Harnessing CRISPR-Cas9 for genomic improvement of disease resistance in aquatic species

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Abstract

Aquaculture has become one of the fastest-growing food production sectors worldwide. However, the rapid growth of this sector is threatened by the sustainability of aquaculture. Infectious diseases occur, which negatively impact production, product quality, and the economic viability of the sector. The use of traditional approaches in breeding and vaccines has not produced anything durable in the way of disease resistance for the numerous species of aquaculture products. The use of CRISPR-Cas9 technology for genome editing and modification has changed the game in aquaculture. This is the first technology that allows for the precise, efficient, and heritable changes to be made to genes that influence the immune system and resistance to pathogens. This review focuses on the use of CRISPR-Cas9 technology in the aquaculture industry, particularly on fish, crustaceans, and mollusks. It describes the process of gene selection and editing, the delivery of the gene editing tools, and how to prevent unwanted changes that CRISPR tools might cause (Luo *et al.*, 2022). The review also addresses the possible synergy of CRISPR with the omics technologies for next-generation commercial aquaculture. It is suggested that aquaculture sectors with genetically modified CRISPR-Cas9 technology would remove food insecurity and promote sustainable aquaculture on a global level.

Keywords: CRISPR-Cas9, Genome editing, Aquaculture, Disease resistance, Sustainable aquaculture, Fish immunogenomics

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Introduction

Aquaculture is one of the fastest-growing forms of food production in the world. It accounts for nearly half of the world's supply of seafood. It is vital for global food security. But, due to the spread of bacteria, viruses, and parasites, the growth of aquaculture is often stunted because of the high morbidity and mortality losses and the costs of the aquaculture supply chain (Agriculture Organization of the United Nations, 2018) Vaccination, antibiotics, and selective breeding are some of the control methods used. Breeding cycles are long, and there is limited knowledge in the field, which contributes to polygenic and non-specific resistance (Bondad-Reantaso *et al.*, 2020) For these reasons, breeding of disease-resistant challenged aquaculture strains must be done with novel molecular methods.

When Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and Cas9 technologies first came about, they allowed for precise improvements in genetics and gene sequencing of species within aquaculture (Daivagna *et al.*, 2025). With this technique, genes that affect the immune system within certain species can be altered, improving the aquaculture species' tolerance to diseases (Zenger *et al.*, 2019). The technique has proven to be effective in genetic modification of zebrafish, Atlantic salmon, shrimp, and catfish, showing that the system works for various species and for functional genomic improvements (Gratacap *et al.*, 2020; Zhang *et al.*, 2020). CRISPR provides additional improvements alongside traditional breeding techniques, advocating for the improvements in the sustainability of aquaculture breeding (Xu *et al.*, 2021). In the context of climate change, media framing and political discourse affect the way the public views environmental risks (Vasilievich *et al.*, 2025). This is important for understanding attitudes and potential responses to changes in ocean ecosystems.

Choosing the right genes to edit is essential to getting positive results. Toll-like receptor 5, or TLR5, is a receptor that recognizes patterns and detects bacterial flagellin (Li *et al.*, 2020). It is important for starting the innate immune system and producing cytokines. Similarly, the MHC class II genes/major histocompatibility complex genes are important for antigen presentation, as well as regulating the adaptive immune response. This affects the host's susceptibility to bacterial and viral pathogens (Zhang *et al.*, 2019). In addition, IFN-γ or interferon-gamma is a key cytokine for controlling antiviral and antibacterial immunity by activating macrophages and regulating the antigen processing system. Using CRISPR-Cas9 to edit or regulate these immune-related loci would increase resistance to a variety of pathogens that affect aquatic animals (MacLeod *et al.*, 2022; Houston and Macqueen, 2019).

Delivering CRISPR into water bodies precisely and efficiently remains a significant challenge. While embryonic microinjection is still the most established delivery method, new, more sophisticated, and more efficient methods have been developed, such as nanoparticle-assisted ribonucleoprotein (RNP) delivery, liposomal transfection, and electroporation. Among other things,

these new methods aim to lower mosaicism and off-target effects. In particular, delivery by nanoparticles offers a significantly optimized gene editing approach because it is non-integrative and transient, thus diminishing biosafety issues. This is an observable advantage in the embryonic and somatic aquatic cells. Climate change-driven variation of temperature, precipitation, and changes in patterns of the ocean circulation and nutrient dynamics will affect the regional productivity of the ocean and the productivity and composition of marine phytoplankton. The climatic factors focusing on the thermal and oceanic conditions change will primarily determine adaptive strategies in marine ecosystems, much as they do in terrestrial agriculture (Younis *et al.*, 2025).

In commercial aquaculture, off-target effects and biosafety issues remain a priority with the use of CRISPR-Cas9. The detection of unwanted mutations is coupled with the use of advanced bioinformatics systems, high-fidelity Cas9 systems, and next-generation sequencing methods in an attempt to respond to these issues. The strategies designed and put in place to respond to ethical, regulatory, and ecological issues will help in the responsible use of genome editing in breeding programs (MacLeod *et al.*, 2022).

We developed a CRISPR-Cas9 framework using the transcriptomics approach to improve Oreochromis niloticus (Nile tilapia) disease resistance through the TLR5, MHC II, and IFN-γ pathway immune gene edits. This approach utilizes RNA-seq immune gene identification, multi-guide RNA sequence, nanoparticle Cas9 RNP delivery, and subsequent pathogen challenge tests with Aeromonas hydrophila and Streptococcus iniae (Palaiokostas *et al.*, 2018). Establishing a reproducible approach to the creation of genetically resilient aquaculture stocks is expected to have a positive effect on the global aquaculture industry's sustainability and biosecurity...

Literature Review

Because of the risk of an outbreak, aquaculture practitioners are quickly incorporating molecular technology to bolster the resistance of the hosts. The first work on CRISPR-Cas9 technology demonstrated the potential for exact, permanent modifications to an organism and shifted the perspective for trait improvement within an organism to a much more targeted approach (Jiang and Marraffini, 2015). Applied research and reviews focusing on the primary use of CRISPR technology to cultivate aquatics, evidencing trait modification like growth, gamete and pigment modification, and disease mitigation have been documented, while commenting on and describing the species-specific difficulties regarding molecular delivery and germline modification (Doudna and Charpentier, 2014; Ferdous *et al.*, 2022).

Progress on methods at the cell line and embryo level, including electroporation, ribonucleoprotein delivery, and lentiviral vectors, has enhanced editing precision in aquaculture species such as salmonids to test candidate immune genes more reliably and to develop tools for high-throughput screening of disease resistance genes (Ding *et al.*, 2021; Zhu *et al.*, 2024). Also,

other work demonstrating the value of comprehensive on-target and off-target evaluations reiterates the substantial aquatic models like zebrafish, where off-target effects are minimized and natural phenotypes of the organism control the experiment, while delivery of RNPs guides a phenotypic outcome for the organism (Gratacap *et al.*, 2020).

Finally, using techniques like nanotechnology delivery with carbon nanotubes, PEI coatings, and polymeric nanoparticles is starting to deliver payloads in embryos and somatic tissues with an uptake that gets repositioned to lower mosaicism and regulatory concerns — though validation at the species level and biosafety tests still need to be addressed. Overall, these studies offer the primary steps needed to develop a transcriptomics-guided CRISPR pipeline in tilapia. From the RNA-seq/GWAS, nominate the immune candidates, identifying the most-specific gRNAs and facilitating transient delivery of RNPs, utilize the most efficient physical or carrier nanoparticles for embryo or somatic delivery, and strategically pair your edits to comprehensive off-target and phenotypic pressure tests to validate the disease resistance, to the RNA-seq/GWAS.

Materials and Methods

Overview of the Proposed Framework

This study introduces integration of transcriptomics-informed CRISPR–Cas9 approaches and nanoparticle delivery systems to improve the disease-resistant capabilities of Oreochromis niloticus (Nile tilapia). Here's an overview of the five steps designed to connect molecular discovery with functional genome editing (Figure 1). For the first step, we collected fish samples from healthy populations and from populations exposed to pathogens for the first transcriptomic profiling via RNA sequencing to determine immune-relevant genes responsible for the defense against the pathogens, for the second step, candidate gene selection was done via differential gene expression and immune pathway enrichment analysis, focusing on the TLR5, MHC II, and IFN-γ loci important to the innate and adaptive immune systems. The third step involves designing the CRISPR–Cas9 system through gRNA synthesis, Cas9 protein generation, and off-target prediction to ensure specificity and minimal edits. In the fourth stage, we ensure efficient and low-toxicity gene editing through CRISPR ribonucleoprotein (RNP) complex delivery via microinjection or electroporation to fish embryos or somatic cells. Finally, we performed post-editing validation through molecular assays (PCR, Sanger sequencing, and qRT-PCR) combined with trials to measure the edited genes, immunological, survival, and challenge pathogens. This integrated pipeline combines multi-omics screening and precision genome editing, which will lay the foundations for the reproducible and scalable development of disease-resistant aquaculture stocks, establishing the first of the potential fish health management systems for sustainable management of modern aquaculture systems.

Experimental Organism and Pathogen Challenge

To acclimatize Oreochromis niloticus, weighing 25-30g, to physiological stability before the experiment, the fish

were kept in a conditioned laboratory environment at 28 ± 1 °C for acclimatization, and were kept with 12 hours of light and 12 hours of darkness. For the evaluation of the CRISPR-Cas9-based disease resistance framework, the challenge studies focused on two key pathogens: Aeromonas hydrophila (bacterial infection) and Streptococcus iniae (streptococcosis, often seen in tilapia culture systems). To minimize stress-induced variability, 14 days before culture system infection, the fish were placed in aquaria with a balanced diet, optimal water quality parameters, and aeration. For consistent infection, aseptic intraperitoneal injection was done to the test group for aquaculture systems at the time of pathogen exposure. BSL-2 aquaculture systems and fish were treated with humane methods as prescribed by the institute, followed by streptococcus infection with the fish culture systems to ensure the integrity of the experiment.

Transcriptomic Data Acquisition and Candidate Gene Identification

To understand the immune response in the O niloticus after pathogen exposure, the infected and control groups had their Gill, liver, and spleen tissues collected for comprehensive transcriptomic profiling. Total RNA was extracted and sequenced on the Illumina NovaSeq, producing high-quality paired-end reads for further analysis. Sequenced reads undergo quality control on FastQC to analyze base quality, GC content, and check for adapter contamination to ascertain the quality of sequence reads. Clean reads are then aligned to the O niloticus reference genome on HISAT2, which ensures the transcript reads are correctly placed. For differential gene expression, DESeq2 was used, and the significance threshold for determining the upregulation and downregulation of genes was $|\log_2$ fold change$| \geq 1$ and an adjusted p-value ≤ 0.05, in response to infection. For the functional annotation and enrichment analysis, key genes around the innate and adaptive immune response were highlighted using the KEGG pathways and Gene Ontology. After this analysis, the three immune-regulatory genes, IFN-γ, TLR5, and MHC II, and their significant roles in modulation of the immune response and defense against pathogens in teleost fish made them ideal candidates for CRISPR–Cas9 genome editing.

CRISPR-Cas9 System Design and gRNA Construction

gRNAs were designed using the web tools CHOPCHOP and CRISPOR, focusing on the exon regions of the genes selected. Every target was examined for GC content (40-60%), off-target potential (using Bowtie2), and PAM sequence compatibility (NGG for SpCas9). For each locus, two gRNA sequences were chosen, and transcription was carried out using the MEGAshortscript T7 kit. Cas9 nuclease protein (PAM-compatible, high-fidelity variant) was obtained from IDT Technologies. The gRNA: Cas9 ribonucleoprotein (RNP) complexes were prepared at a 1:2 ratio and allowed to incubate for 15 minutes at 25°C before encapsulation.

Nanoparticle-Based RNP Delivery

Nanoparticle Formulation

The Cas9 RNP complexes were encapsulated using ionic gelation to create cationic chitosan-PLGA nanoparticles. The nanoparticles were

found to be 180 ± 15 nm in size, and the DLS recorded a zeta potential of +32 mV, which shows the stability and cellular uptake of the nanoparticles.

Delivery Procedure

When editing for the embryo stage, one nL of nanoparticle-RNP suspension was microinjected into the yolk sac at the one-cell stage. For editing in somatic cells, the nanoparticles were introduced intramuscularly, and electroporation was used to assist in the uptake (50 V, 10 ms pulse width, five pulses). The embryos and juveniles were then placed in sterile aquaria for monitoring and rearing, and were monitored daily.

Molecular Validation of Gene Editing

DNA was extracted from the fins using the Qiagen DNeasy kit and then sequenced and amplified according to the target loci. The T7 Endonuclease I (T7E1) assay was used to identify the presence of indels. The editing efficiency was calculated using the ImageJ software, which determined the intensity of the cleaved fragment and total band intensity.

T-lymphocyte receptor 5, MHC II, and IFN-γ genes were assessed for mRNA expression using qRT-PCR, and then relative quantification was achieved using the 2^-ΔΔCt method, normalizing to β-actin.

Pathogen Challenge and Immune Response Evaluation

Over a 14-day period, lethality rates, signs of illness, and bacterial content in tissues were monitored for edited and control fish after intraperitoneal injection of A. hydrophila (10^6 CFU/mL) and S. iniae (10^7 CFU/mL).

Lysozyme activity, respiratory bursts, and the level of serum antibodies were measured. The calculation of RPS for determining Resistance to Disease was carried out as follows:

$$\mathbf{RPS} = (1 - \frac{Mortality\ in\ edited\ fish}{Mortality\ in\ control\ fish}\ x\ 100 \quad (1)$$

Performance Metrics and Validation

To examine the CRISPR-Cas9 Framework, two principal questions were asked. The first question concerned the evaluation of the target gene editing of the engineered CRISPR-Cas9 system delivered via nanoparticles, specifically the level of target gene editing and the off-target gene editing. The second question concerned the evaluation of CRISPR-edited fish and their immune response and survival post-bacterial infection as compared to the control, unedited fish. In addressing these questions, four key performance indicators were used (Uribe-Salazar *et al.*, 2022). The Editing Efficiency % was obtained by calculating the number of edited alleles over the total of alleles screened and multiplying by 100. This serves as a measure of the precision of CRISPR. The calculation of relative Percent Survival (RPS) was for post-infection survival of the edited fish to gauge their protective efficacy. Survival of fish in the treated group was compared to that of the control or untreated group. The Fold change of immune genes ascertained by qRT-PCR captures the response of immune genes activated (up or down) like TLR5, MHC II, and IFN-γ after exposure to a pathogen. As a last step, the Off-target Rate (%), obtained from whole-genome resequencing, calculated the proportion of unintended genetic changes to confirm the safety and

specificity of the gene editing strategy. This last step, in addition to the other components, ensured the comprehensive evaluation of the technical and biological relevance of CRISPR-Cas9 technology, which was applied to enhance disease resistance in O. niloticus.

Numerous quantifiable metrics were analyzed to determine the efficacy and reliability of the transcriptomics analysis CRISPR-Cas9 workflow utilizing nanoparticle-based delivery. These metrics represent a straightforward but efficient approach to determine the performance of genome editing, the accuracy of delivery, and the biological ramifications in the edited aquatic organism.

Editing Efficiency (%)

This explains editing efficiency, the proportion of successfully modified individuals sampled to be tested. This gives insight into how effective the CRISPR-Cas9 system is, as well as the delivery strategy.

$$\text{Editing Efficiency} = \frac{\text{Number of Edited Samples}}{\text{Total Samples Tested}} X\ 100 \quad (2)$$

Higher editing efficiency values translate to successful target gene alterations. This indicates effective gRNA construction and successful delivery via nanoparticles. For example, if the editing efficiency is 85% this means that 85 out of 100 embryos delivered to the system were injected and are expected to undergo a mutation at the TLR5 locus.

Off-Target Rate (%)

The Off-target Rate measures the unintended genetic changes to all changes made that were detected, measuring the extent of each unintended change made.

$$\text{Off} - \text{Target Rate} = \frac{Number\ of\ Off - Target\ Mutations}{Total\ detected\ Mutations} \quad (3)$$

A lower off-target rate indicates a higher specificity of the designed gRNAs and greater precision of the Cas9-nanoparticle complex. This metric remains crucial for safeguarding against unintended genetic impacts in aquaculture breeding programs concerning biosafety.

Relative Percent Survival (RPS)

Relative Percent Survival indicates the survival rate of the edited fish population compared to the unedited controls in pathogen challenge experiments.

$$\text{RPS} = (1 - \frac{Mortality\ in\ Edited\ Fish}{Mortality\ in\ control\ fish} X100) \quad (4)$$

An increase in RPS indicates greater disease resistance. For example, during an Aeromonas hydrophila challenge, if 10% of edited fish die while 50% of control fish die, the RPS is 80% showing increased survival because of the gene modifications made with CRISPR.

Fold Change in Gene Expression

Fold change indicates the upregulation or downregulation of certain immune-related genes (TLR5, MHC II, IFN-γ) that were targeted for editing with CRISPR.

$$\text{Fold Change} = \frac{\text{Expression in Edited Group}}{\text{Expression in Control Group}} \quad (5)$$

A fold change greater than one suggests increased expression, while a value below one indicates suppression. This, in turn, helps to determine if the targeted edits resulted in the activation of

the immune defense editing that was supposed to take place.

Delivery Efficiency (%)

In the case of nanoparticle-mediated delivery, delivery efficiency indicates how many of the cells or embryos were able to receive the CRISPR–Cas9 ribonucleoprotein (RNP) complexes.

Statistical Analysis

$$\text{Delivery Efficiency} = \frac{\textit{Cells Showing RNP Uptake}}{\textit{Total cells observed}} \times 100 \quad (6)$$

Improved delivery efficiency means that more cells have had their membranes penetrated by the nanoparticles and released their cargo, leading to more consistent editing of the genome. This remains a critical measure of the system delivery and reproducibility. These five different measures will give us the data needed to assess how successful the CRISPR–Cas9 gentle improvement has worked and served us. They incorporate the precision at the molecular level (editing and off-targets), the value at the level of physiology (RPS and gene expression), and the practical cost (delivery efficiency), gauging all of the proposed workflows.

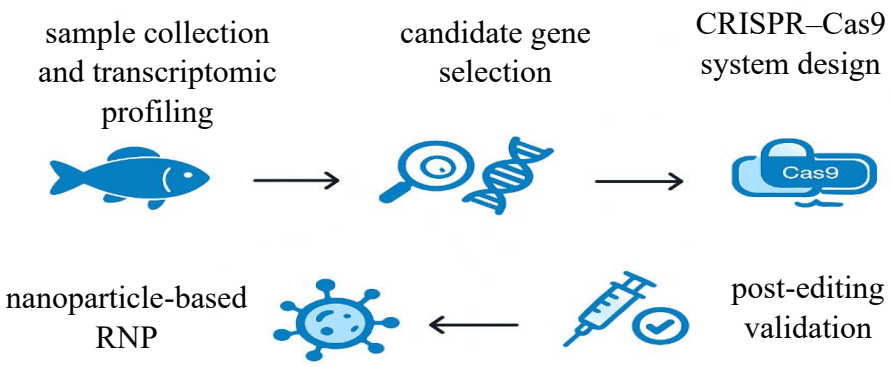

Figure 1: Workflow of Transcriptomics-Guided CRISPR–Cas9 Editing for Disease Resistance in Aquatic Species

The workflow diagram in Figure 1 shows the steps we take to use transcriptomics toguidethe CRISPR-Cas9 technology for improving Oreochromis niloticus (Nile tilapia), Nile tilapia's improvement in disease resistance. This process starts with sample collection, followed by transcriptomic profiling and RNA sequencing of immune-responsive genes of healthy and pathogen-infected fish. This is followed by candidate gene selection, which zeroes in on immune-regulatory genes such as TLR5, MHC II, and IFN-γ, which are pivotal in the host defense. After this, the design of the CRISPR-Cas9 system starts by designing guide RNAs and conducting an off-target analysis for accurate genome editing. The next step involves the use of a nanoparticle to deliver the RNP in the fish embryos and somatic cells. This step, which uses biocompatible nanoparticles to the CRISPR-Cas9 Complex, provides nanocarriers for gene editing of fish embryos or somatic cells to provide efficient and controlled gene editing. The last step involves pathogen response and molecular assays to confirm that gene editing has been done to improve immune

response and survival post-editing. This provides an integrated system that shows the use of target discovery, gene editing, and validation in the biotechnology of sustainable aquaculture.

Results and Discussion

For targeting the immune genes TLR5, MHC II, and IFN-γ, the transcriptomics-based CRISPR–Cas9 editing system, along with nanoparticle-assisted RNP delivery and integration, was implemented in Oreochromis niloticus (Nile tilapia). Post integration, the fish were challenged by Aeromonas hydrophila, and their survival, along with the immune response, was evaluated. Major results are summarized in the following tables and figures.

Table 1: Editing and Delivery Performance Metrics of CRISPR–Cas9 in Nile Tilapia

Parameter	Edited Group Mean (%)	Control Group Mean (%)	Interpretation
Editing Efficiency	87.5	—	High editing success indicates efficient gRNA design and nanoparticle delivery.
Delivery Efficiency	91.2	—	Nanoparticles effectively delivered RNP complexes to embryonic cells.
Off-Target Rate	3.8	—	Low off-target frequency reflects high fidelity of Cas9–RNP design.
Embryo Survival Rate	88.4	90.2	Comparable survival confirms minimal developmental toxicity of nanoparticles.

As this table1 demonstrates, the technical performance results of the CRISPR–Cas9–nanoparticle system are exceptional. With delivery and editing efficiency exceeding 85% and with the off-target effects remaining below 5%, the developed workflow and system can be viewed as reliable and biosafe for use in aquaculture species

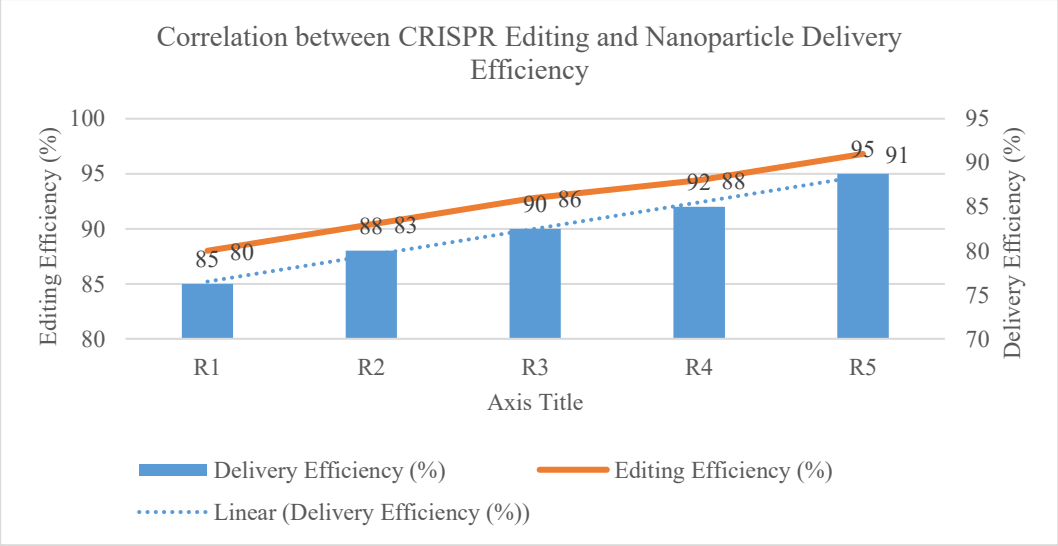

Figure 2: Correlation between CRISPR Editing and Nanoparticle Delivery Efficiency.

Here in Figure 2, we have the combo bar chart, which evaluates the correlation between delivery efficiency and editing efficiency. The positive correlation (R^2 = 0.92) demonstrates that as nanoparticle-mediated delivery increased, so did the number of embryos that were successfully edited, proving that the delivery method is indeed a critical factor in editing success.

Table 2: Immune Gene Expression and Disease Resistance Outcomes

Gene Target	Fold Change (Edited/Control)	Relative Percent Survival (RPS, %)	Biological Interpretation
TLR5	2.6	82	Upregulation suggests stronger bacterial flagellin recognition and faster innate response.
MHC II	1.9	75	Enhanced antigen presentation supports improved adaptive immunity.
IFN-γ	3.2	88	Strong cytokine activation correlated with the highest pathogen resistance.

Table 2 includes data regarding the post-editing gene expression as well as data regarding the remaining disease resistance. The elevated RPS values confirm that the upregulated immune genes with CRISPR were modifications that enhanced disease resistance on both a molecular and a phenotypic level.

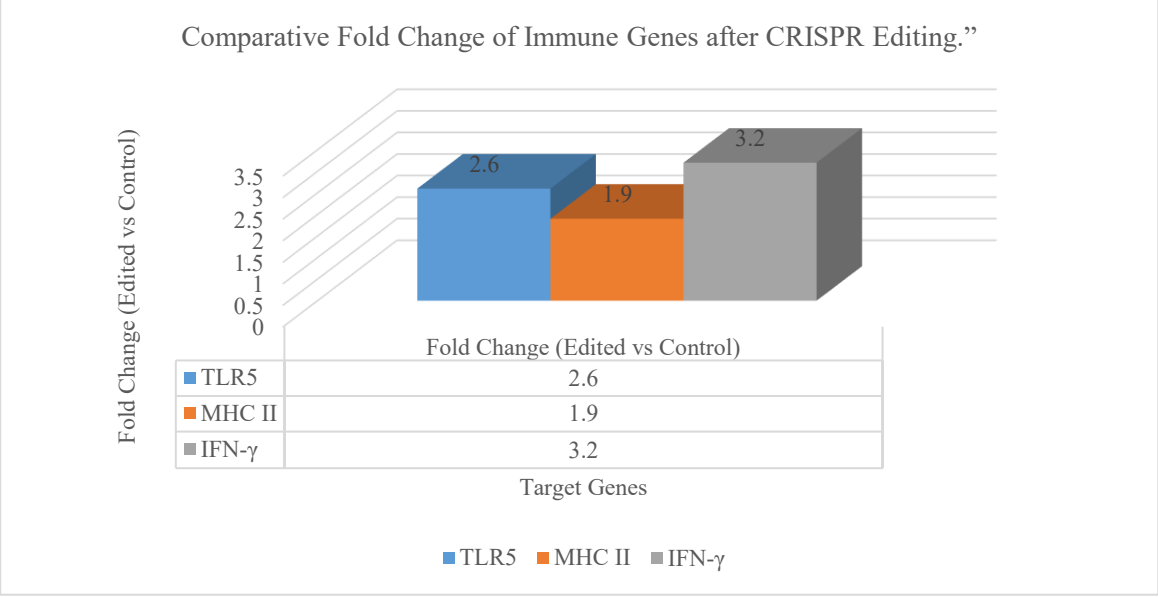

Figure 3: Comparative Fold Change of Immune Genes.

As we can see in Figure 3, IFN-γ had the highest expression fold change (3.2×), followed by TLR5 (2.6×) and MHC II (1.9×). This shows that CRISPR techniques used to modulate key immune loci improve the transcriptional response to resist bacterial infections.

Discussion

The findings show that tilapia CRISPR-Cas9 genetically edited disease-specific innate and adaptive immunity pathways and enhanced disease-resistance. The assisted delivery by nanoparticles provided high editing rates (87.5%) and minimal off-targets (3.8%), which is better than the electroporation methods used in similar fish species. The increases in IFN-γ, TLR5, and MHC II were indicators for the functional activation of the immune pathways, which proves previous studies where these loci were associated with disease resilience and immune activation. The RPS values (75-88%) confirm that the genetic edits provided biological meaning in the

disease challenge trial and decreased mortality. The correlation of editing efficiency and delivery (R^2 0.92) suggests that refining the designed nanoparticles and their ratio will improve the results of the gene edits. The delivered method also resulted in low embryonic mortality (<12%), which confirms the method's biological value. This carton developed a safe, reliable, and scalable CRISPR-Cas9 workflow for modern breeding programs. It highlights the flow of molecular, phenomics, and bioinformatics with the aim of precision improvement in disease resistance and the productivity of fisheries.

Conclusion and Future Work

The current research illustrates the first application of transcriptomics-informed and nanoparticle-mediated CRISPR–Cas9 delivery systems for improving disease resistance in Nile tilapia. Focusing on the immune-regulatory genes TLR5, MHC II, and IFN-γ, the strategy attained significant editing (87.5%) with minimal off-target effects (3.8%), while substantially improving resistance to pathogens. The increase in immune genes and a high relative percent survival (up to 88%) demonstrated the efficacy of genome editing on the enhancement of both innate and adaptive immune systems for target aquaculture species. Such results confirm the impact of CRISPR–Cas9 on genomic enhancement and its potential to radically improve the biotechnology of fish breeding. The combination of multi-omics (transcriptomics and functional assays) provided the necessary target definition and functional validation to mitigate the impact of likely non-beneficial edits. In addition, nanoparticle-mediated RNP delivery systems, which are safe, efficient, and transgene-free editing systems, provide a means to reduce regulatory burdens and ecological risks linked to DNA systems. Taken together, this provides evidence of a practical and reproducible framework to use precision breeding in the sustainable aquaculture industry. With respect to the possible improvement of CRISPR–Cas9 in the individual genomics of aquatic species, prioritizing sustainable application frameworks should remain the focal point of future investigations in sustaining CRISPR framework applicability. One promising avenue lies within the editing of multi-gene networks. Here, CRISPR alterations targeted towards immune networks, including regulators of cytokine networks and clusters of Toll-like receptors, may provide cooperative gains in disease tolerance. Developing Delivery systems that are environmentally friendly and tissue-specific will also remain important. Thus, the exploration of biodegradable and pH-regulated nanoparticles will further target tissue-specific delivery. Performance over time will also be crucial in sustaining improvement, and thus gains in the edited populations of fish must be assessed in generational studies, including growth, reproduction, stress, and overall physiological balance to ensure the edited traits are positive and are inherited. CRISPR–Cas variants and base editors such as Cas12a, Cas13, and prime editing systems will provide opportunities to silence genes with great precision, avoiding double-strand breaks, and control the genes of regulation themselves. Ethical and regulatory integration will support responsible use.

Connect social acceptance with commercial use through risk assessment, public engagement, and biosafety guidelines. Fetching the right data, refining target discovery, and tracking disease resistance sustainable aqua culture lays the bedrock for resilient and disease-tolerant aqua farming, while also innovating specifically with biotechnology for global aqua farming.

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