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Priyadharsini R
Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Theni, Tamil Nadu, India

Selvam R
Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Theni, Tamil Nadu, India

Satheeshkumar P
Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Theni, Tamil Nadu, India

Richard Jagatheesan PN
Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Theni, Tamil Nadu, India

Corresponding Author:
Priyadharsini R
Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Theni, Tamil Nadu, India

A short glance about gene editing technology and its use in livestock

Priyadharsini R, Selvam R, Satheeshkumar P, and Richard Jagatheesan PN

Abstract
At present, advancement has been achieved in genome edited livestock by means of technologies like ZFN, TALEN, and CRISPR-cas 9. The gene editing technologies can cut the precise target sequence in the DNA and able to add, removal, or replacement of gene via disruption at exact sequence of DNA. The recent technology like CRISPR had ability to generate multiple genomic cuts in a simpler way. Genome editing tools have significant applications in livestock production such as disease resistance, to increase the production, for improving the milk quality, elimination of milk allergen, animal health and welfare and bioreactors. This paper discusses about genome editing technologies, its application and ethics in livestock.

Keywords: Gene editing, ZFN, TALEN, CRISPR, Livestock, Production

Introduction
The process of altering or restructuring a gene through use of genetic engineering technology is known as genome editing. Modelling suggests that compared to traditional breeding strategies, utilising genome editing to introduce desirable alleles into livestock could maintain or even accelerate the rate of genetic advancement. The production of native, regionally adapted livestock could be increased by precisely introducing valuable genes into their genetic makeup. Genome editing research so far concentrated on features that improve disease resistance (eg. Tuberculosis, Porcine Reproductive and Respiratory Syndrome virus (PRRSV), Bovine Viral Diarrhoea Virus (BVDV), heat tolerance, parasite resistance, increase production (e.g., myostatin knockout; generation of all-male progeny), elimination of allergen in milk or humanization of bovine milk (e.g., beta-lactoglobulin knockout), animal welfare (e.g., polled, or hornless cattle), pharmaceutical protein production, animal models for human diseases and agricultural uses (Raza *et al*., 2022) [21]. The science of life will undergo a revolutionary upheaval because to this technology. Breeders will only be able to apply genome editing in cattle genetic improvement projects if international agreements are made addressing the governance and regulatory framework of genome editing for food animals.

Genome editing techniques
In the past, gene editing techniques like somatic cell nuclear transfer and pronuclear microinjection were frequently used, but these techniques are not precise cutters and result in mosaicism. The latest genome editing technologies are already being used on livestock, including ZFN (Zinc Finger Nuclease), TALEN (Transcription Activator-like Effector Nucleases), and CRISPR-cas 9 (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9). They precisely cut targets in the genome.

Zinc Finger Nucleases (ZFNs)
Researchers began utilising ZFN in the 2001 to increase genome editing accuracy and reduce off-target modifications. Zinc Finger Nucleases are tiny (20–30 amino acid) proteins that bind to DNA and are controlled by the zinc ion, which is recognised by a 3 base pair sequence. Modifying naturally occurring proteins that were present in eukaryotic animals allowed for the creation of ZFNs.

These proteins can be modified by scientists to cut DNA and bind to particular DNA sequences in the genome (Bibikova *et al*., 2001) [2]. After binding to their target DNA sequence, the ZFNs cut the genome at the specified spot, allowing scientists to either delete the target DNA sequence or replace it with a new DNA sequence through homologous recombination. It is challenging and time-consuming to design, develop, and produce effective zinc finger proteins, and a new ZFN must be designed for each new target DNA sequence, even though ZFNs increased the success rate of genome editing to roughly 10%.

Transcription Activator-like Endoucleases (TALENs)

In 2009, a brand-new gene nuclease known as TALEN was found. The rice-infecting genus Xanthomonas oryzae of plant pathogenic bacteria. A bacterial effector protein called TALENS reduces host resistance. These effector proteins include 33–35 repeating amino acid motifs that bind to host genomic DNA, recognise each base pair, operate as transcription factors, and cause the production of genes that support bacterial assault. Similar to ZFNs, TALENs are derived from naturally existing proteins and have the ability to attach to particular DNA regions (Gaj *et al*., 2013) [8].

While the efficiency with which they may produce genomic modifications is equivalent between TALENs and ZFNs, TALENs tolerate the advantage of greater simplicity. TALEN construction is simpler than ZFN synthesis.

Even though a variety of technologies, including ZFN and TALEN, have made significant advancements in gene manipulation, these early technologies suffer from poorer specificity because of their off-target adverse effects. Therefore, scientists primarily exploited the recently discovered CRISPR and cas-9, which successfully inserted variation at precise locations within genes. It offers improved efficiency, viability, and clinical application across multiple roles.

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) Cas 9

The CRISPER-Cas9 method was developed from a genome editing mechanism utilised by bacteria as an immune system. CRISPER-Cas9 arrays are segments of DNA that are created by virus-infected bacteria by integrating DNA fragments from invasive viruses into their own DNA. The viruses (or ones that are closely related to them) that the bacteria "remember" while creating CRISPR arrays. If they are attacked by viruses again, the bacteria produce RNA segments generated by CRISPER-Cas9 arrays that identify and target the exact position of the viruses' DNA. The virus is then totally rendered inoperable by cleaving that DNA using Cas 9 or a comparable enzyme (Cong *et al*., 2013) [5].

This bacterial immune defence system was modified by scientists to regulate DNA. Similar to RNA segments bacteria make from the CRISPR array, they produce a little piece of RNA with a short "guide" sequence that binds to a specific target sequence in a cell's DNA. The Cas9 enzyme was also linked to this guide RNA. When delivered into cells, the guide RNA recognises the desired DNA sequence, and the Cas9 enzyme, mimicking the action of the bacterial process, slices the DNA at the desired spot. Other enzymes, such Cpf1, can also be employed, however Cas9 is the one that is most frequently used. Researchers employ the cell's own DNA repair machinery to add or remove genetic material after the DNA has been cut.

Double strand breaks (DSBs) are introduced into specific genome areas using CRISPR/Cas9 gene editing techniques, and these DSBs are then repaired by biological processes. Homology-directed repair (HDR) or non-homologous end joining (NHEJ), depending on the state of the cell and the existence of a repair template, are the two major processes used by the cell machinery to repair the DSB (McMahon *et al*., 2012) [13]. The guide RNAs' ability to interact with target sequences via Watson-Crick base pairing gives CRISPR/Cas9 editing its specificity. Introducing CRISPR-Cas9 editing tools into particular cells *in vivo* and limiting or reducing off-target effects, despite its benefits and potential, remain important challenges that are essential for therapeutic applications.

Table 1: Difference between this three genes editing technologies

Properties	ZFNs	TALENs	CRISPR
DNA binding system	Protein-DNA	Protein- DNA	RNA-DNA
Targeted sequence	18-36bp	30-40bp	22bp
Off target events	comparable	comparable	comparable
Multiple targeting	Difficult	Difficult	Easy
Delivery	Easy	Difficult	Moderate
Cost	High	Middle	Low

Applications

To improve the livestock production traits like meat and fibre production, improvement in milk quality, elimination of allergen in milk, reproductive performance, disease resistance, heat tolerance, parasite resistance, methane reduction and animal welfare.

Enhancement in livestock production traits

The MSTN gene, which encodes myostatin, inhibits muscle development and differentiation because it is a negative regulator of skeletal muscle. The function of myostatin was discovered through gene knockout research. Due to a combination of muscle fibre hyperplasia and hypertrophy, myostatin KO mice had roughly doubled body weights in skeletal muscle (McPherron, 1997) [14]. According to studies (Dilger *et al*., 2022; Crispo *et al*., 2015) [7, 6], altering the MSTN gene in sheep, goats, and pigs enhances the animals' ability to grow.

Another potential gene for improving meat output in animals and developing therapeutic approaches for muscle growth is the protein-coding gene FBXO40 (Zou *et al*., 2018) [28]. By using CRISPR Cas 9, Park (2023) [18] created hens lacking the G0/G1 switch gene 2 (G0S2), which is responsible for lipid deposition

Improvement in milk quality

Candidate genes associated with milk yield and fat content are growth hormone receptor gene (Y allele), *DGAT1* (A allele) and stearoyl-coenzyme A desaturase (SCD1) gene (A allele) genes had more single nucleotide polymorphisms which is present in European cattle breeds but absent in indigenous breeds. Insertion of this SNPs or alleles into indigenous breeds by gene editing method will improve the milk yield and fat content of indigenous breeds (Camargo and Pereira, 2022) [3]. By employing Zinc Finger Nuclease technology to direct the human lysozyme gene to the beta casein locus, created a transgenic cow with mastitis resistance.

Elimination of allergen

The primary cause of milk allergy beta lacto globulin, which is present in whey protein, is absent in human milk. As shown by Silaeva *et al*. (2020) [23] and Xu *et al*. (2020) [26], the beta-

lacto globulin gene can be knocked out using the CRISPR/Cas9 system to produce milk that is hypoallergenic in cows and goats, respectively. ZFNs can be used to successfully knock down the main b-lacto globulin gene, which in cattle encodes the main milk protein allergen (Yu *et al*., 2011) [27].

Disease resistance

Because of the Porcine Reproductive and Respiratory Syndrome virus (PRRSV) infection in pigs, pig farms experience enormous financial losses. Therefore, modification in CD 163 receptor gene using CRISPR/Cas 9 gene editing was produced pigs which is totally immune to viral infection (Cigan and Knap, 2022) [4]. Pigs having double-gene knockouts (DKO) for the known receptor proteins CD163 and pAPN are completely resistant to genotype 2 PRRSV and TGEV (Transmissible Gastero Enteritis Virus).

For the first time, gene-edited tuberculosis resistance cattle was created using CRISPR/Cas 9 technology with NRAMP (Natural Resistance-Associated Macrophage Protein) gene insertion by Aruna pal and AK Chakaravarthy (2020) [29]. According to studies, it is possible to create cattle that are resistant to Mycobacterium bovis infection through genome editing (Gao, *et al*., 2017) [9].

Pasteurellosis in cattle caused by *Pasteurella haemolytica* which secrete leucotoxins that bind to CD18 proteins present in the leucocyte surface. Introduction of single amino acid into CD18 proteins by using zinc finger nuclease technology produce transgenic cattle which is resistant to pasteurellosis (Shanthalingam *et al*., 2016) [22]. Gene editing technologies can also successfully fix mutations that cause disease. The highly contagious bovine viral diarrhoea virus (BVDV) can cause serious intestinal and respiratory conditions in cattle. It results in abortion in pregnant cows, and some infected calves who make it to birth will carry the infection for the rest of their lives. Virus binds to cellular receptor (CD46), causing infection in cows. By using gene editing technology modified CD46 receptor results in virus not able to bind. The first CD46 gene edited calf was produced (Workman *et al*., 2022) [25].

Parasite resistance

Ticks, mosquitoes, and flies can directly affect animals and have a negative impact on their growth and productivity. The New World screwworm (*Cochliomyia hominivorax*), which affects cattle in parts of South America, is one of these pests. The Sterile Insect Technique (SIT) programme was used to eradicate this pest. Sex determination in C. hominovorax controlled by transformer (TRA) gene, by altering this gene, it was probable to deleting the sequence of DNA to encourage masculinization of surviving XX flies. The Cochliomyia hominivorax and Lucilia cuprina yellow genes (ChY and LcY), which regulate the pests' natural population, were subjected to site-directed alterations utilising CRISPR CAS 9 (Paulo *et al*., 2019) [19]. In the F2 generation of Holstein and Gir cross cattle, candidate genes for immunity such as Cluster of differentiation 83 (CD83) and Triggering receptor expressed on myeloid cells (TREM) 1 and 2 were also linked to tick resistance. These genes could be used for gene editing to reduce the number of ticks (Mota *et al*., 2018) [15].

Reducing heat stress

In tropical and subtropical climates, heat stress is one of the most manageable issues since it reduces animals' ability to produce milk. Prolactin hormone receptor (PRLR) gene contains the Slick hair coat (SLICK) locus. Animals with this SLICK haplotype, which have short and fine hair, have been found as being able to cope with heat stress (Huson *et al*. 2014). The PRLR gene's exon 11 (SLICK2) region, which is well adapted to hot climatic conditions, has a mutation (C > T) in Limonero and Carora cattle (Porto-Neto *et al*., 2018). Therefore, the introduction of this (SLICK) mutation into the PRLR gene of heat-sensitive breeds will lessen heat stress in animals exposed to hot and humid weather conditions and won't have an impact on their ability to produce milk (Hansen, 2020) [10].

Reducing methane emission

During fermentation process, ruminants produce methane, which is released into atmosphere by the process of eructation. Ruminants are the most important species to contribute methane emissions into atmosphere which had high impact on global warming. By manipulating the genome of methanogenic archaea, removal of genomic sequences of M*ethanosarcina acetivorans* by CRISPR CAS 9 technology reduces methane emission into atmosphere. (Nayak and Metcalf, 2017) [17].

Animal welfare

To protect animals and humans from injury, disbudding and dehorning were commonly used in cattle management practices. They are unappealing, expensive procedures that are increasingly being scrutinised by the public as a problem of animal welfare. Breeding for polled (hornlessness), which is a recessively inherited characteristic, is one way to stop dehorning. This method has not, however, been widely used due to the low genetic quality and dearth of polled dairy sires. Conventional breeding techniques will promote inbreeding and impede genetic improvement if used to reduce the prevalence of the HORNED allele. Mueller *et al*. (2019) [19] study revealed that gene editing may be used to quickly decrease the frequency of the HORNED allele in US dairy cattle populations while preserving the rate of genetic advancement, limiting levels of inbreeding, and concurrently addressing a growing animal welfare issue.

Bioreactors

Transgenic animals also secrete melatonin enriched milk for patients with sleeping disorders. Additionally, transgenic goat can produce proteins into their milk that human haemophilia sufferers require, such as blood clotting factors (Umaraw *et al*., 2015) [24].

Conclusion

The prevention and treatment of human diseases is a major area of focus for genome editing. Genome editing is currently employed in research facilities to study diseases in cells and animal models. Researchers are currently figuring out whether this method is secure and efficient for usage in people. It is being investigated in clinical trials and research for several disorders. Ethical concerns are raised by the change of human genomes by genome editing using techniques like CRISPR-Cas9. Many of the changes induced by genome editing are restricted to somatic cells, which are cells other than germline cells (sperm and ova). These changes only impact certain tissues, and they do not pass down from one generation to the next. However, genetic changes done to embryonic, sperm, or egg cells may be passed on to next generations. Genome editing of germline cells and embryos is now illegal in the United States and many other countries due to ethical and

security concerns. It's possible that this technology will one day rule human culture. Establishing rules and oversight organisations is crucial for this technology. The inhabitants of the world must not suffer as a result of knowledge advancement. Despite the benefits of gene therapy, it's crucial to keep the drawbacks in mind as well.

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