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Genome Editing: A Strategic Tool to Advance Poultry Production in the Tropics

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Authors' contributions

This work was carried out in collaboration among all authors. Author EMK designed the review paper, managed the literature searches and wrote the first and final draft of the manuscript. Authors ABT and FRB managed and corrected the first draft of the manuscript. Author ASS managed the references and corrected the final draft. All authors read and approved the final manuscript.

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ABSTRACT

The output of poultry genetic resources (PGR) can be greatly increased by the use of the very powerful tool known as genome editing. Poultry farming is significant in the tropics because it significantly raises household income and the level of living. Increasing PGR production in the tropics requires overcoming several challenges, such as high rates of disease prevalence and

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resistance, subpar performance, and adverse environmental conditions. However, the application of genome editing technology presents a promising opportunity to address these problems and maximise poultry output in tropical regions.

In poultry production, selective breeding has resulted in notable improvements in output, effectiveness, and product quality. However, modifying characteristics linked to health becomes more difficult. In the tropics, high temperatures, high humidity, and other challenging conditions are often observed, and they can have a negative impact on the well-being and output of PGR. By using genome editing, it is possible to introduce genetic modifications that increase poultry resistance to these conditions.

Optimising meat yield and quality by genome editing provides a targeted and effective way to introduce disease resistance. Through precise DNA modification, researchers may make specific genetic alterations in organisms through precision genome editing. Benefits including disease resistance, increased welfare and feed conversion efficiency, and better adaption to tropical climates can be inserted into poultry species by scientists through the use of genome editing technology. Particularly in the tropics where infectious diseases can have a major negative influence on flock health and productivity, disease management is an essential component of chicken farming.

Keywords: Genome editing; poultry production; tropical climate; gene modification; CRISPR/Cas9.

1. INTRODUCTION

An integral part of the living organism's molecular makeup is protein. Protein mass is highly significant in healthy individuals; it makes up 10.6 kg, or 15.1%, of total body mass. Undernutrition in proteins can lead to a variety of detrimental outcomes. Protein from a range of food sources, including both plant- and animal-based sources, can successfully combat undernutrition. But it's crucial to remember that protein's nutritional worth depends on both its amount and quality. Therefore, it makes sense in theory to include high-quality protein obtained from animals in one's diet because it is essential for fostering normal human growth, development, and wellbeing in general [1]. Poultry products, encompassing both meat and eggs, hold a distinct position within the realm of animal protein sources. Due to its high-quality protein content, poultry meat and eggs serve as a primary source of nutrition for a significant portion of the global population. In addition to providing a rich source of high-quality protein, they also contribute essential vitamins and minerals. Net protein utilization (NPU) serves as a metric for assessing the quality of protein. According to [2] eggs possess a net protein utilization (NPU) value of 97%. Cereals lack essential amino acids that are crucial for human nutrition, including lysine, threonine, the sulphur-containing amino acids (methionine and cysteine), and occasionally tryptophan. Due to their abundance and affordability, eggs and poultry meat are rich sources of essential amino acids. Therefore, there is a growing perception that it is becoming less of a luxury and more of an essential commodity in everyday life. Moreover, it is noteworthy that the consumption of these goods is not subject to any significant societal taboos. With the continuous growth of the global human population, there will be a corresponding increase in the demand for livestock products. Nevertheless, the poultry farming industry and global food supply face significant challenges due to climate change and the occurrence of viral disease outbreaks. The adverse effects of climate change, such as heat stress, contribute to reduced feed intake and weakened resistance to infections. Additionally, RNA viruses, including the avian influenza virus, pose a substantial threat by causing widespread mortality among poultry populations, resulting in substantial economic repercussions. Moreover, it is worth noting that RNA viruses possess the capacity to undergo evolutionary changes that enable them to become zoonotic pathogens, so posing a direct threat to human well-being. In order to effectively mitigate the impact of environmental changes, it is imperative to develop novel breeding strategies that facilitate the introduction of favourable traits and enhance the resilience of cattle in the face of potential challenges.

Selective breeding in the chicken industry has led to significant advancements in production, efficiency, and product quality. However, the improvement of health-related traits has not been as easily achievable. Genetic selection approaches are consistently applied to enhance production parameters, in conjunction with state-of-the-art production facilities and protocols [2].

Genome editing is employed as a means to augment the population of animals within the breeding herd that possess advantageous genetic variations. This enables the preservation of important genetic variations that would otherwise be lost due to their tendency to be inherited with deleterious variants. Therefore, it is anticipated that genome editing will offer a broader scope for enhancing advantageous characteristics in cattle populations, including productivity, health, fertility, and safety [3]. Various genome-editing techniques have been effectively employed in a wide range of animals, such as mice [4], monkeys [5], pigs [6], sheep [7,8], goats [9], among others. This technology enables geneticists and medical researchers to manipulate specific regions of the genome through the processes of addition, deletion, or alteration of segments within the DNA sequence. Given its unique reproductive biology, poultry requires specialized approaches in order to achieve heritably modified characteristics. The utilization of genome editing techniques is anticipated to have a substantial influence on the value and prospective advancement of poultry. Furthermore, the utilization of precise editing techniques in the endogenous genome, without the integration of exogenous DNA, has the potential to emerge as a contemporary breeding strategy for the creation of genetically modified organisms intended for human consumption.

High temperatures, high humidity, and other difficult circumstances are frequently observed in tropical environment, and they can have a detrimental effect on the health and productivity of chickens. It is feasible to add genetic alterations that improve poultry tolerance to these environments by genome editing. Researchers can create poultry breeds that flourish in tropical climates by discovering and manipulating genes linked to heat stress tolerance. This will improve the breeds' overall performance and output.

Genomic editing has emerged as an efficient method to confer valuable features including as disease resistance, meat output, meat quality, egg weight, egg number, and qualities that enhance animal welfare. This article provides a comprehensive analysis of genome-editing technology as a means to improve poultry products. During our discussion, we also examined the various issues that arise in relation to genome-edited animals, as well as the potential future applications of this technology.

2. IMPROVEMENT OF ECONOMICALLY ADVANTAGEOUS TRAITS IN FARM ANIMALS

The primary objective of animal breeding is to enhance genetic features that contribute to economic value, including growth, disease resistance, meat production and quality, vis-a-vis reproductive traits. Over the course of recent decades, significant advancements have been achieved in the augmentation of economic qualities, such as growth and reproduction, through the utilization of selective breeding and crossbreeding techniques. Nevertheless, the utilization of traditional breeding techniques has proven to be both expensive and time-consuming. Furthermore, many polygenic characteristics, such as disease resistance, have not experienced significant enhancements. The advancement of genetic manipulation technology in large animals, specifically the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) system mediated genome editing, has facilitated the efficient modification of desired traits in agricultural animals within a single generation. The CRISPR system has sparked a paradigm shift in the realm of genetic manipulation, significantly broadening its scope and offering invaluable resources for the advancement of animal biotechnology research and cattle breeding. Significant progress has been achieved in the field of genetic manipulation, encompassing gene editing techniques such as base editing [10] and prime editing [11]. Additionally, breakthroughs have been made in the areas of transcriptional control and post-transcriptional engineering, utilizing tools based on the CRISPR system. In recent years, the utilization of CRISPR techniques has brought about a significant transformation in the domain of animal breeding. The utilization of these methods has demonstrated significant potential in not only decreasing the duration of selection and lowering production expenses, but also in enhancing traits that are challenging to attain or not easily modifiable by conventional breeding techniques in farm animals.

2.1 Genome Editing in the Poultry Industry

The process of genetically modifying chicken is relatively more challenging when compared to other livestock due to the distinct physiological characteristics of avian eggs in contrast to mammalian oocytes. The impracticability of

isolating and transferring a chicken yolk was evident in this scenario. However, [12] proposed a novel method involving ovo electroporation of editing reagents. It should be noted that this technique led to mosaicism, with editing occurring only in a subset of cells. This limitation arises from the fact that the chicken embryo is already at an advanced stage of development when the egg is laid, as opposed to the zygote stage [13]. Consequently, the likelihood of producing genetically modified birds using this methodology was low. Subsequently, a novel approach referred to as sperm transfection-assisted gene editing emerged, involving the utilization of lipofected sperms that are subjected to editing reagents prior to artificial insemination [14]. However, it was ultimately the progress made in chicken stem cell research that demonstrated the most promising prospects for manipulating the chicken genome. Similar to fibroblast cells found in mammals, primordial germ cells (PGCs), which are stem cells that eventually differentiate into germ cells, can be extracted from the blood of developing chicks in-ovo and subsequently grown in vitro. The chick embryo is observed by means of an aperture in the eggshell, which subsequently necessitates resealing until the chick undergoes hatching. The successful demonstration of genome editing in primordial germ cells has been achieved by multiple groups [15,16]. Notably, one of these groups has successfully generated edited birds [17]. Genome editing in chickens can be achieved using three distinct methods: electroporation in ovo, sperm lipofection, or separation and editing of primordial germ cells. In each of these methodologies, the outcome will be characterized by heterozygosity or mosaic patterns, necessitating breeding in order to produce homozygous avian specimens.

2.2 Genome Editing Techniques in Poultry Production

Genome editing is a process that initiates a double-strand break in the DNA molecule, leading to alterations in the nucleotide sequence through the activation of DNA repair mechanisms. At present, there are three distinct genome editing technologies available, namely zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), and clustered regularly interspaced short palindromic repeats/CRISPR-associated protein (CRISPR/Cas) [18]. The area of molecular biology has been greatly enhanced by genome editing tools, with special emphasis on the CRISPR/Cas system. One notable benefit of the CRISPR/Cas system is in its capacity to efficiently and expeditiously generate genetically modified animals by means of direct administration of the genome editing tool into a single-cell fertilized egg originating from mice and many other vertebrates. On the other hand, the application of direct injection of genome editing tools in chickens is currently not feasible. Additionally, the establishment of in vitro fertilization methods for chickens remains incomplete due to the challenges associated with handling their one-cell fertilized eggs, which are characterized by high levels of egg yolk. Furthermore, it has been observed that the generation of transgenic mice using pluripotent stem cells, specifically embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), has been successful. However, the process of inducing chicken embryonic stem cells (ESCs) to achieve germinal transmission has proven to be challenging [19,20]. While the generation of chicken iPSC-derived offspring has been achieved in a recent study conducted by [19], the widespread use of this method has not been observed. As a result, the development of genome-edited chickens has seen a delay in comparison to other animal species, such as mice.

3. PROGRAMMABLE GENOME EDITING TECHNOLOGY BASED ON CRISPR/CAS9

The initial iterations of programmable genome-editing tools consisted of zinc finger nucleases (ZFNs), which were then succeeded by transcription activator-like effector nucleases (TALENs), and eventually supplanted by CRISPR/Cas9 technology. Using genome-editing techniques, researchers have the ability to deliberately create a double-strand break (DSB) at a specific location, resulting in the deactivation of a target gene or the insertion of foreign gene cassettes by introducing a donor DNA template. Furthermore, the utilization of base-editing and prime-editing methods facilitates a higher degree of efficacy and accuracy in genome alteration, eliminating the need for a donor plasmid.

3.1 Base Editing Technologies

In recent times, there has been a significant advancement in the field of genome modification with the development of CRISPR/Cas9-mediated base editing technology. This technology allows for a more accurate and targeted approach to

modifying the genome. The cytosine base editor (CBE) is comprised of modified Cas9 (either nickase Cas9 or dead Cas9), cytosine deaminase, single-guide RNA (sgRNA), and uracil N-glycosylase inhibitor (UGI). This molecular tool has the ability to change cytosine (C) to thymine (T) or guanine (G) to adenine (A) without causing a double-strand break (DSB), as demonstrated by [21]. The adenine base editor (ABE), which is an alternative base editing method, is comprised of a nickase Cas9 enzyme that has been genetically modified. The approach described by [22] involves the utilization of coli tRNA adenosine deaminase (TadA*) and sgRNA to catalyze the conversion of adenosine to guanosine (or thymine to cytosine) through the process of adenosine deamination in DNA. DNA base editing can nevertheless result in the occurrence of indel mutations, albeit at significantly reduced frequencies. As a result, base editing demonstrates a higher degree of precision in genome editing outcomes compared to conventional CRISPR/Cas9 technology, with a relatively low occurrence of off-target effects. Additionally, this approach does not necessitate the use of exogenous donor template DNA. Given these benefits, base-editing technology is extensively utilized not only in the agricultural sector and fundamental scientific investigations, but also for medicinal intentions [23].

3.2 Prime Editing Technologies

Base-editing technologies that have been recently demonstrate high efficiency in performing four types of nucleotide-to-nucleotide conversions, namely C to T, G to A, A to G, and T to C. Nevertheless, the capabilities of these technologies are constrained when it comes to executing all 12 categories of conversions. Additionally, achieving accurate alteration of insertion and deletion mutations (indel) becomes challenging without the introduction of a double-strand break (DSB) or a donor template. The authors of the study conducted by Anzalone et al. (2019) propose a novel prime-editing technology that addresses the limitations of existing genome-editing methods. This technology incorporates a nickase Cas9 (H840A), prime-editing extended guide RNA (pegRNA), and a mutated Moloney murine leukemia virus reverse transcriptase (M-MLV RT). By utilizing these components, the researchers demonstrate the enhanced potential of this approach to achieve precise and reliable modifications to the genome. The introduction of mutations in the M-MLV RT component results in enhancements in processivity, thermostability, and binding affinity between the DNA and RNA substrates. Consequently, these improvements lead to an increase in prime-editing efficiency. Furthermore, in conjunction with the pegRNA, the utilization of an additional sgRNA that generates a nick on the non-edited strand can augment the efficiency of editing. The prime-editing technology discussed herein represents a very sophisticated kind of genome-editing technology that has been deemed the most advanced to date. This cutting-edge technology possesses the remarkable capability to achieve exact modifications inside the genome, while concurrently minimizing the occurrence of off-target consequences, surpassing the capabilities of traditional genome-editing technologies. Although prime-editing technology has several benefits, it is currently limited by the high size of the protein structure, which hinders its efficient transport in vitro or in vivo. Moreover, it is unable to produce precise alterations in big indel mutations. Hence, in order to extend the applicability of this strategy, additional research is required to devise more refined systems capable of surmounting existing constraints and enhancing both efficiency and specificity [24].

3.3 Pgcs Mediated Genome Editing in Poultry

In the context of chicken development, primordial germ cells (PGCs) are localized inside the central region of the area pellucida during the Eyal-Giladi and Kochav (EGK) stage X. Subsequently, these PGCs undergo migration towards the germinal crescent following the creation of the primitive streak, as described by [25]. Following this, primordial germ cells (PGCs) undergo circulation through the blood arteries of the developing embryo and then migrate to and establish themselves within the embryonic gonads. As a result of the distinctive migratory trajectory of avian primordial germ cells (PGCs), these cells can be extracted from different developmental stages of the embryo and maintained in culture while retaining their germline potential. Upon injection into the blood vessels of recipient embryos, these cultivated primordial germ cells (PGCs) establish residence in the gonads, hence leading to the generation of a germline chimera) [26]. Offspring that have undergone genome editing can be created through the injection of genome-edited primordial germ cells (PGCs) into a germline chimera. In recent times, the successful implementation of genome editing in chickens has been achieved

by the utilization of cultivated primordial germ cells (PGCs) and the CRISPR/Cas9 system. This breakthrough has facilitated several applications in the field.

The initial instance of a genetically modified chicken utilizing the CRISPR/Cas9 technology was documented in the year 2016. The present investigation involved the targeted disruption of ovomucoid (OVM), a prominent allergen found in egg white, in chickens by the utilization of CRISPR/Cas9 technology. The aforementioned work provided evidence that the CRISPR/Cas9 system can be effectively employed in chicken primordial germ cells (PGCs) to induce targeted mutagenesis, leading to the successful generation of hens with modified genomes. The researchers in the study conducted by [27] utilized the CRISPR/Cas9 system to delete the myostatin (MSTN) and G0/G1 switch gene 2 (G0S2) in chickens.

One of the limitations associated with HDR is its comparatively lower frequency in comparison to NHEJ, as well as the necessity for the donor vector to possess extended homology arms [28]. Therefore, the utilization of NHEJ-mediated knock-in, which has demonstrated higher efficiency compared to HDR, together with the use of uncomplicated donor vector architectures, has been observed in a wide range of species. The application of non-homologous end joining (NHEJ) for knock-in purposes in chicken was initially documented in 2018. The study conducted by [29] involved the precise insertion of a green fluorescent protein (GFP) expression cassette into a specific genomic region situated between DNAJ homolog subfamily A member 1 (DNAJA1) and DNA replication regulator and spliceosomal factor (SMU1) on the Z chromosome. This targeted modification led to the successful generation of chickens expressing GFP, which can serve as a valuable avian sexing model.

3.4 Genome Editing in Poultry using other Methods

While cultured primordial germ cells (PGCs) have proven to be effective in performing genome editing in poultry, it is important to acknowledge the limitations associated with this method. Within the realm of avian species, it has been observed that the cultivation of primordial germ cells (PGCs) in vitro has only been accomplished successfully in the case of chickens, thus far, over extended periods of time. In many avian species, such as quail, the culturing of primordial germ cells (PGCs) over multiple generations is not feasible, hence posing challenges in the process of identifying and amplifying genome-edited PGCs. Furthermore, the utilization of PGC-mediated techniques necessitates a significant investment of time, as it involves the careful selection of genome-edited PGCs, the process of microinjection, and the subsequent raising of G0 germline chimeras until they reach sexual maturity in order to get offspring with edited genomes. Hence, the development of innovative approaches for the generation of genetically modified poultry is imperative.

One approach utilized in the production of genome-edited avian species is known as Sperm Transfection–Assisted Gene Editing (STAGE). This method entails the direct transfection of spermatozoa with both Cas9 messenger RNA (mRNA) and single-guide RNA (sgRNA). The utilization of transfected sperm for insemination enables the direct production of genome-edited offspring, which represents a significant benefit of STAGE in comparison to PGC-mediated genome editing. [30] demonstrated the successful implementation of genome editing in chicken embryos using the STAGE technique. However, it was observed that the efficiency of generating genome-edited offspring was rather low, indicating the need for further enhancements in this regard.

An alternative approach entails the direct administration of plasmids into the vasculature of developing embryos. The introduction of Tol2 transposon and transposase plasmids into recipient embryos via lipofectamine has the potential to induce genetic modification in circulating primordial germ cells (PGCs) and generate transgenic chickens. According to a recent study, it has been found that the co-injection of a Tol2 transposon plasmid carrying Cas9 and sgRNA expression cassettes, along with a transposase plasmid using lipofectamine, can result in the production of G1 progeny that exhibit stable expression of Cas9 and sgRNA. In the study conducted by [31], it was observed that the approach employed resulted in the production of both transgenic and non-transgenic progenies with genome-edited characteristics in the G2 generation.

In summary, the use of CRISPR/Cas9 technology has proven to be effective in the field of poultry genome editing, leading to the creation of diverse genome-edited poultry strains with

various intended applications. As elucidated in the subsequent section, the utilization of genome-edited poultry is anticipated in several sectors, including agriculture and biomedicine.

4. DEVELOPMENT OF DISEASE RESISTANT BREEDS USING GENOME EDITING TECHNOLOGY

Enhancing the resistance or tolerance to diseases is the most important use of genome editing in agricultural animals. Infectious diseases affecting farm animals not only result in significant economic losses within the animal husbandry sector, but also pose a substantial risk to human health and food security. The issue at hand has long been a formidable challenge within the industrial sector, causing concern among animal breeders and veterinary professionals. However, the feature of disease resistance is intricate and influenced by multiple genes, making the conventional genetic selection for disease resistance breeding a more expensive, time-consuming, and ineffective approach. Furthermore, the widespread implementation of vaccines is both time-consuming and inefficient. Likewise, the utilization of vaccinations and medications has somewhat diminished the imperative for disease resistance selection programming. Transgenic and gene targeting techniques have been effectively employed in the development of antiviral animals, namely in the case of prion protein-free chickens [32]. In a study conducted by [33], it was observed that pigs were genetically modified to produce anti-foot-and-mouth disease virus (FMDV) small hairpin RNA (shRNA). In recent times, there has been significant advancement in genome editing technology, namely the utilization of CRISPR/Cas for gene knock-out/knock-in and precision modification. These advancements have greatly enhanced the efficacy of animal breeding for disease resistance.

In order to induce disease resistance traits in poultry birds, [34] performed genome editing on chicken DF-1 fibroblasts. This approach facilitated the investigation of the involvement of **chicken Na+/H+ exchange type 1** (chNHE1) in viral interactions within the avian system. The avian leukosis virus has been the subject of extensive research for several decades. However, despite these efforts, there remain significant gaps in our scientific understanding of this virus. These gaps pertain to various aspects, such as its ability to induce tumour formation, its impact on immune suppression, and its mechanisms of immune evasion [35]. It is worth mentioning that the oncogenic processes of Avian Leukosis Virus (ALV) and Marek's Disease Virus (MDV) exhibit dissimilarities. The MDV virus possesses an oncogene that has the capability to directly initiate the development of tumours within the organism. On the other hand, ALV viruses integrate with particular cellular genes through their proviral DNA. The insertion of the viral promoter in close proximity to these genes leads to an increased expression of the genes, ultimately resulting in the creation of neoplasms [36]. As a consequence of the swift progression of ALV, the current absence of efficacious therapies and vaccines, along with potential inadequacies in other biosecurity measures, persists. Hence, conventional breeding techniques can be employed to cultivate resistance or tolerance to diseases.

Exogenous retroviruses (ERVs) could be regarded as a plausible genetic determinant, as suggested by [37]. [37] have identified over 400 endogenous retroviruses (ERVs) within the poultry genome. While knowledge has been acquired regarding a subset of these ERVs, the majority of them remain uncharacterized. Endogenous retroviruses (ERVs) present in the host organism exhibit both advantageous and disadvantageous characteristics. The stochastic integration of endogenous retroviruses (ERVs) within genes has the potential to generate commercially valuable features. However, this integration event may also render the host organism more vulnerable to exogenous viral infections and heighten the likelihood of recombination events involving exogenous viruses. As an illustration, the integration of ev21 into the genetic material resulted in the development of a commercially advantageous characteristic in chickens, namely the slow-feathering plumage phenotype. However, this genetic modification also notably heightened the host's vulnerability to ALV-J infection [38]. Nevertheless, the proliferation and maturation of fast-feathering hens lacking the ev21 gene exhibited no discernible disparities compared to their counterparts, so suggesting that ev21 does not possess the characteristics of an indispensable gene for the host organism. Indeed, it is possible to selectively remove individuals from the genome who possess related endogenous retroviruses (ERVs) as required. In order to harness the potential benefits offered by these unique endogenous retroviruses (ERVs), it is imperative to enhance

the stringency and efficacy of biosecurity protocols, as well as implement comprehensive disease mitigation strategies.

Viruses are capable of entering target cells solely through the process of attaching to certain receptor proteins found on the surface of host cells. The incorporation of proviral DNA of Avian Leukosis Virus (ALV) is a stochastic and unpredictable occurrence. This observation underscores the significance of inhibiting or disrupting the interaction between viruses and cellular receptors, and furthermore implies that viral infections can be averted by the alteration or removal of host cell receptors. Fortunately, comprehensive knowledge regarding the receptors linked to various avian leukosis virus (ALV) subgroups in chicken has been acquired. This knowledge encompasses the genetic composition of these receptors, the specific amino acid locations to which the virus binds, and the existence of alleles that confer resistance to ALV. The genomes of Chinese indigenous chicken breeds exhibit a high frequency of Tva and Tvb resistance alleles, which contributes to their significant genetic selection potential [39]. The examination of the resistance of subgroup C will be deferred to a subsequent stage, as its occurrence within domestic chicken populations is infrequent. Enhancing resistance to subgroup J is presently the foremost priority. According to [40], the genetic sequence of Na+/H+ exchange type 1 (NHE1) in domestic chicken breeds exhibits a high degree of conservation. This conservation may perhaps explain the prevalence of subgroup J in China. This gene may potentially function as a therapeutic target or a significant genetic locus for the purpose of selecting disease resistance in breeding programs.

Transcriptome sequencing technology enables the identification of additional interferon-stimulated genes (ISG) and immune-related genes within the chicken genome, hence enhancing the potential for enhancing disease resistance in poultry. These genes can be identified and their genotypes (SNPs) can be utilized for the purpose of selective breeding. Selective breeding can serve as a means of coordinating gene editing efforts, since it enables the exact modification of specific loci found in genome sequencing data. This approach facilitates the introduction of new alleles that are linked to economically significant features. The integration of genomics and gene editing methodologies has been shown to enhance the efficiency of chicken breeding programs aimed at developing disease-resistant strains [41,42]. Over the past few decades, various gene editing technologies have been developed, with the PGC mediated method and CRISPR/Cas9 system being the predominant approach [41,42].

[19] employed CRISPR/Cas9 genome editing techniques to deliberately induce frame-shifting indel mutations within the Tva, Tvc, and NHE1 genetic loci. These loci are responsible for encoding receptors specific to the ALV subgroups A, C, and J, respectively. In DF-1 cells, the phenotypes created by homozygous frame-shifting indels at all three loci resulted in complete resistance to the appropriate subgroup virus. Chicken primordial germ cells (PGCs) were successfully modified using the CRISPR/Cas9 gene editing technique, resulting in the creation of a genetically altered chicken line known as NHE1 ΔW38 hens. These chickens were found to be resistant to ALV-J, a common viral infection in chickens. This achievement was documented in the scientific literature by [43], [44] and [45]. The mutation NHE1 ΔW38 confers resistance to HPRS-103 in chickens, and the deletion of W38 does not adversely affect chicken growth and health, as demonstrated in studies by [19], [46], and [43]. Additionally, employing the identical approach, a transgenic strain of commercially bred chickens that exhibit resistance to ALV-A/K was successfully acquired after a period of 9 years. According to the study conducted by [44]. The findings indicate that the viral receptor of Avian Leukosis Virus (ALV) is dispensable in the growth cycle of chickens, and its elimination can confer resistance. Simultaneous editing of the binding receptor sites of various subpopulations can potentially bestow resistance to multiple Avian Leukosis Virus (ALV) subgroups in chicken. Additionally, the utilization of CRISPR/Cas9 gene editing technology has the potential to decrease both the duration and financial resources required for the breeding of chicken with enhanced disease resistance.

4.1 Improving the Well-Being of Animals

The application of genome editing techniques in livestock holds the potential to enhance the resistance or tolerance of farm animals to infections, as well as improve their production performance. Additionally, it has the capacity to mitigate unnecessary animal suffering, hence potentially fostering public acceptance and support for the utilization of genome editing approaches in food chain production.

In modern livestock production, the daily handling of poultry birds with their sharp beaks and nails presents a significant hazard, both in terms of potential harm to one another and to the farmers involved. The practice of physical beak trimming in birds is implemented with the intention of safeguarding both animals and farmers from inadvertent harm. However, it is important to acknowledge that this procedure is linked to the experience of stress and pain in avian species. Most poultry birds are known to possess naturally occurring structural variations that result in a blunt beak.

4.2 Application of Genome-Edited Poultry in Many Industries

In subsequent years, it is anticipated that the global population would persistently expand, thereby leading to a proportional rise in the demand for animal-derived food products. According to the Food and Agriculture Organization (FAO), it is projected that the global population will reach 9.7 billion by the year 2050. Furthermore, the FAO predicts a substantial increase in the demand for animal food products, estimating a rise of 70% [45]. In anticipation of this heightened demand, it is imperative to enhance economic qualities such as productivity, disease resistance, and heat tolerance. Researchers can utilize genome sequencing technology to identify genetic variations that play a role in enhancing economic features. This DNA information can then be applied in selective breeding practices. The integration of genome editing techniques with selective breeding practices can be achieved due to its ability to accurately modify specific genomic regions that have been identified through the analysis of genome sequencing data. This enables the introduction of new genetic variations associated with economically significant features, all while avoiding the incorporation of transgenes. Numerous investigations have employed genome-editing techniques in cattle to bestow advantageous characteristics, such as disease resistance and heat tolerance, with the aim of enhancing productivity. It is anticipated that this line of research will witness significant acceleration in the forthcoming years [18].

Genome editing techniques have been employed in the field of chickens to enhance traits such as muscle productivity, feed conversion ratio, and disease resistance. The myostatin (MSTN) gene is responsible for encoding a protein that acts as a suppressor of muscle development. Studies have shown that the elimination of MSTN by knockout techniques leads to a substantial increase in muscle mass across several animal species [47]. Therefore, the modulation of MSTN represents a significant focus for enhancing livestock productivity [8]. The successful elimination of MSTN and G0S2 has been achieved in the context of poultry. According to a study conducted by [26], it was shown that the absence of myostatin (MSTN) in chicken and quail resulted in a substantial increase in muscle mass. Additionally, in G0S2-knockout chicken, a reduction in fat composition was seen. In recent times, there has been significant progress in the advancement of genome sequencing technologies, which have been extensively utilized in the field of poultry breeding. The primary objective of employing these technologies is to identify genetic markers that have an impact on productivity within poultry populations [48]. Simultaneous editing of these genomic markers is achievable through the utilization of the CRISPR/Cas9 system. The integration of genomics and genome editing techniques is poised to significantly expedite the progress of chicken breeding.

Viral infections in chicken are associated with substantial economic losses and has the capacity to drastically diminish poultry productivity. The process of genome editing has the potential to bestow resistance against viral infection by altering essential host components responsible for viral entrance or replication [49]. In order to acquire admission into target cells, viruses engage in the process of binding to host cell receptor molecules. According to [50], the prevention of viral infection can be achieved through the precise deletion of the receptor, as the virus-receptor interaction is known to exhibit a high degree of specificity. Therefore, the utilization of CRISPR/Cas9-mediated genome editing is a viable approach for the creation of avian models that are resistant to diseases by the precise targeting of host receptors. [51] conducted preliminary studies on DF1 chicken fibroblasts, wherein they employed CRISPR-Cas9 technology to achieve accurate gene editing of the chNHE1, avian leucosis virus (ALV) subgroup receptor *tva, tvb*, and *tvc* genes. This genetic manipulation resulted in the acquisition of resistance against infection by ALV subgroup J (ALV-J), A (ALV-A), B (ALV-B), and C (ALV-C), respectively. The findings from these initial investigations demonstrate the successful development of genetically modified hens by genome editing techniques. These chickens were specifically engineered to possess a

targeted deletion at residue 38 (W38) in the chNHE1 gene, resulting in resistance to ALV-J infection. The involvement of host factor ANP32A in supporting the viral polymerase **(**Vpol) activity of the influenza A virus (IAV) has been identified as crucial [52]. The presence of an additional 33 amino acids between the leucine-rich repeats and C-terminal acidic region in chicken ANP32A has been observed. [52] found that the deletion of these 33 amino acids leads to a considerable disruption of IAV replication in avian cells. According to [52], there is speculation on the potential production of an avian influenza virus (IAV)-resistant chicken through the exact deletion of these 33 amino acids using genome editing techniques. The findings of this study suggest that the identification of host variables that play a crucial role in viral entrance or replication can lead to the effective development of disease-resistant lines by genome editing techniques. Moreover, the concurrent editing of these host variables holds the potential to facilitate the development of chickens that exhibit resistance to numerous illnesses [52].

4.3 Bioethical Concerns in the use of Genome Editing in Animal Production

Despite the fact that, genome editing plays positive roles in animal production such improvement of animal welfares, sustainability and optimization of animal protein security, there are still some ethical concerns against this vital technology. Some of these concerns are presented in Table 1 and addressing them requires concerted efforts of the main stakeholders like researchers, scientists, farmers, consumers, commercial entities, vis-à-vis animal welfare groups.

4.4 Prospects and Challenges

The utilization of genome editing technology presents innovative tools and approaches for the purpose of changing animal genomes, hence creating novel opportunities for the fields of livestock breeding and animal husbandry. It is anticipated that more uses will be devised, leading to the imminent availability of genome-edited livestock-derived meat for consumption. Nevertheless, the issue of off-target effects remains a significant problem in the context of animal production for agricultural purposes. Off-target mutations have the potential to cause knock-out events or silent alterations in protein coding-genes, as well as disrupt transcriptional control. Mutations occurring in protein-coding areas have the potential to result in the production of proteins with abnormal structures. Consequently, these aberrant proteins may contribute to the development of food allergies. Translational regulatory alterations can potentially exert significant effects on various aspects of animal physiology, including but not limited to health, reproduction, and growth performance. Subsequently, there has been a notable surge in interest surrounding low-risk genome editing techniques, particularly DNA-free genome editing. According to [56], the implementation of DNA-free genome editing approaches has the potential to significantly mitigate the occurrence of off-target mutations. Somatic cell nuclear transfer (SCNT) has the potential to be employed in the eradication of off-target mutations prior to the breeding of genetically engineered animals (GEAs). The SCNT technique enables the pre-production verification of donor cell genotype and identification of off-target mutations in live animal production. Additionally, it has the potential to mitigate the development of genetic mosaicism and decrease the overall expenditure associated with the production of genome altered animals. These factors are of particular importance when considering their application in large domestic animals with extended generation intervals.

Table 1. Potential risks and bioethical concerns of genome editing technology in farm animals

Risks	Bioethical concerns	Authors
Unintended negative effects on animal health, welfare and environment	Unintended consequences	[39]
Narrow range of desirable traits, diseases and pests vulnerability	Loss of genetic diversity	[53]
Unnatural and disrespectful to animals	Socio-cultural	[54]
Competition with native species, introduction of novel diseases into the environment	Environmental impact	[55]

5. CONCLUSION

In conclusion, genome editing holds great potential for enhancing poultry productivity in tropical areas. Scientists are able to introduce traits that improve feed conversion efficiency, increase resistance to disease, and increase tolerance to tropical settings through meticulous genetic engineering. The application of this technology not only raises the general productivity and profitability of chicken farming in these regions by reducing the need for antibiotics and minimising their adverse effects on the environment, but it also promotes sustainable practices. The advancement of genome editing technology offers promising prospects to tackle the increasing need for superior quality protein and transform chicken farming in tropical regions.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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