Differences between tetraploid and diploid gamete cells in rainbow trout (*Oncorhynchus mykiss*) production and their effects on reproductive success

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Abstract

In trout (*Oncorhynchus mykiss*) production, differences between tetraploid and diploid gametes have important effects on growth rates and reproductive success. Tetraploidy is a condition in which the chromosome number of individuals is doubled from the diploid level and is generally associated with higher growth rates and superior phenotypic traits. It has been observed that tetraploid individuals have the potential to increase productivity in trout farming because they can produce more eggs and sperm. In addition, the reproductive success rates of tetraploid and diploid individuals were compared in terms of factors such as sperm and egg quality. The genetic and biological advantages of tetraploid individuals were revealed and the potential benefits of these individuals in trout farming were discussed. The findings obtained indicate that the application of tetraploid individuals in trout production may guide future strategies. In this review, the role of tetraploid and diploid gametes in trout production was comprehensively examined. The methods used in the production of tetraploid individuals, especially chemical and physical techniques, were detailed. The effectiveness of these methods and the points to be considered during application were emphasized.

Keywords: Triploid, Tetraploid, Trout, Production, Phenotypic

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Introduction

Trout (*Oncorhynchus mykiss*) is one of the most widely cultivated freshwater fish species worldwide. Aquaculture is gaining more and more importance in terms of food security and sustainable agricultural practices. In particular, trout farming has high economic potential both commercially and individually (Bostock *et al*., 2010; FAO, 2020). Trout has become a popular foodstuff worldwide due to its rich nutritional values and taste. Therefore, trout production is constantly evolving with the necessity of providing healthy food sources.

Increasing genetic diversity and increasing reproductive success are of critical importance in order to increase efficiency and quality in trout production. Genetic diversity ensures that populations are resistant to environmental changes (Weber and Hostuttler, 2012; Martínez et al., 2014). In production systems, diversity of genetic structure is necessary to ensure sustainable farming by increasing resistance and adaptation to diseases (Gjedrem, 2000; Beaumont *et al.,* 2010). In this context, tetraploidy applications offer a new perspective in trout production.

Tetraploidy is a condition obtained by doubling the chromosome number of individuals from the normal diploid level. Tetraploidy is used especially in fish farming to increase growth rates and obtain better phenotypic characteristics (Diaz and Neira, 2005; Bonnet *et al*., 2007; Qin *et al*., 2022). Studies show that tetraploid trout have higher yield and growth potential due to their genetic structure. The capacity of tetraploid individuals to produce more eggs and sperm reveals that these individuals have the potential to increase productivity in trout farming (Kang *et al*., 2018; Samad and Ghani, 2021).

Although tetraploid individuals have many advantages, there are also difficulties that can be encountered in the production of these individuals. Tetraploidy may bring some genetic problems, which may affect the overall health of populations. Therefore, tetraploidy applications should be managed carefully (Arai, 2001; Liu *et al*., 2022).

On the other hand, diploid individuals help preserve genetic diversity. Diploid individuals play an important role in preserving genetic diversity by exhibiting characteristics closer to natural reproduction processes (Weber and Hostuttler, 2012; Carter *et al*., 2021). Since diploid trout have a more balanced genetic structure, they can better adapt to natural selection processes. Therefore, preserving diploid individuals and strengthening their genetic structure is critical for sustainable trout production (Liu and Zhang., 2010; Martínez et al., 2014).

Studying tetraploidy and diploid in trout production is important to understand the potential benefits in the field of trout farming. The aim of this review is to examine the differences between tetraploid and diploid reproductive cells and to evaluate the effects of these differences on reproductive success in trout production.

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The methods used in the production of tetraploid individuals will be explained in detail and the advantages and disadvantages of these methods will be emphasized. In conclusion, this study is expected to reveal the potential benefits of using tetraploid individuals in the field of trout farming.

Differences between tetraploid and diploid reproductive cells

Tetraploidy is a condition characterized by the chromosome number of individuals being doubled from the normal diploid level. In fish farming, especially in trout production, tetraploidy applications have attracted considerable attention due to their potential to increase production efficiency and genetic diversity (Blanc *et al.*, 2005; Bonnet *et al*., 2007; Martínez et al., 2014). Tetraploid individuals are generally associated with higher growth rates, larger sizes, and better adaptability (Weber and Hostuttler, 2012; Qin *et al*., 2022). Under some conditions, these individuals may perform better than diploid individuals. Tetraploid trout generally have the capacity to produce more eggs and sperm. This is an important advantage for increasing productivity in trout farming (Blanc *et al.*, 2005; Benfey, 2018; Fraser *et al*., 2021). Studies show that tetraploid individuals have significantly higher growth rates than diploid individuals, depending on their genetic makeup (Li *et al*., 2016). For example, some studies have shown that tetraploid trout grow 20-30% faster than their diploid counterparts (Gu *et al*., 2024).

Tetraploid individuals are generally seen to be more robust and healthy. These individuals can be more resistant to stress and diseases, and this feature provides a significant advantage in rearing conditions (Liu *et al*., 2022). However, tetraploidy practices can affect genetic diversity and lead to some genetic problems (Gjedrem, 2000; Yamaha *et al.,* 2007; Martínez et al., 2014). Therefore, tetraploidy practices need to be managed carefully.

Diploid individuals play an important role in maintaining genetic diversity. These individuals exhibit characteristics closer to natural reproduction processes, which is critical for maintaining genetic diversity (Rasmussen and Morrissey, 2007; Carter *et al.*, 2021). Diploid trout are better adapted to natural selection processes. Genetic diversity ensures that populations are resistant to environmental changes, which is important for trout farming (Piferrer *et al*., 2009; Weber and Hostuttler, 2012).

The reproductive potential of diploid individuals differs from tetraploid individuals depending on their genetic structure. Diploid individuals are generally more successful in natural reproduction processes because they have a more balanced genetic structure (Delvin and Nagahama, 2002; Bonnet *et al*., 2007). In addition, diploid trout have the potential to increase their reproductive success by providing more genetic diversity. This allows natural selection and adaptation processes to

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occur more effectively (Samad and Ghani, 2021; Chen *et al*., 2022).

Another important difference between tetraploid and diploid trout is the phenotypic characteristics of individuals. Tetraploid individuals are generally characterized by larger sizes and higher biomass, while diploid individuals generally remain smaller in size (Leeds and Weber, 2019; Qin *et al*., 2022). This helps tetraploid individuals gain an advantage in terms of marketability and economic value.

In trout production, examining tetraploidy and diploidy helps understand the genetic and phenotypic differences between these two groups. The methods used in the production of tetraploidy individuals should be carefully selected in order to both increase productivity and preserve genetic diversity (Song *et al.*, 2012; Wang and Shen, 2018). In addition, the potential benefits and disadvantages of tetraploidy applications should be emphasized.

Tetraploid and diploid reproductive cells have important differences in trout farming. Tetraploid individuals attract attention with their higher growth rates, more egg and sperm production depending on their genetic structure. On the other hand, diploid individuals can better adapt to natural selection processes by preserving genetic diversity. This is important in terms of developing optimum production strategies, taking into account the advantages and disadvantages of both groups in trout production.

Methods applied for tetraploid trout production

Tetraploid trout production is a method applied to increase genetic diversity and increase production efficiency in trout populations. This process aims to obtain tetraploid (4n) individuals by changing the genetic structures of trout. One of the commonly applied methods to obtain tetraploid is to block cell division of eggs in vitro with high temperature or chemicals such as colchicine. This process prevents mitotic division of normal diploid (2n) eggs and provides the formation of individuals with double-layered chromosome sets (Piferrer *et al*., 2009; Braasch and Postlethwait, 2012). In addition to genetic differences, the cultivation of tetraploid trout allows for more productive and durable hybrids to be obtained by crossing with diploid individuals. These hybrids may be advantageous in terms of growth rates, resistance to diseases and adaptation to environmental conditions (Arai and Fujimoto, 2018; Iqbal et al., 2020). In addition, tetraploid individuals have an important place in commercial fish production because they have the potential to produce larger and more productive fish than diploid individuals. However, the production and maintenance of tetraploid individuals is a process that requires special knowledge and technical skills and therefore requires careful management (Bartley *et al*., 2000; Preston, 2014). Methods for obtaining tetraploid provide significant development in aquaculture and various techniques are used. In this

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section, the temperature, chemical and pressure methods applied in the production of tetraploid trout will be discussed in detail (Chen *et al*., 2022; Ahmad *et al*., 2023).

Chemical methods

The use of colchicine and 6-DMAP stands out among the most common chemical methods for tetraploid trout production. These chemicals affect the mitosis processes of fertilized eggs and provide tetraploidy formation (Alexander *et al.*, 2006; Hu *et al.*, 2020).

Colchicine application

Colchicine is an alkaloid that inhibits mitosis and is widely used in obtaining tetraploidy (Arai, 2001; Yamaha *et al.*, 2007; Everson, 2015). The application process is as follows:

Fertilized eggs are immersed in a 0.01-0.1% colchicine solution. This process prevents mitosis and allows the chromosome number of the eggs to double (Nichols, 2009; Iqbal et al., 2020).

The eggs are kept in this solution for approximately 20-30 minutes and 1-6 hours after fertilization. This period is a factor that directly affects the success of the tetraploid individuals obtained (Nichols, 2009; Zohar, 2021).

At the end of the colchicine application period, the eggs are transferred to the normal water environment and continue the development phase. At this stage, the growth and health status of the individuals obtained are carefully monitored (Mizuno *et al*., 2002; Song *et al.*, 2012; Gu *et al*., 2024).

Dimethylaminopurine application

6-dimethylaminopurine (6-DMAP) is also an effective chemical for creating tetraploidy (Ahmedifar *et al.*, 2021). Application protocol:

Fertilized eggs are immersed in a 0.5-1.0% 6-DMAP solution approximately 20-25 minutes after fertilization (Peachey and Allen, 2016; 2016; Rathod *et al*., 2023; Zhang *et al*., 2023).

They are usually kept for 6-12 hours. During this period, the mitosis processes of the eggs are inhibited (Mizuno *et al*., 2002; Leeds and Weber, 2019).

After the application, the eggs are left to develop in normal water conditions. Studies have shown that this method increases the formation of tetraploid individuals (Mizuno *et al*., 2002; Wang *et al.*, 2024).

Temperature methods

Heat treatments are among the methods of obtaining tetraploidy and are carried out with applications made at certain temperature ranges. The protocol is as follows:

Fertilized eggs are kept at 30-35°C (Leeds and Weber, 2019; Tao *et al*., 2019). Heat is usually applied for a period of 30 minutes to 1 hour. The duration has been optimized to increase the efficiency of obtaining tetraploidy (Nichols, 2009; Wang and Shen, 2018 ; Zhang *et al*., 2023).

At the end of the period, the eggs are transferred to normal water conditions and continue their development phase. Heat treatments have positive effects on the growth rates of the obtained

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individuals (Leeds and Weber, 2019; Gu *et al*., 2024).

Pressure methods

High pressure treatments are another effective method used in the production of tetraploid trout. This application is carried out in order to increase the chromosome number of the eggs (Mizuno *et al*., 2002).

Before the fertilized eggs enter the first mitosis phase, this period is approximately 20-30 minutes. The eggs are kept under 300-400 atm pressure for 30-60 minutes (Bonnet *et al*., 2007; Nichols, 2009). This pressure affects the cell structure of the eggs and supports the tetraploidy formation process.

After the pressure application, the eggs are transferred to normal conditions and continue their developmental stage. Studies have shown that this method significantly increases the formation of tetraploid individuals (Liu *et al*., 2022; Zohar, 2021).

After the production of tetraploid individuals, a careful monitoring process is required on the growth performance and reproductive behavior of these individuals (Mizuno *et al*., 2002; Liu *et al.*, 2017; Hu *et al.*, 2020). Monitoring is of critical importance to increase the health and productivity of tetraploid individuals.

Advantages of tetraploidy application

Increased growth rate and yield

Tetraploid individuals generally grow faster than diploid individuals. Studies have shown that tetraploid trout exhibit better growth performance than their diploid counterparts (Nichols, 2009; Li *et al*., 2016). This provides higher yield and lower production costs commercially, making tetraploidy an attractive alternative for the aquaculture industry.

Reproductive success

Tetraploid individuals increase genetic diversity, allowing for more resistant and healthy individuals to be obtained. Tetraploid trout tend to be more resistant to certain environmental conditions and diseases (Nichols, 2009; Wang *et al.*, 2004). In addition, the fact that these individuals are under less stress during their reproductive periods increases their reproductive success (Rinchard *et al*., 2019). The reproductive success of tetraploid individuals is important in terms of increasing genetic diversity and obtaining productive hybrids. When tetraploid trout are crossed with diploid individuals, the hybrid offspring can grow faster and be more resistant to diseases. However, it has been observed that tetraploid individuals often have lower reproductive efficiency (Hu *et al*., 2019; Lu *et al*., 2022). To overcome this problem, special care techniques and environmental control measures can be applied to improve the genetic fitness of tetraploids.

Genetic diversity

Genetic diversity is the presence of different genetic variations within a population, and this diversity is a critical factor for the species' ability to adapt to environmental changes, disease resistance, and long-term survival.

Tetraploid trout production can play an important role in increasing this genetic diversity (Iqbal et al., 2020; Wang et al., 2024). Tetraploid (4n) fish have twice as many chromosome sets as normal diploid (2n) fish, which can increase genetic diversity and allow hybrid offspring to exhibit superior characteristics.

When tetraploid individuals are crossed with diploid individuals, the hybrids resulting from the mixture of genetic material can grow faster, be more resistant to environmental stresses, and can be raised with higher yields. This feature can help create more productive and durable populations, especially in commercial fish production. In addition, crossbreeding operations with tetraploid fish can create new genetic diversity and thus contribute to the prevention of genetic degradation (Huang *et al*., 2017; Zhang *et al*., 2023). Although tetraploid trout production has significant potential in terms of preserving genetic diversity, this process also involves some difficulties. Tetraploid individuals generally have low reproductive ability because the excess chromosome set can affect gamete formation. However, methods such as genetic selection and artificial breeding techniques can be used to increase genetic diversity in tetraploid fish. In this way, it may be possible to raise the species more efficiently and sustainably. (Mizuno *et al*., 2002; Zhou and Gui, 2017).

Disadvantages

Difficulties in the production process

Tetraploid trout production involves some difficulties since chemical and physical methods must be used. The application of these methods can lead to complications and potential health problems in the production process (Iqbal et al., 2020; Zhang *et al*., 2023). In particular, the fact that the applied chemical substances can negatively affect health makes the process of obtaining tetraploidy risky.

The production of tetraploids is a process that must be carefully controlled in high-tech laboratory environments. Eggs must be exposed to chemicals (e.g., tetraploidy induction with colchicine) or made tetraploid by methods such as high temperature. These processes, if not managed carefully, can lead to undesirable genetic deterioration or low reproductive efficiency. In addition, special care conditions, water quality and feeding regime are also important for the healthy rearing of tetraploid fish (Piferrer *et al*., 2009; Zhou and Gui, 2017; Ahmad *et al*., 2023).

Market acceptance

The commercial acceptance of tetraploid trout may encounter some market and consumer reactions. Consumers' lack of knowledge and concerns about tetraploid products may negatively affect the market share of these products (Bonnet *et al*., 2007; Preston *et al.*, 2014). Therefore, it is important to increase consumer awareness and emphasize the benefits of such products.

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Genetic effects

The production of tetraploid trout is a genetically complex process. Tetraploid individuals have twice the number of chromosome sets compared to normal diploid (2n) fish. This may affect gamete (egg and sperm) production. Tetraploid fish are often infertile, meaning they cannot reproduce on their own. Therefore, tetraploid individuals must be crossed with diploid individuals. However, these crosses can sometimes fail to provide the expected genetic diversity. In addition, problems such as genetic incompatibility and chromosome losses may prevent the healthy development of juvenile fish (Preston *et al.,* 2014; Carman *et al*., 2022).

Tetraploidy creates changes in the genetic structure, which in some cases can lead to undesirable genetic variation. Such genetic changes can have negative effects on the ecosystem and may also affect the long-term performance of tetraploid individuals (Nichols, 2009; Martínez et al., 2014).

Conclusion and recommendations

In order for tetraploid trout production to be successful, some fundamental problems need to be overcome. Chemical applications used during the production of tetraploids may adversely affect the health of the fish. The cost of these processes may limit the large-scale tetraploid production in commercial fish farming. In addition, the fact that environmental factors can affect the growth and survival rates of tetraploid individuals necessitates taking measures to prevent the release of these species into natural ecosystems.

Tetraploid trout production offers an important area in terms of increasing genetic diversity and improving commercial production efficiency. However, reproductive problems, physiological difficulties, high costs and environmental stresses experienced during the production process limit the wide-scale application of this method. Although changes in the genetic structures of tetraploid individuals potentially provide the emergence of more productive and resistant hybrids, this process requires further research and technology development.

In conclusion, tetraploid trout production is an important step in developing sustainable and efficient production methods in the aquaculture industry. However, for this technology to be applicable on a commercial scale, the difficulties experienced in the production processes need to be solved. The success rate of tetraploid fish production can be increased by integrating genetic engineering, environmental management and new breeding techniques. However, it is important to conduct further scientific research and application to improve the productivity of tetraploids.

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