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Harnessing CRISPR/Cas9 for Poultry Improvement: Applications in Egg Albumin and Industrial Innovation

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A B S T R A C T

Egg albumin, a protein-rich component of egg, has considerably drawn interest in various sectors because of its special qualities and numerous applications. The common egg albumin modifying techniques covered in this article are gene editing, base editing including RNA base editing and cytosine base editing, and CRISPR technology. CRISPR/Cas9 technology revolutionizes genetics research in animal models, including poultry. It allows scientists to manipulate gene functions for transcriptional regulation, targeting, epigenetic modification, gene therapy, and drug delivery. These peptides indicate antibacterial, antioxidant, and antihypertensive properties, making them an intriguing potential option for nutraceutical and functional food compositions. The enormous potential application of egg albumin proteins as functional components is highlighted in this review, which also stresses the significance of effective extraction methods. CRISPR-mediated techniques can improve genetic variants in chicken populations, allowing for genetic information introduction or regulation. However, some drawbacks must be addressed before it can be used in the chicken industry. To investigate innovative ideas, additional research and development work is required.

Keywords: *Egg Abumin, CRISPR/CAS9, Ovotransferrin, Conalbumin, Ovalbumin*.

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INTRODUCTION

Egg white, also termed egg albumin and/or ovalbumin, is a nutritive, and protective gelatinous substance that surrounds the yolk. Global annual production of eggs is about seventy million tonnes. The nutritive benefits of eggs make them a desirable and inexpensive complete food. Eggs are widely consumed as a whole or in the form of egg products. The organoleptic qualities of eggs involve their utilization in innumerable food products such as pulps, seafood, meat, dairy foodstuffs, *etc*. Egg albumin proteins; ovalbumin and conalbumin (constituting 70% of egg white) because of their heat coagulation capability, are considered as an important constituent of food products (Imani et al., 2023) . Egg albumin also contains a smaller concentration (up to 1.8% mannose) of carbohydrates. Both enzyme-modified and unmodified proteins can be utilized by the food and pharmaceutical industries. Lysozyme is primarily utilized as a food preservative, whereas Ovo-transferrin functions as a metal transporter, an antimicrobial, or an anticancer medication (Lambré et al., 2023).

Domestic chicken egg has the lowest concentration of albumin while hybrid chicken has the highest concentration. The major protein constituent of egg albumen includes ovalbumin, ovotransferrin ovomucoid lysozyme, and ovomucin up to 54%, 12%, 11%, 3.5%, and 3%, respectively. Other proteins which constitute negligible quantities include Avidin, Cystatin, Ovo-macroglobulin, Ovo-flavoprotein, and Ovo-glycoprotein. Egg albumen proteins with efficient characteristics can be extracted by using various techniques (Abeyrathne et al., 2013). Peptides produced by ovotransferrin, a major protein responsible for various bioactivities, find their tremendous potential as antimicrobial, antioxidant, and anti-hyperactive anticancer agents The structural perspective indicates that hen ovotransferrin constitutes single polypeptide with amino acid residues (686), encompassing an isoelectric point (6) and a molecular mass of 77.7 kDa. Conalbumin, a monomeric transferrin, accounts for 13% of egg white protein, crucial for iron homeostasis. Gene duplication and fusion result in a protein with homologous portions binding iron atoms, used in pharmaceutical, medical, and food processing industries (Moreau et al., 2022).

Ovalbumin, which accounts for more than 50% of the egg white proteins, is comprised of a 45 kDa monomeric phosphoglycoprotein, that has one carbohydrate unit, two phosphoserine residues, one disulfide link, and four SH groups. There are three -sheets and nine -helices in it. The denaturation of ovalbumin takes place at a temperature near 84°C. In-vitro production of S-ovalbumin involves cell incubation at 55°C, 100 mM Na_3PO_4, pH 10, and accelerated conversion rate. When using protein fractionation to create lysozyme and ovalbumin, chick egg white (CEW) is the most affordable supply (Legros et al., 2021).

Table 1: Therapeutic and nutraceutical Advantages of egg albumin.

Applied Characteristics	Essential components	References
Anti-microbial Activity	Lysozyme, oval-albumin, ovotransferrin, ovomucin, Avidin	(Jabalera et al., 2022)
Anti- carcinogenic or anti-tumor property	Lysozyme, ovotransferrin, ovomucin, igY	(Aljabali et al., 2020)
Anti-inflammatory property	Phospholipids, lutein, HDL, phosphivitin	(Salim et al., 2023)
Anti -adhesive	Bioactive peptides	(Zhang et al., 2021)
Anti-oxidative activity	Cystatin, Ovomucin, Ovalbumin, Lysozyme, Ovotransferrin, Carotenoids, Phosvitin, Ovoinhibitor, Phospholipids, Zeaxanthin/Lutein, Selenium, Vitamin E,	(Zhang et al., 2021)
Hypotensive property	Lysozyme, Bioactive peptides hydrolysates of egg yolk, HDL, Ovomucin	(Lee & Paik, 2019)
Anti-viral efficacy	Ovomucin, Lysozyme, IgY, Bioactive peptides, Ovotransferrin	(Lee & Paik, 2019)

Genome-Centric Modification of Egg Albumin

Genome editing technology makes it simple and quick to introduce advantageous genetic alterations. Genome editing may precisely change the DNA sequence (genome) of particular DNA segments (genes) to improve livestock output without resulting in any unwanted problems. Protein medications function more effectively due to a particular N-glycosylation structure seen in chicken egg white proteins. Gene targeting techniques have been developed to change specific gene locations (gene loci) in the genome, however, the conventional approach of applying a targeted gene vector and connecting it with homology-based recombination is ineffective and contains methodological issues.

Genome editing technologies like TALENs enable gene knockouts in chickens, allowing for the production of mutant offspring by knocking out genes (Zhou et al., 2018). Gene editing techniques involve Zinc Finger Nuclease (ZFNs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated or linked protein 9 (CRISPR or Cas9) are also considered well-liked and innovative approaches for genome editing. The two domains are obligatory for precise and error-free gene and genome editing. The two genome editing techniques *i.e.*, ZFNs and TALENs, are considered of less potential as compared to CRISPR/Cas9 Technique which is due to the latter’s simplicity, flexibility, efficiency, and cost-effectiveness (Park et al., 2014).

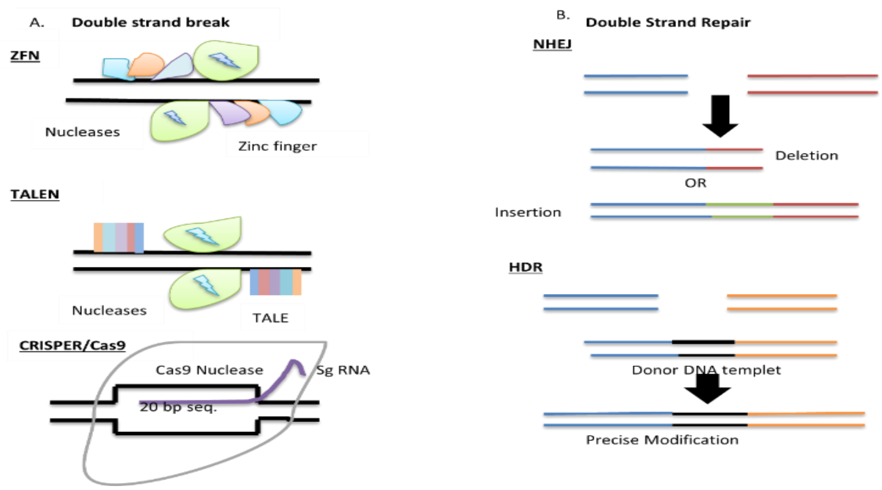

Figure 1: Gene Editing Tools for the modification of Egg albumin.

Prime Editing Technique

A novel gene or genome-editing technique known as "Prime editing" was created by the combination of reverse transcriptase enzyme adjoined with the Prime Editing gRNA known as pegRNA (Khwatenge & Nahashon, 2021)This technique allows for the editing or "search and replace" of nucleotide bases in mammal cells without triggering a double-strand breakdown and with minor collateral mutilation to the DNA donor template. Prime Editing (PE) limits BEs, although BEs can effectively implant four conversion alterations or mutations (C to T or G to A, A to G, and T to C). The 12 transitional modifications (C/A, C/G, G/C, G/T, A/C, A/T, T/A, and T/G) can all be installed in the genome through Prime editing. BEs demand that the target site alter PAM in a certain location. This is why the presence of numerous Cytosine (C) or Adenine (A) bases results in unintended bystander mutations. Due to the presence of many Cytosine(C) or Adenine (A) bases in the "edit window" or the absence of a PAM spot after 15 to 2 about the target edit, undesired bystander mutations develop. When an edit is undesirable or the targeted location does not have positioned PAM, PE can edit. With minimal off-targeted editing, the mutations that cause Tay-Sachs disease and sickle cell anemia have been fixed, and PE can precise up to 88% of identified damaging mutations. Prime editing is an immature base editing technology. More research on animal and plant models is necessary to transform primary editing into therapeutic gene editing (Khwatenge & Nahashon, 2021) summarized in Figure 2.

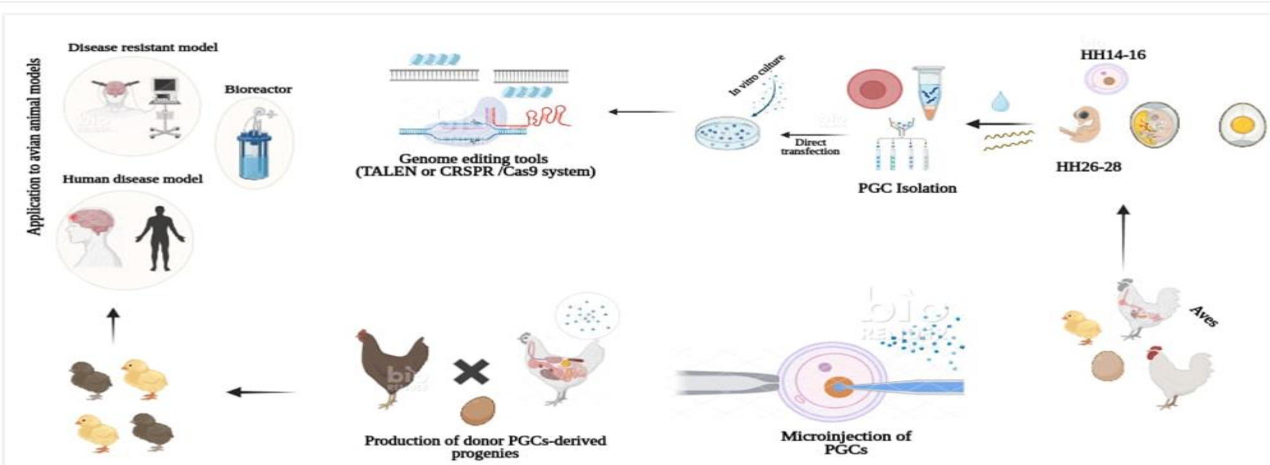

Figure 2: Comparative description of new & traditional strategies in the modification of Poultry egg albumin.

Transgenic Engineering of Mediated Poultry Genome

Germline alterations are crucial for hybrid animals and birds, with transgenic technologies enabling genetically altered chicken lines. The first transgenic chicken was created in 1987 using the ALV vector, and replication-defective lentiviruses improved germline transmission. However, viral vectors still have downsides. Transposons enhance transgene delivery to embryos and cultured PGCs by allowing DNA sequences to jump from a plasmid to a cell's genome, enabling effective integration without silencing, unlike viral vectors Aro et al., 2023). The potential application of egg LDL as cryopreservation has been given in Table 2.

Table 2: Potential Applications of Egg LDL as Cryopreservatives.

Extender Applied (%)	Organism Species	Functional Characteristics	References
LDL (4%)	Epididymal Spermatozoa (Canine)	Sperm mobility (Post-thaw), Integrity in the plasma membrane, and integrity in acrosome	(Ichikawa et al., 2022)
LDL (20%)	Collared peccary (*Pecari tajacu*)	higher sperm mobility (post-thaw) membrane undamaged spermatozoa (frozen-thawed)	(Rees & Liu, 2018)
LDL with sucrose (10%)	Hare semen	Improved sperm fertilization, reduced conception, and fertility rates.	(Kim et al., 2023)
LDL (2%–3%) supplemented with Glycerol (2.5%)	Equid semen	Improved post-thaw sperm eminence and mobility.	
LDL (6%–10%) with glycerol	Rhesus monkey (*Maccacca mulatta*)	Post-thaw motility was comparable.	(Kwon et al., 2018)
LDL (10%)	Buffalo bull semen	Improved freeze-thaw mobility, fertility, and freeze-thaw efficiency.	

The avian embryo is a particularly accessible archetypal for learning growth and genome editing. Important discoveries regarding toxicity tests, gene editing, elucidating the activities of genes, cell transplantation, and cell tracking have been made using chicken whole embryo cultivation. Eggshell windowing, either with or without a seal, is a straightforward method for manipulating chickens; the latter has shown modest enhancement in hatching rates (Zhou et al., 2023). A substitute eggshell mechanism improves chick and quake development by enhancing egg-laying characteristics, enabling chicken ex ovo-culture methods for tomography and microsurgery applications in non-natural artificial environments (Nishijima et al., 2021).

Crispr/Cas Engineered Modification System of Ovalbumin

CRISPR/Cas gene editing technology evolved from innate bacterial defenses against plasmids and viruses. They have been designed for eukaryotic cell gene editing. Six types have been discovered, with type II being the most researched and experimentally validated in 2008. Cas9 protein, CRISPR RNA gene, insert, duplicate sequences and double RNA director find and cleave target DNA. With the advancement of the CRISPR technique, this is now conceivable to manufacture programmable genome-edited chickens by combining it with an in vitro culture arrangement meant for PGCs. First, gonads or embryonic blood can be used to obtain PGCs in chickens. Following the CRISPR/Cas9 system's delivery, poultry having modified genomes are made by microinjecting directly extracted or in vitro cultivated PGCs to recipient embryos' blood vessels. Recent studies show chicken ovalbumin exon 2 genes can be knocked in with CRISPR or Cas9 for hIFN knock-in (Hussin et al., 2022; Nishijima et al., 2021). Numerous studies involving transgenic species connected to poultry reported developing the primary or first CRISPR-mediated chick. These studies are addressed in the following section. Integrating this unique genome editing technology with genomic analytic tools like CRISPR is the up-to-date inclination in employing the CRISPR or Cas9 technique in hen species aimed to boost their target particularity, productivity, & decrease off-target consequences (Zhou et al., 2018). A schematic diagram showing CRISPR CAS9 delivery in the cell for genome editing has been given in Figure 3.

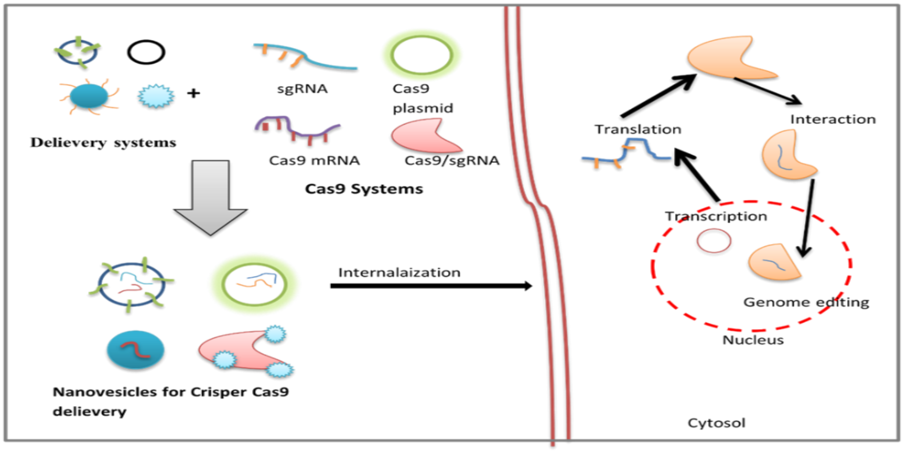

Figure 3: Schematic diagram showing CRISPR CAS9 delivery in the cell for genome editing.

Potential Industrial Applications of Modified Egg Albumin

Egg white, a vital dietary component, holds significant industrial value due to its unique properties and diverse applications. The following sections explore various industrial uses of genome-centric engineered ovalbumins. Eggs serve as a crucial protein and amino acid source globally. For instance, in Nigeria, extruded snacks made from flour blends (maize, rice) and egg white showed a 20% to 50% increase in protein content compared to control samples. Despite increased moisture content during storage, advanced packaging can mitigate this issue, enhancing food security in developing regions (Sun et al., 2017) Exogenous proteins, such as egg white, are often added to improve food processing. A study on oat bread with added Wheat Gluten (WG) or egg white (EW) demonstrated that the addition of Trans-Glutaminase (TGase) enhanced noodle quality, including springiness and cooking properties. The rheological properties were notably improved with TGase, highlighting the benefits of using egg white in food systems (Farzaneh et al., 2018).

The advent of 3D printing technology for personalized food production has introduced new possibilities. Research on 3D printing using egg white proteins, soy, and cricket proteins revealed that while egg albumin reduced certain rheological properties, it remained essential for precise, multi-layered food designs. Soy and cricket proteins showed improved printability and structural consistency (Ezaki et al., 2023). With the consumption of egg white reaching 1.6 million tons in 2020, sustainability concerns have risen. Innovations include plant-based alternatives like "V Loved the Boiled" and the development of edible coatings using chitosan (CS) combined with egg albumin. This approach addresses water resistance and antimicrobial limitations of egg albumin (Cherkaoui et al., 2022). Hydrolyzed egg albumin yields peptides with notable health benefits, including ACE inhibitory, antibacterial, antioxidant, and antihypertensive properties. These bioactive peptides offer potential for functional foods and nutraceuticals, enhancing egg albumin's value beyond its traditional uses (Ricroch, 2019). Microspheres made from egg albumin for oral drug administration, such as paracetamol, have been developed using emulsion and capillary extrusion techniques.

CONCLUSION

Chickens and other poultry models have become increasingly valuable in developmental biology and biomedicine due to recent advancements in genetic engineering technologies. The CRISPR/Cas9 technology, in particular, has revolutionized genetic research by enabling precise genome editing in poultry. This tool allows for the direct modification of genes associated with desirable traits, such as improved feed conversion efficiency, accelerated growth rates, and enhanced disease resistance. Such advancements offer substantial benefits for the poultry industry, including increased productivity and better adaptability to changing environmental conditions. However, there remain several technical and ethical challenges that need to be addressed to fully leverage these technologies in poultry breeding and production. Future studies could focus on optimizing gene-editing protocols to minimize off-target effects and understanding the long-term impacts of genetic modifications on animal health and welfare.

CONFLICTS OF INTEREST

Authors disclose no conflicts of interest.

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