



ISSN: 2456-2912

NAAS Rating (2026): 4.61

VET 2026; 11(7): 260-263

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www.veterinarypaper.com

Received: 27-04-2026

Accepted: 30-05-2026

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Targeted gene editing strategies in modern cattle breeding

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DOI: <https://www.doi.org/10.22271/veterinary.2026.v11.i7d.3633>

Abstract

Gene editing technology is a groundbreaking biotechnology tool that offers significant opportunities and challenges in livestock breeding. It enables precise genome modification through efficient insertion, deletion, or substitution of DNA sequences, leading to changes in phenotypic traits and faster genetic improvement. Substantial progress has been achieved in improving key economically important traits in cattle, including disease resistance, meat quality, lactation performance, and sex control. This work summarizes recent advancements in gene editing for cattle breeding and discusses the associated challenges and future directions for its development.

Keywords: Gene editing, cattle breeding, DNA sequences, lactation performance

Introduction

The rapid growth of the global population has made cattle farming increasingly important for ensuring food supply and meeting human nutritional needs. However, traditional breeding methods are slow and less efficient in improving genetic traits and production performance. Advances in biotechnology have enabled a shift from conventional breeding to molecular approaches, including gene editing. Gene editing technology is now widely used to enhance the economic value of cattle through precise genetic modifications, improving growth, productivity, and disease resistance. It also accelerates breeding by increasing the frequency of favorable alleles at quantitative trait loci (Jenko *et al.*, 2015) [9]. Despite its potential, large-scale application faces challenges such as off-target effects, animal welfare concerns, and differences in public awareness and regulatory policies across regions. Addressing these issues is essential for wider adoption. Overall, gene editing holds great promise for improving livestock productivity and meeting the rising global demand for animal protein.

Major gene editing technologies

Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 are the principal gene editing technologies used for precise genome modification. These tools function by creating double-strand breaks (DSBs) at specific DNA sites, which are then repaired by cellular mechanisms such as non-homologous end joining (NHEJ), often leading to insertions or deletions, or homology-directed repair (HDR), enabling precise sequence changes (Fig. 1). ZFNs are constructed by fusing zinc-finger DNA-binding motifs with the FokI endonuclease, allowing sequence-specific DNA recognition and cleavage. TALENs operate in a similar manner, combining TALE-based DNA-binding domains with the FokI nuclease, where their modular repeat structure allows flexible targeting of a wide range of DNA sequences (Carlson *et al.*, 2012) [3]. Despite their specificity, both ZFNs and TALENs depend on protein-DNA interactions, requiring time-consuming redesign of binding domains for each target site and facing limitations in efficient delivery into cells.

In contrast, the CRISPR/Cas9 system, derived from the bacterial adaptive immune system, represents a more advanced and user-friendly approach.

It utilizes a single guide RNA (sgRNA) to direct the Cas9 nuclease to specific genomic regions, enabling accurate and efficient DNA cleavage. Compared to ZFNs and TALENs, CRISPR/Cas9 is simpler to design, more cost-effective, and highly efficient, making it the most widely adopted gene editing tool (Hsu *et al.*, 2013) [6]. It has been extensively applied in livestock improvement for enhancing traits such as growth, productivity, and disease resistance, as well as in

biomedical research. For example, CRISPR/Cas9 has been used to knock out specific genes in pigs, providing a promising strategy for xenotransplantation by reducing immune rejection risks. Overall, these gene editing technologies, particularly CRISPR/Cas9, have revolutionized modern genetics and hold great potential for future advancements in animal breeding and medicine (Butler *et al.*, 2016) [2].

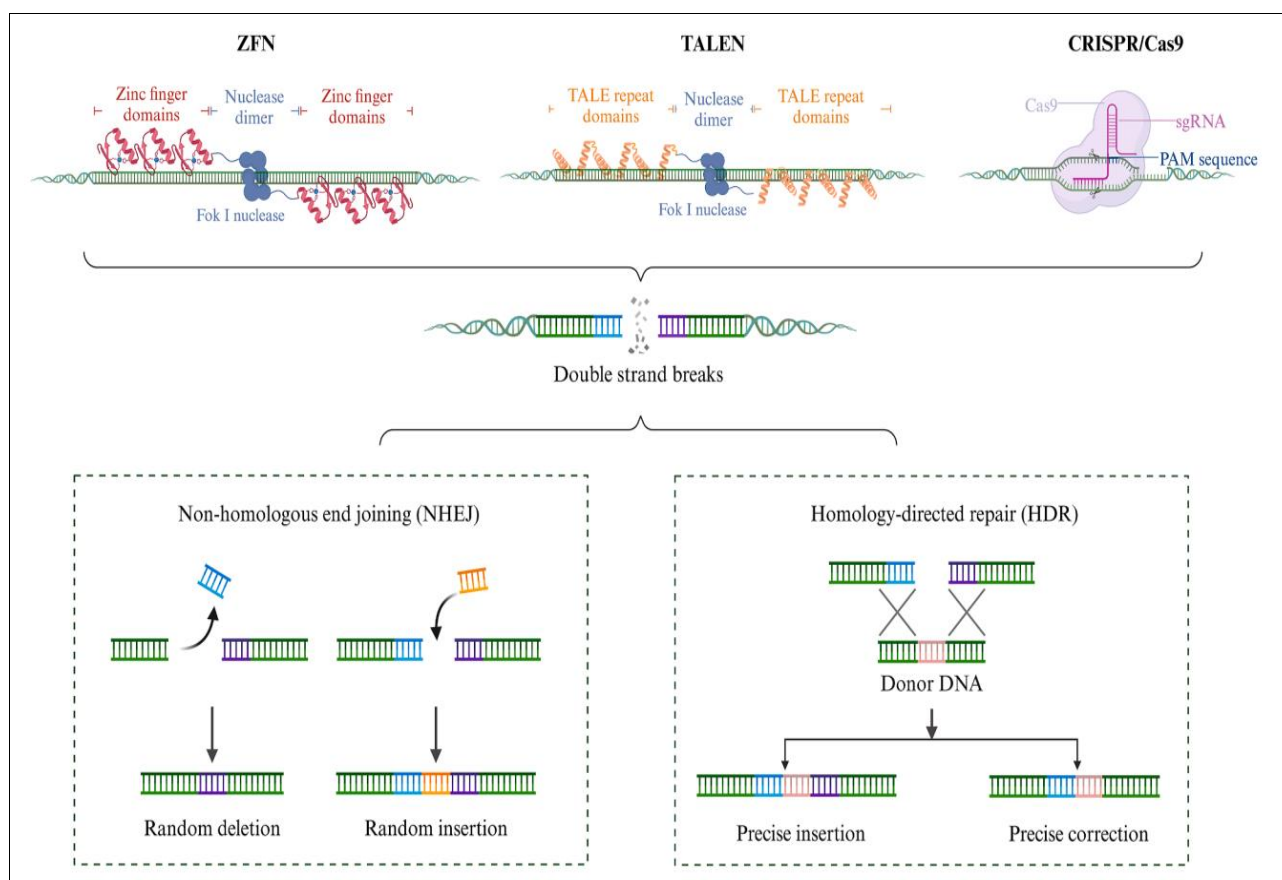


Fig 1: Working Principles of ZFN, TALEN and CRISPR/Cas9 in gene editing

Base editors (BEs) are advanced CRISPR/Cas9-derived tools that enable precise single-base conversions without creating double-strand breaks. They are mainly classified into cytosine base editors (CBEs), which convert C-G to T-A, and adenine base editors (ABEs), which convert A-T to G-C. Continuous improvements have enhanced their efficiency and reduced off-target effects. In organisms such as mycoplasmas and many bacteria, where NHEJ and HDR pathways are inefficient, CRISPR/Cas9 functions mainly as a counterselection tool; however, BEs overcome this limitation. Compared to conventional CRISPR/Cas9, base editors cause less genomic disruption and present lower risk, making them highly promising for applications in animal breeding research (Luo *et al.*, 2022) [10].

Prime editors (PEs), developed in 2019, represent an advanced gene editing tool capable of generating all 12 types of point mutations as well as precise insertions and deletions without the need for double-strand breaks (DSBs) or donor DNA templates. Although their initial editing efficiency was low, continuous optimization has significantly improved their performance. Along with CRISPR/Cas9 and BEs, prime editing is now considered one of the most promising and widely used gene editing technologies due to its affordability, ease of use, high efficiency, and reduced off-target effects (Choi *et al.*, 2022) [4].

The CRISPR/Cas12 system has recently emerged as a powerful gene editing tool, significantly improving editing accuracy and efficiency. Compared to Cas9, Cas12 is smaller and has broader sequence recognition, and targeted amino acid modifications can further enhance its *in vivo* editing performance. Smaller variants such as Cas12f and Cas12n are particularly promising for gene therapy due to their compatibility with adeno-associated virus (AAV) delivery (Badon *et al.*, 2024) [1]. In addition, a novel RNA-guided endonuclease system, termed OMEGA, has been identified, consisting of transposon-associated TnpB and IscB, which are considered evolutionary precursors of Cas12 and Cas9, respectively. These proteins are much smaller, about one-third the size of Cas12 and Cas9-making them suitable for developing miniaturized gene editing tools and single AAV-based *in vivo* delivery. CRISPR/Cas12 is already widely applied across biological research, while the OMEGA system holds strong potential for future breakthroughs (Wang *et al.*, 2024) [15]. Overall, continuous advancements in gene editing technologies have led to their widespread application in cattle genetic breeding (Table 1), with significant potential to enhance livestock productivity, support sustainable agriculture, and advance medical research.

Table 1: Application of gene editing technology in cattle breeding

Application	Target gene/Exogenous gene	Generation method	Gene manipulation tools	Effect
Disease resistance	PRNP (Target gene)	SCNT	RNAi	Reduce prion protein expression
	PRNP (Target gene)	ZMI	CRISPR/Cas9	BSE resistance
	SP110 (Exogenous gene)	SCNT	TALEN	Tuberculosis resistance
	NRAMP1 (Exogenous gene)	SCNT	CRISPR/Cas9	Tuberculosis resistance
	LYZ (Exogenous gene)	SCNT	ZFN	Mastitis resistance
	LYZ (Exogenous gene)	SCNT	ZFN	Mastitis resistance
Improved meat production	MSTN (Target gene)	SCNT	ZFN	Enhanced meat production
	MSTN (Target gene)	SCNT	CRISPR/Cas9	Enhanced meat production
	MSTN (Target gene)	SCNT	CRISPR/Cas9	Improved production & quality
	IGF2 (Target gene)	SCNT	CRISPR/Cas9	Improved production & quality
Improved milk quality	LALBA (Exogenous gene)	SCNT	Expression vector	Improved nutritional value
	LYZ (Exogenous gene)	SCNT	Expression vector	Improved nutritional value
	LYZ (Exogenous gene)	SCNT	Cre-LoxP	Improved nutritional value
	BLG (Target gene)	SCNT	ZFN	Hypoallergenic milk
Sex control	SRY (Target gene)	SCNT	TALEN	Sex-reversed cattle
	SRY (Exogenous gene)	ZMI	CRISPR/Cas9	Targeted bull creation

The application of gene editing technology in cattle breeding

Enhancement of disease resistance

Enhancing disease resistance is a major goal in livestock breeding, as epidemics cause significant economic losses and threaten both animal and human health. Traditional methods such as vaccination and antibiotics have limitations, including environmental pollution and antimicrobial resistance. Disease resistance is a complex polygenic trait, and conventional breeding is slow, costly, and risky. In contrast, gene editing technologies enable precise modification of disease-related genes, improving resistance efficiently and reducing breeding costs, thereby supporting sustainable livestock production. Bovine spongiform encephalopathy (BSE), or mad cow disease, is a fatal neurodegenerative disorder linked to misfolded prion proteins encoded by the PRNP gene. Studies have shown that knockout or reduced expression of PRNP confers resistance to BSE without affecting normal development. Gene editing approaches such as RNA interference and CRISPR/Cas9 have been used to generate PRNP-modified cattle with reduced prion levels. Additionally, the PrP G127V variant has demonstrated strong resistance to prion diseases, and its introduction into cattle using CRISPR/Cas9 has shown successful germline transmission. However, challenges such as mosaicism still need to be addressed (Park *et al.*, 2020) [12]. Bovine tuberculosis, caused by *Mycobacterium bovis*, is a chronic zoonotic disease affecting cattle productivity and human health. Gene editing strategies targeting the SP110 and NRAMP1 genes have shown promising results. Insertion of the murine SP110 gene using TALEN produced cattle resistant to infection, while overexpression or targeted insertion of NRAMP1 using CRISPR-based approaches enhanced resistance with minimal off-target effects. These modifications are heritable and demonstrate the potential of gene editing for disease control (Gao *et al.*, 2017) [5]. Mastitis, a common and economically important disease in dairy cattle, reduces milk yield and quality. Gene editing has been used to enhance resistance by introducing antimicrobial proteins such as lysostaphin and human lysozyme into the bovine genome. These proteins are secreted in milk and exhibit strong antibacterial activity against *Staphylococcus aureus*. Such genetic modifications improve udder health, milk quality, and overall dairy productivity, highlighting the potential of gene editing to improve animal welfare and economic outcomes in livestock production (Jabed *et al.*, 2012) [7].

Improvement of milk quality

Improving milk quality through gene editing focuses on making cow's milk more similar to human breast milk to better meet human nutritional needs. This involves increasing beneficial proteins such as α -lactalbumin and lactoferrin. α -Lactalbumin is abundant in human milk (25-35%) but low in cow's milk (2-5%). Gene-edited cattle expressing recombinant human α -lactalbumin have been successfully produced using ZMI and SCNT, with milk showing similar physicochemical properties to human milk and improved nutritional value (Jackson *et al.*, 2004) [8]. Lysozyme is another important protein with strong antimicrobial properties and a key role in human immunity. Its concentration in human milk is much higher than in cow's milk. Gene editing has been used to produce cows expressing human lysozyme, with levels and activity comparable to natural human milk. These advancements not only enhance milk's nutritional and immune benefits but also support large-scale production and improve resistance to diseases like mastitis (Piccinini *et al.*, 2005) [13]. Milk allergy, mainly caused by β -lactoglobulin, is a significant issue in infants. Conventional processing methods are costly and may not fully eliminate allergenicity. Gene editing offers an effective alternative by reducing or eliminating β -lactoglobulin using RNAi or ZFN technologies. This results in hypoallergenic milk with improved protein composition and reduced allergenic potential without introducing foreign DNA (Saha *et al.*, 2001) [14]. Overall, gene editing technologies enhance milk nutritional quality, improve safety by reducing allergenicity, and provide economic benefits to the dairy industry while supporting human health.

Sex control

Sex control is highly important in cattle farming, as it directly affects productivity and economic returns. Male calves are preferred in beef production due to better feed efficiency, while female calves are essential in dairy systems for increased milk production. Traditional methods such as sperm sorting, embryo sexing, and reproductive manipulation have limitations. Gene editing offers a more precise approach by targeting sex-determining mechanisms. For example, CRISPR/Cas9 has been used to insert the eGFP gene into the Y chromosome, enabling sex identification through fluorescence. The SRY gene, a key regulator of male development, has been widely studied, with knockout and knock-in strategies demonstrating its critical role in sex determination. Techniques like TALEN and HMEJ-based

CRISPR approaches have successfully produced sex-reversed or targeted-sex cattle (Owen *et al.*, 2021) ^[11]. Although these methods show strong potential, challenges related to efficiency and accuracy still limit their large-scale application, and further advancements are needed for commercial use.

Conclusion

Gene editing technology has emerged as a powerful tool for accelerating genetic improvement in cattle by enabling precise and efficient modification of economically important traits. Its applications in enhancing disease resistance, improving milk quality, increasing meat production, and enabling sex control highlight its transformative potential in modern livestock breeding. Despite these advancements, challenges such as off-target effects, ethical concerns, regulatory limitations, and technical constraints still need to be addressed. With continued technological improvements and supportive policies, gene editing is expected to play a crucial role in achieving sustainable livestock production, improving animal welfare, and meeting the growing global demand for high-quality animal products.

Conflict of Interest

Not available

Financial Support

Not available

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How to Cite This Article

Gangwar M, Diwakar V, Chelani L, Barwar R, Deb D, Verma MK. Targeted gene editing strategies in modern cattle breeding. *International Journal of Veterinary Sciences and Animal Husbandry*. 2026;11(7):260-263

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