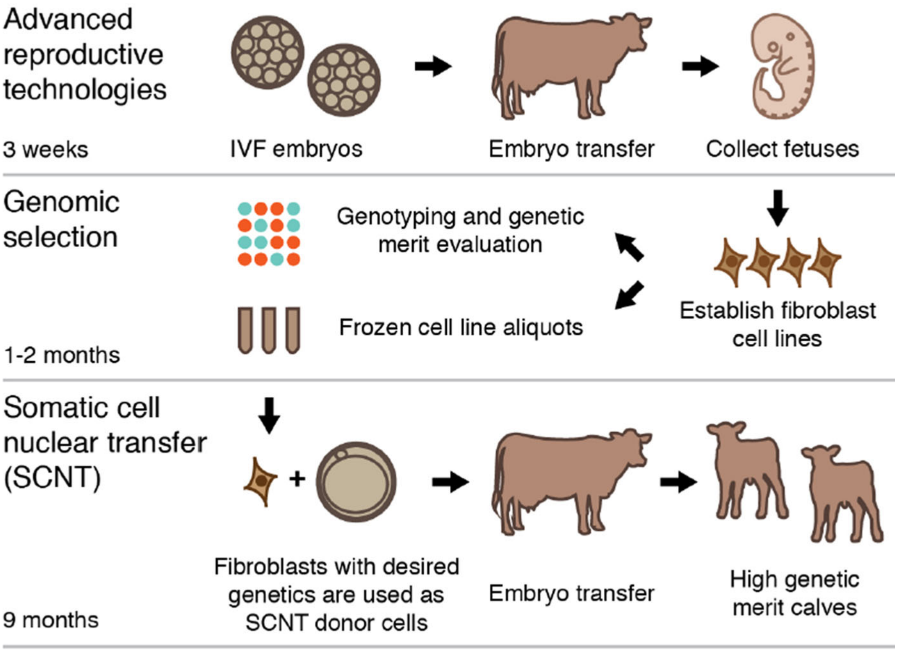
Fig. 4 US cattle inventory 1961–2015. Cattle numbers (Million head; blue, left axis) versus beef production (Million Tonnes; red, right axis). (FAO 2018)

Approaches or technologies that can improve one of these components can accelerate the rate of genetic progress towards the breeding objective. A number of advanced reproductive technologies and breeding methods are being routinely combined to accelerate the rate of genetic improvement in the cattle breeding sector. Figure 5 shows how in vitro fertilization (IVF), genomic selection, and somatic cell nuclear transfer can work together to increase the intensity of selection, the reliability of the genetic merit estimate, and decrease the generation interval.

Genome editing in cattle genetic improvement

Genome editing could be integrated into genomic selection programs to alter the genetic variation and/or generation interval in order to accelerate the rate of genetic gain. Figure 6 shows how genome editing could seamlessly integrate into existing breeding programs. To date, genome editing research in cattle has focused primarily on disease resistance [e.g. tuberculosis (Wu et al. 2015; Gao et al. 2017)], production [e.g. myostatin knockout (Proudfoot et al. 2015), generation of all-male offspring (Van Eenennaam, unpublished data)], elimination of allergens

Fig. 5 Production of high genetic merit calves. Image from Kasinathan et al. (2015)



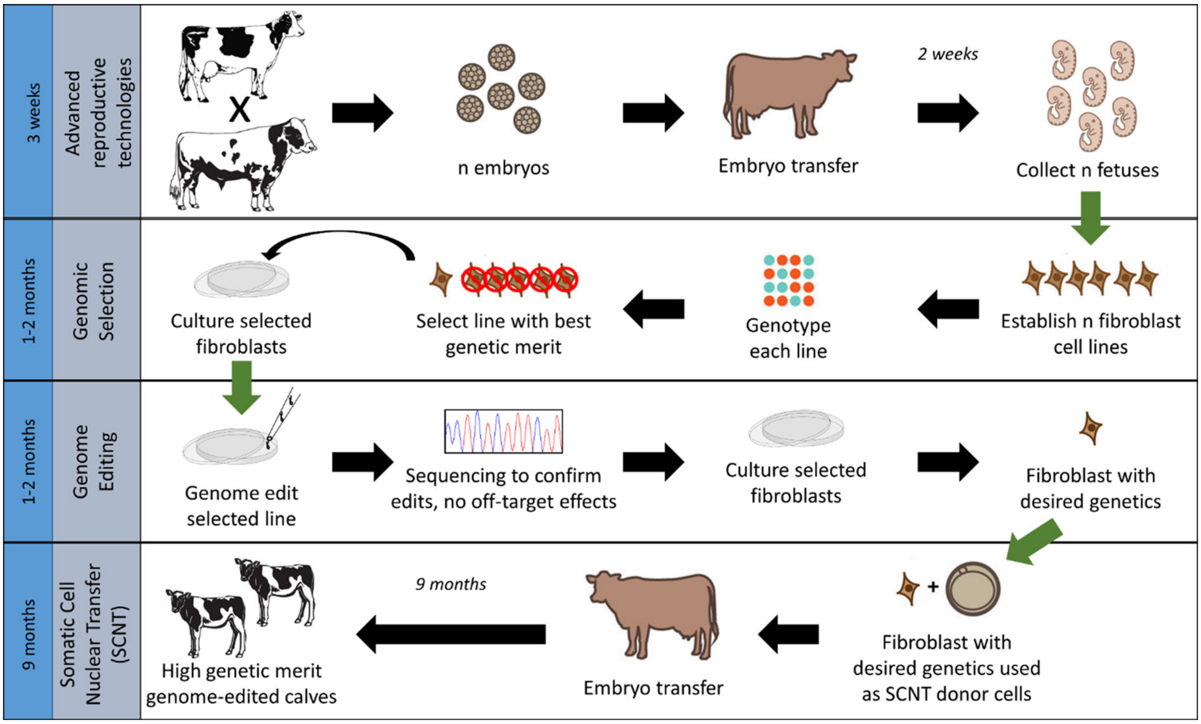


Fig. 6 Production of high genetic merit calves using a range of biotechnologies and showing where genome editing might fit into the process. Image from Van Eenennaam (2017)

[e.g. beta-lactoglobulin knockout (Yu et al. 2011)] and welfare traits [e.g. polled or hornlessness (Carlson et al. 2016)] (Table 1). Genome editing could be used to precisely introduce useful alleles

Table 1 Examples of proposed and actual targets for genome editing in cattle

Target	Targeted trait/goal	References
Intraspecies *POLLED* allele substitution	No horns/welfare trait	Carlson et al. (2016)
Intraspecies *SLICK* allele substitution	Heat tolerance	Porto-Neto et al. (2017)
Myostatin (MSTN) gene knockout	Increased lean muscle yield	Proudfoot et al. (2015)
Beta-lactoglobulin gene knockout	Elimination of milk allergen	Yu et al. (2011)
Prion protein (PRNP) knockout	Elimination of prion protein	Bevacqua et al. (2016)
Intraspecies *CALPAIN* & *CAPASTATIN* allele substitution	Improved meat tenderness	Casas et al. (2006) (not reduced to practice)
Insertion of lysostaphin/lysozyme transgene	Resistance to mastitis	Liu et al. (2013, 2014)
CD18 gene edit	Resistance to bovine respiratory disease	Shanthalingam et al. (2016)
Insertion of SP110, NRAMP1	Resistance to tuberculosis	Wu et al. (2015) and Gao et al. (2017)
Intraspecies SRY translocation onto X chromosome	All male offspring	Owen et al. (unpublished data)
NANOS gene knockout	Infertile males (for gonial cell transfer)	Ideta et al. (2016)

(e.g. heat tolerance, disease resistance) and haplotypes into native locally-adapted cattle breeds, thereby helping to improve their productivity (Dikmen et al. 2014).

Computer modeling has revealed how the use of genome editing to introduce 1–20 beneficial edits impacting a quantitative trait could maintain or even accelerate the rate of genetic gain accomplished by conventional breeding programs. The data shows that it is a superior approach to the lengthy process of introgressing those same alleles from distant breeds (Fig. 7, Jenko et al. 2015).

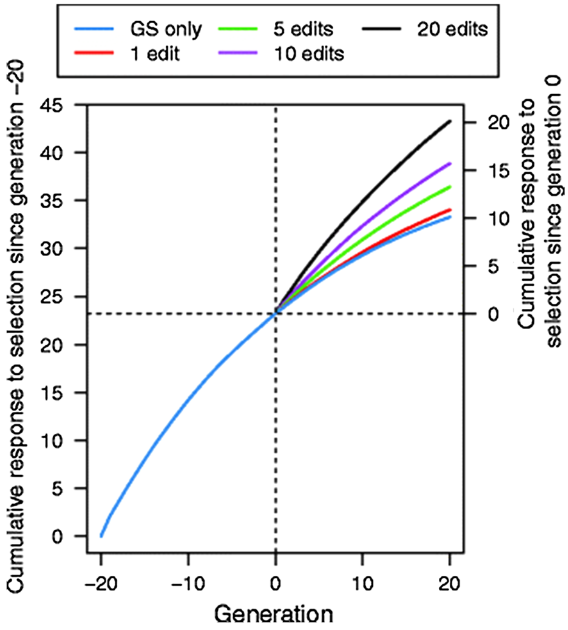


Fig. 7 Cumulative response to selection across 21 generations of recent historical breeding based on genomic selection only (GS only) and 20 generations of future breeding based on GS only or GS plus the promotion of alleles by genome editing when different numbers of quantitative trait nucleotides were edited. Image from Jenko et al. (2015)

It should be noted, however, that the scenario modeled in Fig. 7 simulated editing a quantitative trait that had 10,000 known quantitative trait nucleotides (QTN). In reality, breeders do not currently have a comprehensive understanding of which edits would be impactful on quantitative traits, i.e. those controlled by many genes. Genome editing is particularly suited to addressing qualitative traits that are controlled by a single gene like *POLLED* (hornlessness). In the short term, therefore, 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. In this regard, genome editing can be represented as a cherry on top of the ice cream sundae of an existing breeding program, synergistically allowing the precise introgression of beneficial genetic variants, while still building on the genetic

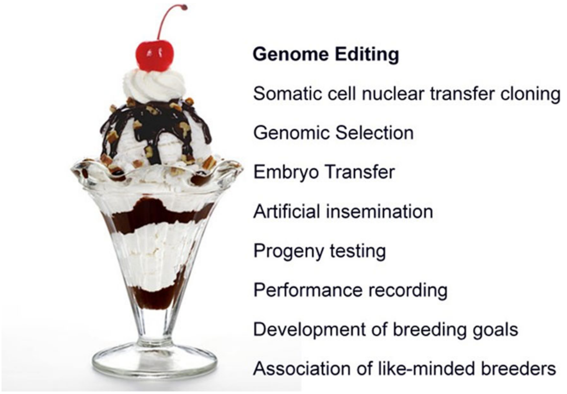


Fig. 8 Genome editing can be envisioned as the cherry on top of the ice cream sundae of progress made using traditional breeding techniques and programs

progress that is achieved every generation using traditional breeding methods (Fig. 8).

Regulations

As with earlier genetic engineering approaches, whether breeders will be able to employ genome editing in cattle genetic improvement programs will very much depend upon global decisions around the regulatory framework and governance of genome editing for food animals. On January 18, 2017, the United States Food and Drug Administration came out with a draft guidance on the regulation of genome edited animals entitled, “Regulation of Intentionally Altered Genomic DNA in Animals” (Food and Drug Administration 2017). The new guidance removes the presence of a recombinant DNA (rDNA) construct as the regulated article that meets the definition of a drug, replacing it instead with “intentional genomic alterations” produced using modern molecular technologies. It is proposed that the presence of any “intentionally altered genomic DNA” produced using genome-editing tools would trigger mandatory, premarket new animal drug evaluation, irrespective of product risk or novelty of the genomic alteration.

One procedural problem with the proposed guidance is differentiating between “intentional genomic alterations”, off-target genome-editing alterations, and de novo mutations (Van Eenennaam 2018). In one analysis of whole-genome sequence data from 234 taurine cattle representing three breeds, more that 28 million variants were observed, comprising insertions, deletions, and single-nucleotide variants (Daetwyler et al. 2014). Another recent study found that on average every new animal will have around 65 de novo mutations, of which approximately five will be small insertion/deletions and the remaining 60 will be single-nucleotide substitutions (Harland et al. 2017).

In contrast, Argentina’s proposed regulatory approach is to ask the same question of edited plants and animals, “Is there a new combination of genetic material in the final product?” If not, then they do not trigger the GE regulatory approval process that was initially put in place for plants and animals containing rDNA constructs containing new combinations of DNA that could potentially present a hazard in the form of a new food allergen or toxin (Whelan and Lema 2015).

From a risk perspective, it does not make a lot of sense to regulate genome edited polled calves differently than naturally-occurring polled calves carrying exactly the same allelic DNA sequence at the *POLLED* gene. Animal breeders need certainty that if they use genome editing to develop products that are no different from those that could have been obtained using conventional breeding, they will not be faced with additional layers of regulatory scrutiny. This would require proportionate regulations based on any novel risks inherent in the product, rather than arbitrary regulation of products based solely on human intent being the basis for the modification, or the processes that were used to create them (Carroll et al. 2016).

Conclusions

Significant improvements in the efficiency of milk and beef production have historically been accomplished through conventional breeding of superior individuals with an eye towards specific breeding objectives. Genome editing is a tool that is well-suited for modifying qualitative, single-gene traits at comparatively rapid rates and could be used in conjunction with conventional selection approaches to address issues such as disease resistance and improved welfare traits. The availability of this technology for use by animal breeders hinges on the regulatory framework imposed, which will likely vary by country. From a risk-based perspective, it makes little sense to regulate genome edited animals differently than conventionally-produced animals carrying the same allelic DNA at the targeted locus simply because they were produced using genome editing. Regulations should be fit-for-purpose, proportional, and based upon novel product risks, if any, rather than being triggered by the use of an arbitrary set of breeding methods.

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