



A Review

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Investigating the Impact of Embryonic Gene Editing on the Growth Rate and Fat Content of Crucian Carp

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Abstract With the growing population and increasing food demand, the aquaculture industry is facing significant challenges. As a vital economic fish species, the growth rate and fat content of crucian carp are crucial factors determining its quality and market competitiveness. The emergence of gene editing technology provides a new avenue for improving the quality of fish. This study summarizes the research progress on the impact of gene editing on the growth rate and fat content of crucian carp embryos and explores the potential applications of gene editing technology in crucian carp aquaculture. Furthermore, the study proposes recommendations for future research directions and developments, including in-depth studies of gene function, long-term monitoring and assessment, advancement of regulatory and ethical standards, and interdisciplinary cooperation, to promote the sustainable application of gene editing technology in the aquaculture industry.

Keywords Crucian carp; Gene editing; Growth rate; Quality improvement

Crucian carp (*Cyprinus carpio*), as a pearl in the aquaculture industry and a gem among economic fish, has always been highly favored (Figure 1). Its wide adaptability, rapid growth, and high edible value grant it a special status in the global aquaculture industry (Xiao and Luo, 2010, Guangxi Fisheries Science and Technology, (4): 12-18). However, to better meet the growing demand for food, it is crucial to improve breeding efficiency and product quality (Liu et al., 2017), and the key to these two aspects is the growth rate and fat content of crucian carp. The growth rate of fish directly determines the length of the farming cycle, which in turn affects the efficiency of aquaculture. Fish that grow quickly can reach market size faster, reducing farming costs and improving production efficiency. In the fiercely competitive global fish market, the enhancement of fish growth rate has become a key factor in seizing market share (Gui et al., 2016). The fat content of crucian carp directly influences its edible quality and market value. Different markets and consumers have varying preferences for fat content. Some markets prefer fish with more fat because it enhances taste and flavor, while others prefer fish with lower fat content. Therefore, the ability to adjust the fat content of crucian carp to meet diverse market demands can provide a competitive advantage to the aquaculture industry.

With the continuous development of biotechnology (Dong and Luo, 2023), gene editing technology has emerged as an innovative approach to improving fish quality (Basavaraju et al., 2002). This technology allows researchers to precisely edit the genes of crucian carp to achieve goals such as altering growth rate and fat content. Through gene editing, the aquaculture industry could potentially undergo revolutionary changes, enhancing production efficiency, meeting diverse market demands, and promoting the sustainable development of aquaculture (Blix et al., 2021).

Despite the enormous potential of gene editing technology in improving crucian carp quality, there are currently some key issues and challenges that require further research and resolution. Researchers need to delve into how gene editing can accurately regulate the growth rate and fat content of crucian carp to ensure the effectiveness of this technology. Additionally, it is necessary to investigate the specific impacts of gene editing on the health and survival of crucian carp, ensuring that fish are not adversely affected during the gene editing process. Successfully applying gene editing technology to practical farming operations is also a critical issue, involving the feasibility of

the technology and how to meet market demands while ensuring environmental sustainability. These issues will be crucial focal points for future research and practice, playing a vital role in the development of the aquaculture industry.



Figure 1 Crucian carp

The main purpose of this study is to explore in-depth the effects of embryo gene editing technology on the growth rate and fat content of crucian carp, discussing the prospects of gene editing technology in crucian carp farming. This includes potential benefits, challenges, and future directions. Moreover, the study emphasizes how to apply gene editing technology to actual farming operations to improve the growth rate and adjust the fat content of crucian carp to meet diverse market demands. In summary, this research aims to provide scientists, agricultural practitioners, and policymakers in the aquaculture field with the latest information and insights on embryo gene editing in crucian carp. It seeks to drive innovation and sustainable development in aquaculture to meet the ever-growing demand for food. The study also underscores the importance of public education and involvement, ensuring that the application of technology aligns with societal expectations and values, providing crucial support for the sustainable development of the future aquaculture industry.

1 Gene Editing Technology Overview

1.1 Principles and applications of the CRISPR-Cas9 system

The principles and applications of the CRISPR-Cas9 system have brought about revolutionary changes in the field of life sciences (Ai et al., 2017). This system originates from the immune defense mechanisms of bacteria and archaea, and has been finely modified to become a powerful gene editing tool. At the core of the CRISPR-Cas9 system is the Cas9 protein, acting as a "molecular scissors" (Figure 2), capable of cutting DNA molecules at specific locations (Roy et al., 2022).

To achieve gene editing, the first step involves designing guide RNA (gRNA), a short RNA molecule with a sequence complementary to the target gene's DNA sequence. The guide RNA combines with the Cas9 protein to form a complex. This complex then recognizes and binds to the specific location of the target gene, triggering a double-strand break in the DNA. When the cell attempts to repair this break, it may introduce genetic changes, thus achieving editing. The CRISPR-Cas9 system has been widely applied in various organisms, including the crucian carp. In studies of gene editing in crucian carp embryos, scientists can precisely design guide RNA to selectively edit genes related to growth rate and fat metabolism. The high precision and efficiency of this system make gene editing in crucian carp feasible, providing unprecedented opportunities for improving crucian carp quality.

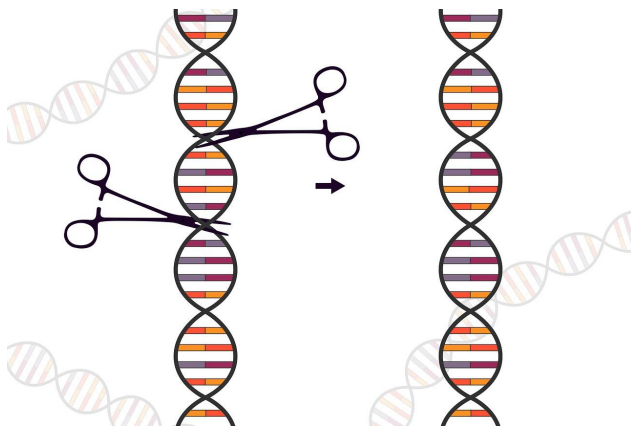


Figure 2 Gene editing technology

1.2 Methods and techniques for gene editing in crucian carp embryos

Editing genes in crucian carp embryos is a complex and precise process that requires carefully designed and tightly controlled operational steps. The design of guide RNA is the starting point for crucian carp gene editing. Guide RNA must highly specifically match the DNA sequence of the target gene, thus requiring in-depth genomic knowledge and the support from computational biology tools. The preparation of guide RNA and Cas9 protein must be carried out in the laboratory. This requires high-purity and highly active complexes to ensure their effectiveness inside embryos. The prepared CRISPR-Cas9 complex must be precisely injected into crucian carp embryos. This is an operation that requires high technical skills and accuracy to ensure the successful introduction of the complex into the embryos. Subsequent verification of successful editing is necessary to ensure the desired genetic changes have occurred. This typically involves extracting DNA from edited embryos and conducting gene sequencing to confirm the editing effects (Liu et al., 2004).

In crucian carp embryo gene editing, selecting the appropriate target genes is crucial. Typically, genes closely associated with growth rate and fat metabolism are chosen as editing targets. Selecting key genes that control crucian carp growth rate and fat metabolism requires in-depth genetic and physiological research. This may involve a comprehensive analysis of existing literature and experimental validation. Additionally, special attention must be paid to the design of guide RNA to ensure its complete match with the target gene's DNA sequence. This requires precise design and analysis of the guide RNA sequence.

By gaining a deep understanding of the CRISPR-Cas9 system and the methods and techniques of gene editing in crucian carp embryos, this study provides a scientific basis for applying gene editing to improve carp quality. These key technologies and steps contribute essential theoretical and practical foundations to the study of gene editing in crucian carp embryos.

2 Growth Rate Control Mechanisms

2.1 Gene regulation of growth hormones and growth factor

The growth rate of crucian carp is complex influenced by various biological processes and gene regulations. Among them, growth hormones and growth factors playing a crucial role in the control of fish growth speed.

Growth hormone (GH) is a multifunctional protein hormone that plays a significant role in the growth of crucian carp. The pituitary gland secretes growth hormone, which initiates a series of complex signal transduction pathways by binding to the growth hormone receptor (GHR). These pathways activate cell division and growth, leading to the rapid development of the fish. Through gene editing techniques, it is possible to precisely control the expression level of the GHR gene, enhancing or attenuating the signal transduction of growth hormones, thereby regulating the growth rate of crucian carp. Insulin-like growth factors (IGF) are another set of molecules that play a crucial role in the regulation of fish growth. They function in cell differentiation, proliferation, and growth, usually secreted by the liver. After binding to the IGF receptor, IGF activates multiple signaling pathways,

further promoting cell proliferation and growth. Gene editing can be employed to explore ways to regulate the IGF signaling pathway, affecting the growth rate of crucian carp.

2.2 Regulation of genes related to muscle development

Muscle development in crucian carp significantly influences its growth rate. Key genes associated with muscle development can be precisely controlled through gene editing.

MyoD is a type of gene closely associated with the differentiation and development of muscle cells. These genes encode transcription factors that promote the differentiation of muscle cells. By editing the MyoD gene or related genes, it is possible to enhance or inhibit the muscle growth of crucian carp, thereby affecting overall growth rate. Muscle fiber protein is one of the key proteins constituting muscle fibers. Editing the expression of muscle fiber protein genes can alter the quantity and quality of muscle fibers, thus influencing the growth rate and body development of crucian carp.

Through gene editing technology, researchers can accurately regulate these genes and signaling pathways related to growth rate, so as to achieve accurate control of the growth speed of crucian carp. This providing a powerful tool for improving the quality and production efficiency of crucian carp, also opens up new prospects for the sustainable development of aquaculture industry.

3 Regulation Mechanisms of Fat Content

3.1 Regulation of genes related to fat synthesis

Fat content is a crucial indicator of the quality of crucian carp, influenced by various gene regulations and metabolic processes. When studying the regulatory mechanisms of fat content, researchers must focus on genes related to fat synthesis.

Fatty acids are the main components of fat and are synthesized through the fatty acid synthesis pathway. In crucian carp, some key genes encoding enzymes play a vital role in the production of fatty acids. Using gene editing techniques, the expression of these genes can be regulated, affecting the rate of fatty acid synthesis and subsequently influencing the accumulation of fat in crucian carp. After the synthesis of fatty acids, they are typically stored in adipocytes. Some proteins encoded by fat storage protein genes play a crucial role in the storage and release of fatty acids. By editing these genes, it is possible to affect fat storage and distribution, thereby regulating the fat content of crucian carp.

3.2 Regulation of genes related to fat degradation

In addition to fat synthesis, fat content is also regulated by the process of fat degradation. Fat degradation is the process of releasing and metabolizing fatty acids stored in adipocytes.

Fatty acid oxidation is a critical step in fat degradation, occurring in the mitochondria. Some genes related to fatty acid oxidation encode oxidases that catalyze the breakdown of fatty acids and generate energy. By editing these genes, the rate of fatty acid oxidation can be adjusted, affecting the extent of fat degradation. Hormones such as thyroid hormones, insulin, and adrenaline play essential roles in the process of fat degradation. These hormones regulate fat degradation by influencing fatty acid oxidation and release. Gene editing technology can be used to study the mechanisms of action of these hormones and how to influence fat degradation by regulating related genes.

Through gene editing technology, researchers can precisely regulate genes and signaling pathways related to fat synthesis and degradation, achieving accurate control of fat content in crucian carp. This not only contributes to improving the quality of crucian carp but also holds the potential to enhance the efficiency and sustainability of aquaculture.

4 Impact of Crucian Carp Embryonic Gene Editing on Growth Rate

4.1 Early findings and observations

Early studies have shown how genetic modification of crucian carp through embryonic gene editing technology

significantly affects its growth rate. These studies have yielded a series of crucial observations and findings that profoundly affect the growth rate of crucian carp.

One notable effect is the increase in growth rate. Research indicates that by editing specific growth-related genes, such as those related to growth hormone receptors or genes promoting muscle development, the growth rate of crucian carp can be significantly enhanced. This implies that gene-edited crucian carp can reach the required weight and size for the market in a shorter time. Furthermore, gene editing contributes to shortening the growth cycle of crucian carp, meaning they can attain marketable size more rapidly, thus enhancing aquaculture efficiency and economic benefits. This holds immense potential for the aquaculture industry as it can accelerate production cycles to meet market demands. Gene editing also aids in improving the uniformity of growth within the crucian carp population. This means that under the same breeding conditions, crucian carp are more likely to exhibit consistent growth, reducing size disparities between individuals. This is a significant advantage for the aquaculture industry, as it enhances product quality and market competitiveness.

Early research has clearly indicated that embryonic gene editing technology has a significant impact on the growth rate and growth cycle of crucian carp, while also improving the uniformity of the population's growth. These findings provide strong support for the quality improvement of crucian carp and the development of aquaculture. However, further in-depth research and exploration are needed to better understand the application prospects and potential challenges of this technology.

4.2 Correlation analysis between gene editing targets and growth rate

To gain a deeper understanding of the impact of gene editing on the growth rate of crucian carp, researchers have started exploring the correlation between editing targets and growth rate.

Choosing suitable gene editing targets is crucial. Researchers need to carefully consider which genes have the most significant impact on growth rate and determine the most effective editing strategy. This may involve editing genes related to growth hormones, muscle development, or other growth-related signaling pathways. The extent of editing is another critical factor. Gene editing can be partial or complete, and the degree of editing may affect the change in growth rate. Therefore, it is necessary to study the effects of different levels of gene editing on the growth rate of crucian carp and identify the editing level most suitable for breeding requirements. Additionally, the growth rate of crucian carp is regulated by multiple genes, which may interact with each other. Therefore, studying the interactions between genes is an important step in understanding the regulation of growth rate. These interactions may produce different effects in combinations of different gene editing targets. Thus, researchers need to delve into these interactions to comprehensively understand the regulatory mechanisms of growth rate.

By thoroughly investigating the impact of embryonic gene editing on the growth rate of crucian carp, humanity can better understand the potential applications of this technology. This not only contributes to improving the quality of crucian carp but also opens up new opportunities and possibilities for the sustainable development of the aquaculture industry. In-depth research into the effects of gene editing on the growth rate of crucian carp will provide robust support for future research and practices, driving innovation and sustainable development in the field of aquaculture.

5 Influence of Crucian Carp Embryos Gene Editing on Fat Content

5.1 Summary of previous research findings

Previous studies have revealed the impact of gene editing in crucian carp embryos on the fat content and summarized a series of significant results. Earlier experiments and observations have shown that by editing specific genes related to fat metabolism, the internal fat content of crucian carp can be significantly reduced. These genes typically involve the synthesis, storage, or degradation of fatty acids, and editing them can effectively regulate fat content.

Gene editing can also lead to changes in the composition of fatty acids. Editing relevant genes can influence the relative proportions of various fatty acids in the fat tissue. This is crucial for improving the quality and health properties of crucian carp fat, as fatty acid composition directly affects its edible value.

Regulating fat content through gene editing may also bring a range of health benefits. Reducing fat content can lower the risk of obesity-related diseases in crucian carp, such as fatty liver and diabetes. This contributes to the production of healthier crucian carp products. This is essential for consumer health and helps meet the growing demand in modern society for food that is both healthy and safe. Therefore, gene editing in crucian carp embryos not only holds economic significance for the aquaculture industry but also has a positive impact on the food industry and consumer health. These research findings suggest that gene editing technology has the potential for widespread application in improving crucian carp fat content.

5.2 Correlation analysis between gene editing targets and fat content

To gain a deeper understanding of the impact of crucian carp embryos gene editing on fat content, researchers are conducting correlation analyses to explore the relationship between editing targets and fat content.

Editing key genes involved in fat metabolism has become a focus. Researchers typically choose to edit genes closely associated with fat metabolism, such as fatty acid synthase, fatty acid oxidase, and hormone receptors. By editing these genes, targeted interventions can be made in the fat metabolism process, thereby influencing changes in fat content. This precise editing strategy provides a powerful tool for regulating crucian carp fat content. The timing and extent of editing are crucial for regulating fat content. Researchers need to determine the most suitable developmental stage for editing and the degree of editing required to achieve the desired regulation of fat content. Research in this area helps identify the most effective editing strategies.

Gene editing not only focuses on fat content but also aims to enhance fat quality, ensuring the controllability and stability of fat content. By editing, fatty acid composition can be controlled to make it more suitable for consumption and processing. This is competitive in the market for producing high-quality crucian carp products that are both market competitive and beneficial to health, such as those with suitable fat content and a healthy fatty acid composition. Improving fat quality makes products more in line with the needs of different markets and consumers.

By delving into the impact of gene editing in crucian carp embryos on fat content, a better understanding can be gained of how to precisely regulate this important quality characteristic to meet the needs of different markets and consumers. This provides new opportunities for the aquaculture industry and encourages researchers to better utilize gene editing technology to enhance the quality and sustainability of crucian carp, realizing broader application prospects.

6 Influencing Factors and Challenges

6.1 The influencing factors gene editing efficiency and stability

In the research of gene editing in crucian carp embryos, one of the crucial determining factors is the choice of editing tools, along with editing efficiency and stability. These factors directly impact the success of the editing process. The selection of suitable gene editing tools is essential for achieving efficient and stable edits. Currently, the CRISPR-Cas9 system is one of the most widely used tools, but researchers need to optimize the system to adapt to the specific biological characteristics of crucian carp. Only through thorough optimization can we ensure that the editing tools achieve maximum effectiveness in crucian carp.

The choice of editing targets also plays a significant role in editing efficiency. Different genes may exhibit varying editability, with some being more amenable to editing than others. Therefore, before embarking on gene editing in crucian carp embryos, it is crucial to carefully select target genes and consider their biological functions. This selection process should undergo thorough research and scrutiny to ensure that the chosen editing targets are wise and scientifically sound choices.

The state and developmental stage of cells are also critical factors influencing editing efficiency. Therefore, during editing, it is essential to ensure that editing occurs at the most opportune time and cell state. This can be achieved through precise timing control and the selection of appropriate cells, thereby increasing the probability of successful edits. Crucian carp may exhibit different biological characteristics at different developmental stages, affecting the applicability of editing.

Therefore, in order to achieve success in gene editing research on crucian carp embryos, it is essential to consider these points comprehensively. This includes choosing appropriate editing tools, wisely selecting editing targets, and ensuring editing occurs at the most suitable cell state and developmental stage. The integrated consideration of these factors will contribute to improving editing efficiency and stability, laying a solid foundation for the success of gene editing in crucian carp embryos.

6.2 Impact of genetic diversity and environmental factors on gene editing effects

In crucian carp gene editing research, researchers must carefully consider the potential influence of genetic diversity and environmental factors. Given the genetic differences among crucian carp individuals, different individuals may react differently to gene editing. Therefore, researchers need to conduct editing experiments in different individuals to determine the universality of editing effects and obtain more comprehensive data.

Environmental factors also play a crucial role in crucian carp gene editing. Aquaculture environmental factors, such as water temperature (Dong and Somero, 2009), water quality, and feed, can have significant impacts on the growth and metabolism of crucian carp (Figure 3). These environmental factors may directly affect the physiological state of crucian carp, potentially interfering with the effects of gene editing. Therefore, researchers need to consider and manage these factors to ensure the stability and consistency of editing.



Figure 3 Aquaculture environment

Considering the genetic diversity and environmental factors of crucian carp will contribute to a more accurate assessment of gene editing effects, providing a solid foundation for the sustainable development of aquaculture. Integrating these factors into experimental design and data analysis will yield more reliable results for future research, while also aiding in a comprehensive understanding of the potential applications of gene editing in crucian carp improvement.

6.3 Feasibility and safety considerations for genetic improvement of fish

Research on genetically improved fish must carefully consider its feasibility and safety to ensure the success and sustainability of its applications. Before conducting gene editing research, the goals and feasibility must be clearly defined. This includes determining whether editing can achieve the desired quality improvements and whether it is applicable to commercial aquaculture. Ecological and environmental risk assessments are also indispensable. Introducing genetically improved fish may have potential impacts on the natural environment. Therefore, ecological and environmental risk assessments are necessary to understand potential risks of escape and hybridization, with corresponding measures taken to mitigate these risks. This includes implementing appropriate

control measures to prevent genetically improved fish from entering natural water bodies, thereby reducing their contact with wild populations. Regulatory and ethical issues are also factors that require careful consideration. Research on genetically improved fish involves regulatory and ethical considerations. Researchers must comply with relevant regulations and ethical principles, taking into account issues such as animal welfare and human health in their research.

Considering these factors and challenges is an integral part of conducting crucian carp embryos gene editing research. Only with a comprehensive understanding and resolution of these issues can gene editing technology be better applied in crucian carp aquaculture and other aquaculture fields, contributing to quality improvement and sustainability.

7 Summary

As the latest research on gene editing technology continues to emerge in the cultivation of crucian carp, this technology presents extensive prospects for the aquaculture industry. It opens new possibilities for improving quality, enhancing production efficiency, and boosting the resistance and adaptability of crucian carp. This study comprehensively analyzes and summarizes the impact of gene editing on the growth rate and fat content of crucian carp, influencing factors and challenges, as well as the application prospects and outlook.

Early studies on gene editing in crucian carp embryos indicate that editing genes related to growth rate and fat content can significantly enhance the quality and production efficiency of crucian carp. Improvements in growth rate, shortened growth cycles, and enhanced growth uniformity have all positively impacted the aquaculture industry. Simultaneously, the regulation of fat content provides the potential for producing healthier crucian carp products, offering new opportunities for the sustainable development of crucian carp aquaculture.

However, in gene editing research on crucian carp embryos, editing efficiency and stability are critical factors. To increase the probability of successful editing, appropriate editing tools must be selected, editing targets chosen wisely, and editing must occur at the most suitable cell state and developmental stage. Genetic diversity and environmental factors also influence editing outcomes, requiring comprehensive consideration and management. Furthermore, the feasibility and safety considerations of genetically improved fish involve target setting, ecological risk assessments, regulation, and ethical issues to ensure responsible and safe technology application.

Gene editing technology brings various application prospects to crucian carp aquaculture, allowing researchers to further explore the impact of gene editing on other quality traits such as flesh texture, disease resistance, and color. This enables researchers to comprehensively improve crucian carp quality to meet evolving market demands. However, ethical and legal issues must be addressed in practical applications to ensure responsible and safe technology use. To better realize the application of gene editing technology in crucian carp and other economically important fish, it is recommended to deepen understanding of crucian carp gene function, monitor effects continuously, actively participate in regulation and ethical standard development, and enhance interdisciplinary cooperation. By implementing these recommendations, gene editing technology can be better applied to meet the growing demand for food while ensuring the sustainability of aquaculture and ecosystem health.

In conclusion, the study of gene editing in crucian carp embryos represents a significant innovation in aquaculture. Through continuous exploration and research in this field, sustainable growth in fish farming is expected to be achieved and provide robust support for meeting global food demand. The future development of this field requires collaboration from scientists, agricultural practitioners, government, and the public to collectively drive sustainable and innovative development in aquaculture.

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