Increasing livestock farming sustainability using genome editing technology

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Farmed animal agriculture is facing big challenges in today's world. Genome editing technology now offers some solutions, and these need to be melded into the other approaches and strategies that can be deployed to produce a sustainable food system. If we embrace these technologies, and do so within a basic justice framing, we can achieve food security for all, while providing enhanced welfare and reduced environmental footprint contributing to a fair and sustainable carbon-zero future.

Man has been rearing animals for food for thousands of years. Farmers will tell you that for a healthy, productive animal you need access to good brood stock. Therefore, it is not surprising that technologies that give the breeders advantage in producing improved brood stock have value in agriculture.

We now know that key to many physical attributes is the underlying genetic make-up. This understanding has only emerged over the last couple of hundred years; prior to this any generation-on-generation improvement relied largely on luck. For example, if a deadly disease came through a region, any animals that survived would form the foundation for the next generation. However, without knowing what underlay that resilience, there would be no selection for the genetic variation conveying it, so genetic drift would minimize any beneficial effect over a short period of time. Furthermore, numerous environmental stressors acting at a local or regional level (e.g., disease, drought, war) could result in the loss of specific local breeds, with subsequent repopulation through importation of new animals. As in the past, farming in many parts of the modern world is performed on a small scale, with small number of animals in local settings hindering genetic improvement without incurring deleterious effects from inbreeding. In such settings, there is limited ability to consistently improve the genetics of farmed animals over time and produce what we now call elite or nucleus populations.

In parallel, the historical geographical isolation of societies and the resulting slow exchange of animals between these peoples has led to breed differences across all farmed animal species, with each breed exhibiting specific characteristics (traits) that confer benefit to that animal in their specific environment. The result is that, within a given species, geographical isolation has produced a multitude of breeds/isotypes that differ in form and function. This is particularly evident when comparing the productivity of western breeds to those in low- and middle-income countries (LMICs). For example, today, the average milk production from indigenous cattle in India is approximately 1000 litres/animal/year increasing to 3000 litres/animal/year for animals cross-bred with high yielding varieties such as Holstein Friesian, which in the USA average over 7000 litres/animal/year. However, there appears to have been a trade-off to achieve this position, since many breeds indigenous to LMICs show a greater resistance to disease than those that predominate in western farming communities. Until recently, it was not possible to effectively utilize the beneficial genetic variation that is present between breeds in breeding decisions outwith the breed where the desirable genetic variation exists.

Advanced breeding technologies in combination with intricate breeding strategies based on probability now enable the coherent design of elite brood stock for many farm animal species. We no longer rely on chance, and instead breed from select individuals based on the genetics that underlie the traits we value. However, incorporating new traits that are not encoded within the gene pool of the elite brood stock is difficult, as crossing with less productive animals that bear the trait of interest would inevitably dilute the overall value of the resultant stock for multiple generations. The development of genome editing technology, for the first time, allows us to introduce a specific genetic variant underlying a trait of interest identified in one breed directly into another, without the requirement for multiple generations of back-crossing. Using this approach we can now incorporate into breeding regimes genetic variation which has been previously either impossible or very hard to access. Animal breeders and farmers around the world can benefit from these technological advances – the 'genie is out of the bottle' and will stay so.

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Genome editing

Since the generation of the first transgenic mouse in 1974 there have been concerted efforts to genetically modify the genomes of livestock species. For decades this was limited to the random integration of transgenes, and while academic successes have been too numerous to mention here, there are few of these that have led to commercial success. The few exceptions, where regulatory approval has been granted for a transgenic animal destined for human consumption, include an Atlantic salmon with a growth hormone transgene and a pig lacking an immunogenicity factor.

While randomly integrated transgenes have clear applications, precise modification of livestock genomes largely remained out of reach until the advent of genome editors. Application of these site-specific nucleases to create a genomic break at a user-specified locus can induce the formation of small insertions or deletions (indels) to disrupt gene function, facilitate precise changes at the target site to alter gene expression profiles or improve the efficiency of gene knock-in.

Programmable nucleases have transformed the field of genome engineering. The first to be developed into a truly transformative genome editing tool was the zinc finger nucleases (ZFNs), quickly followed by the Transcription Activator-Like Effector Nucleases (TALENs). Both involved tethering the DNA binding activity with an endonuclease. While construction of functional ZFNs was challenging, simplicity of reagent design and easily accessible plasmid kits for rapid assembly resulted in broader uptake of TALENs.

The DNA binding domains of both ZFNs and TALENs are proteins. The CRISPR/Cas (clustered regularly interspaced short palindromic repeats – CRISPR-associated protein) systems are different. Based on adaptive defensive systems of bacteria and archea, CRISPR/Cas utilizes a guide RNA to direct the Cas enzyme to cleave the cognate nucleotide sequence. CRISPR/Cas9 from *Streptococcus pyogenes* is currently the most commonly used of these systems and typically involves a bipartite guide, consisting of a CRISPR RNA (crRNA) that recognizes the approximately 20 nucleotide target site by Watson–Crick base pairing and a trans-activating CRISPR RNA (tracrRNA) that hybridizes with the crRNA and complexes with the Cas9 nuclease. A variety of alternative CRISPR/Cas systems have now been developed, and the toolbox continues to expand. Online design tools that identify potential off-target sites and predict on-target activity are freely available, and commercial suppliers offer a variety of CRISPR/Cas reagents for purchase. CRISPR/Cas has been widely adopted in research labs globally, and is now so ubiquitous it has entered into the general lexicon.

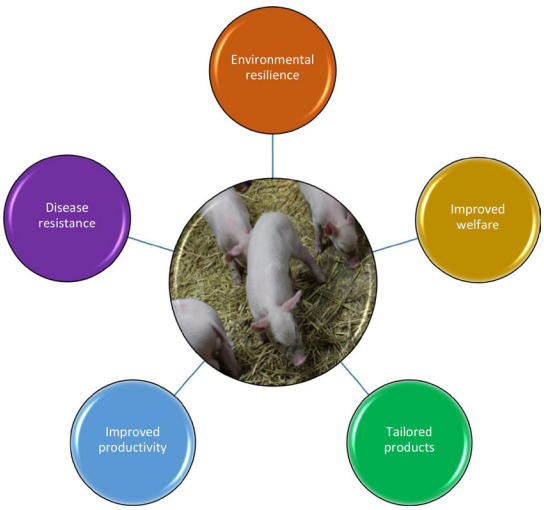

Figure 1.

Genome editing and farmed animals

Genome editing offers myriad possibilities for livestock agriculture (Figure 1). The ability to make specific changes to the genome allows incorporation of variation that is absent, rare or problematic to identify within the breeding population and would therefore be difficult to propagate through conventional husbandry. Genome editing technology has now been successfully applied in most major livestock species, with applications falling into three broad baskets: biomedical, welfare and production traits.

Improved welfare

Livestock welfare is a complex and often polarizing issue. Most, however, would agree with the premise that improved welfare is a laudable goal. One of the most widely discussed editing projects of recent years aimed to achieve just that. In western agriculture, most dairy cattle have horns, while most beef breeds do not. Horns are often considered undesirable as they can result in injury to the farmer, to other cattle or even to the bearer of the horns. As such, disbudding of calves by cauterization or physical dehorning of older animals is common practice, and even with appropriate anaesthesia and analgesia this can be a painful and stressful procedure for the animals. While horned is considered the 'normal' state for bovids, it is recessive to the polled (hornless) state. Despite the fact that the genetics underlying the celtic polled (Pc) trait in cattle is well understood, breeding strategies to introgress this allele into elite germlines would inevitably result in dilution of genetic merit. This is where editing comes to the fore. By breaking the bovine genome proximal to the duplication underlying Pc and supplying an exogenous plasmid repair template, the Pc allele was introduced into cells from horned dairy bulls. These edited cells were then cloned to produce calves which were phenotypically polled, demonstrating the feasibility of this approach.

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Novel disease-resistance traits can also contribute to improved welfare, reducing the risk of animals becoming unwell. Editing to generate a functional knockout of the gene encoding porcine CD163, the putative receptor for porcine reproductive and respiratory syndrome virus (PRRSV), was shown to result in pigs that were completely resistant to this disease. This has the potential to alleviate a disease that causes major losses for swine producers and is a contributor to antimicrobial use in this sector. CD163 is involved in several important biological functions, including haemoglobin/haptoglobin recycling and innate detection of bacteria. As a refinement to ablation of whole gene function, deletion of a single exon encoding the protein domain to which PRRSV binds resulted in pigs with a truncated CD163 that retained its haemoglobin recycling capacity, but that were resistant to infection. Further characterization of these animals will be required en-route to commercialization.

Altered production traits

When it comes to monogenic production traits, few are as visually dramatic as the increased muscling associated with perturbation of myostatin expression. Myostatin is a negative regulator of skeletal muscle growth, with mutations that reduce its expression resulting in enhanced muscle mass. Natural genetic variants have been selected for agricultural purposes, with examples including both Belgian Blue and Piedmontese cattle that completely lack myostatin expression and Texel sheep which have a mutation within the 3′-UTR that is thought to supress expression by acting as a microRNA binding site. Given the striking phenotype associated with mutations to this gene it is of little surprise that multiple research groups have applied editors to modify its expression in a range of agricultural species. Knockouts have been demonstrated in cattle, pigs, sheep, goats and fish. In an alternative approach, modification of the myostatin signal peptide in pigs also resulted in increases to both the number of muscle fibres and the overall muscle mass. Knockout of myostatin in tilapia results in fish with substantial improvements to growth rate, feed conversion rate and time to market. Argentina's National Advisory Commission on Agricultural Biotechnology have ruled that as these fish contain no foreign DNA they are exempt from GM regulations. A similar approach has since been taken by other countries, including Japan, where two edited fish species (tiger puffer and red sea bream) have now been approved for human consumption.

Meat is, of course, only one of the products we harvest from livestock. Milk is a key component of many agricultural systems, and the whey protein β-lactoglobulin (BLG), absent from human milk, is a major allergen in the milk of livestock. While it is difficult to remove BLG from milk using standard biochemical methods, researchers have successfully edited both goats and cattle such that they lack functional BLG genes, with the aim of reducing allergenicity.

Contributing to food security for all

Food security and sustainability go hand in hand. We need more food in the right place at the right time if we are to support our growing population. Genetic technologies offer some solutions and these need to be melded into the other approaches and strategies that can be deployed to produce a sustainable food system. The international regulatory landscape of these technologies is fast evolving, with initial products on the market, and this pipeline is expanding. Future applications will go beyond disease resistance and include altering offspring gender balance, which could have significant welfare impacts the poultry and dairy farming. If we embrace these technologies, and do so within a basic justice framing, we can achieve food security for all, while providing enhanced welfare and reduced environmental footprint contributing to a fair and sustainable carbon-zero future. ■

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Simon Lillico is a core scientist at The Roslin Institute. He has a B.Sc. in zoology (Edinburgh), a Masters in parasitology (Liverpool) and a PhD in parasitology (Glasgow). Simon joined The Roslin Institute in 2002 to produce transgenic hens that produced high-value therapeutic proteins in their eggs, and transgenic livestock as models of human diseases. In recent years, Simon has been at the forefront of the application of genome editors in various livestock species, creating either disease-resistant/-resilient strains or more accurate models of human diseases. His collaborations have included key developers of the tools, R&D companies in the livestock arena, breeding companies and international academic institutions. Simon holds several patents in this field and is the Editor-in-Chief of Transgenic Research.

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