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SEX CONTROL IN RAINBOW TROUT (*Oncorhynchus mykiss*)

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Abstract

In recent years, the decrease in precipitation due to global warming or the change of precipitation regime has led to the review of production methods in agricultural activities in the world and in Turkey. Accordingly, scientific studies continue to develop appropriate methods that can keep up with today's conditions in the agricultural, animal and fishery production sectors in order to use water resources more efficiently. From the point of view of fish production, sustainable production in both marine and inland waters, obtaining more products per unit area, more efficient use of resources, reusing the water used in production and discharging it are ensured to conserve resources. Biotechnological studies continue to increase day by day in order to protect resources, reduce losses in production amount and improve product quality. In this review, biotechnological methods applied in fisheries today are emphasized for target production by eliminating product losses.

Keywords: 17α-Methyltestosterone, rainbow trout, androgenesis, ginogenesis, triploidy.

Introduction

The need for fish in the world and in our country is met by hunting and aquaculture from sea and inland waters (Özgür, 2011). While the contribution of aquaculture, which has been on the rise, to global production was 25.7% in 2000, it increased to 46.8% in 2016. Although aquaculture can no longer catch up with the high annual growth rates it showed in the 1980s and 1990s, it has grown faster than other leading food production sectors with an annual growth rate of 5.8% in the period 2001-2016 (FAO, 2018).

Aquaculture in Turkey started in the 1970s with rainbow trout. It is still at the forefront of inland fish farming (Özden et al., 2003). Today, biotechnology and genetic engineering applications are used for healthy individuals. Biotechnology is used in aquaculture in several different ways. These are sex control, chromosome manipulation, gene transfer and hybridization. In terms of fish breeding, they reported that individuals with superior characteristics will be raised, especially with gene transfer (Başçınar et al., 2009).

The increasing demand for aquatic products and the limited level of fishery production lead to a rapid development in aquaculture. Therefore, it is foreseen that the aquaculture sector will have the greatest potential among production systems in meeting the nutritional needs in the coming years (Atar et al., 2009).

Reported that biotechnological methods have an important place in the emergence of this potential over time. Thanks to the rapid advances in genetics in the last 20 years, biotechnological methods applied in fish culture have developed and although some of them can be applied in practice, some of them can only be done in laboratory conditions (Turan, 2000).

The main purpose of such studies is to obtain healthy and high-yielding products at a more affordable cost. In order to achieve this yield, resistant to diseases, high feed efficiency, fast growing etc. individuals need to be trained. It is necessary to focus on production techniques and fish breeding studies in fish farming (Özdemir, 2018).

Factors Affecting Sex Control

We can say that the main factors affecting sex formation are genetic and environmental factors, besides, developing bioengineering and biotechnology and phenotypic factors also affect sex formation (Başçınar et al., 2009).

Özden et al. (2003) reported that the genetic determination of sex in fish is determined by sex determination genes. However, they reported that it is not only dependent on the sex chromosomes, but also the environmental conditions. These environmental factors, especially during early larval development, temperature, pH and stock density affect sex development in fish. In addition, phenotypically, gender transformations can be achieved with later external intervention (Hatef et al., 2019).

Sex development in most fish usually begins soon after hatching. In response to the embryo's genetic makeup, modifications at this point program the gonads to become either ovaries or testicles. This period in which the phenotypic sex can be changed may differ according to the species. If a larva absorbs anabolic steroids after the marsupial stage, the development of the germ cells may be diverted (Shepherd et al, 1990; Shepherd et al, 1992).

The purpose here is that one or both of the individuals reach early sexual maturity and as a result; have shown that sex control practices are made due to negative changes in growth, feed utilization rate, behavior, health, body and meat color (Başçınar et al., 2009). However, in some species, gender transformation studies are carried out in order to prevent male or female individuals from reaching sexual maturity at different times (potoperiod) and to facilitate production (Köprücü, 2018).

Sex reserved Applications

Gender transformations can be achieved by using different methods in aquaculture. The main purpose here, as mentioned before, is to produce individuals such as monosex, triploid, tetraploid in order to raise productive healthy generations and to provide a controlled production, to bring them to market size quickly and not to lose yield due to breeding activities. such studies will also contribute positively to fish production (Özdemir, 2018).

Various techniques can be used in breeding to produce single breed or sterile populations. Examples of these are monosexuality, sterilization, hybridization, gynogenesis, androgenesis, polyploidy, and sex reversal (Dunham, 2004).

Chemicals Used in Sex-reserved fish

Changing the phenotypic sex without genetically changing sex or providing permanent sex transformation by artificial (androgens and estrogens) means. As a result of these processes, all female, all male and sterile fish can be produced (Yılmaz et al., 2013).

For this purpose, androgens used in masculinization;

* 17α-Methyltestosterone
* 11-Ketotestosterone
* 19-nor-Ethynyltestosterone
* Fadrozole (Aromatosis Inhibitor)

Steroid hormones (estrogens) used in feminization studies are;

* Estradiol-17β *Estrone
* Estriol *Ethynylestradiol

Feminization

Feminization is carried out by administering estrogen to the larvae that have started to feed. As can be seen in Figure 1. Administration of estrogens is the same as for androgens, but a higher dose is needed as the turn towards feminization is less than towards masculinization. The point to be considered in the use of estrogen is not to exceed the recommended doses. High levels of estrogen cause liver damage and mortality in Salmonidae species (Sheperd et al., 1990).

In salmon production, the postlarvae are fed by adding 20 mg of Estradiol-17β to a kg of food for 40-60 days when the first feeding is started (Turan et al., 2011).

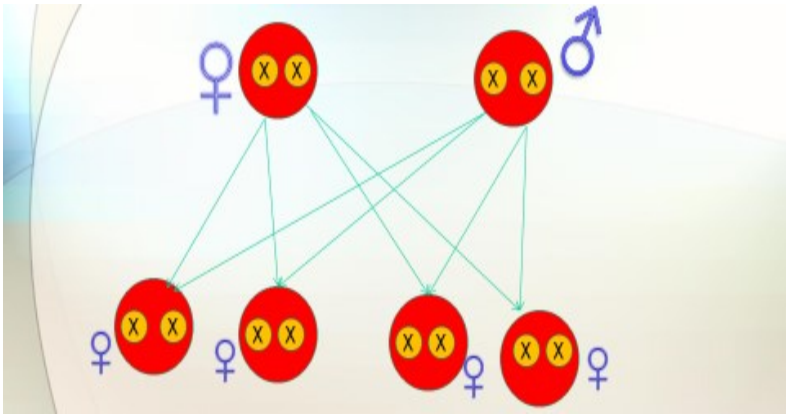

Figure 1. Cross of a sex-reversed male and female fish (Original)

Masculinization

In masculinization of fish, 17α-methyltestosterone, a derivative of the natural hormone testosterone, is usually applied. Of the androgens, 19-nor-ethinyltestosterone is the most effective, but the artificial analogue of methyltestosterone is inexpensive and readily available, so it is widely used in practical masculinization processes (Purdom, 1993). The sex differentiation of hormone-fed fish is shown in picture 2.

Picture 1. A and B (XX) male gonad image (Original)

Ginogenesis

Gynogenesis is ensuring that fish receive their chromosomes only from the mother, and embryonic development in eggs is triggered by sperm and continues without the contribution of sperm hereditary material in any way. The purpose of gynogenesis is the production of consanguineous lines and single-sex populations (Özden et al., 2003; Güner et al., 2016). In gynogenesis, spermatozoa whose genetic material has been destroyed are used for fertilization of eggs. Γ-rays, X-rays and Ultraviolet (UV) are used to neutralize the genetic material of spermatozoa. Ultraviolet is preferred mostly because it is cheap and useful (Manickam, 1991, Güner et al., 2016). Ginogenesis is schematized in Figure 3.

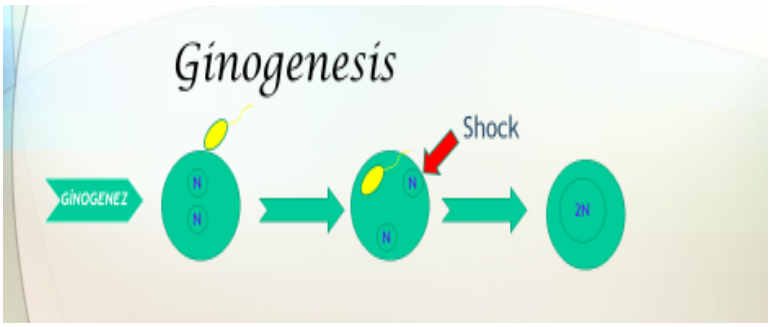

Figure 2. Schematization of female genetic material (Original)

Androgenesis

Androgenesis, unlike gynogenesis, is the continuation of the fertilized embryo development from the chromosome set of the spermatozoa, after the elimination of the genetic material of the egg. Androgenesis is the elimination of the genetic material of the egg with cobalt-60$^{\gamma}$ rays

between 102-105 x-ray doses, then its fertilization and the continuation of the embryo development from the chromosome set of the spermatozoa (Güner et al., 2000).

When the androgenetic zygote will undergo the first division, shock (hydrostatic pressure, heat shock, colchicine, nitrous oxide) is applied and cell division is prevented. The individuals thus formed have chromosome sets from the father and are 100% homozygous (Turan, 2000). Androgenesis is schematized in Figure 4.

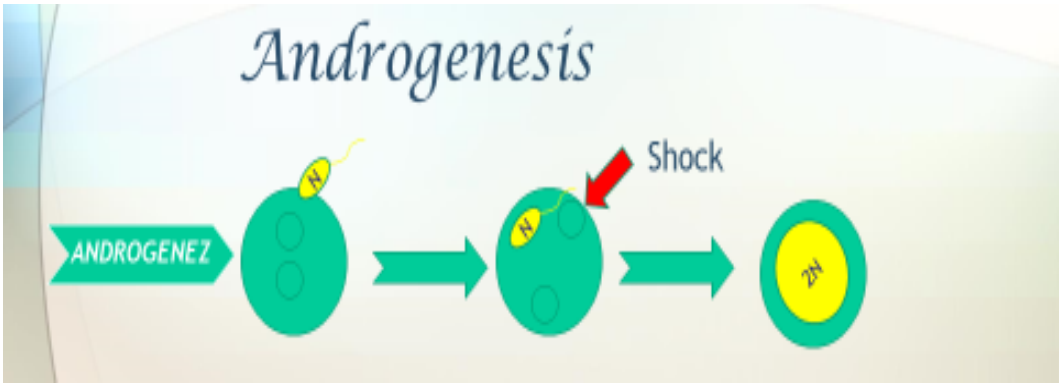

Figure 3. Schematization of male genetic material (Original)

Diploid and Triploid

Sex-reversed functional male (XX) fish with a normal female will result in a female fish, while crossing a normal male and female will result in a male or female individual. Triploidization applications are mostly used for sterile salmonid production and offer many practical advantages for the aquaculture industry. The most important benefit of such manipulations is that metabolic energy is used for somatic growth rather than gamete production. At the same time, sterile fish retain their bright silvery color and are considered a higher quality by the consumer (Fenske et al., 2004) (Figure 5).

In addition, sterile salmon produced by triploidization are morphologically the same as diploid fish when they reach adult size and their functions are normal under intensive culture conditions (Teskeredzic et al., 1993).

Achieving triploidy can be achieved by blocking the second meiosis and preventing the expulsion of the second polar cell after fertilization (Yeşilayer et al., 2008).

Methods used in triploidization;

- Heat (Temperature and Cold shock)
- Pressure
- Chemicals (such as Colchicine, Cytokalacin, Diazot Monoxide (Purdom, 1993).

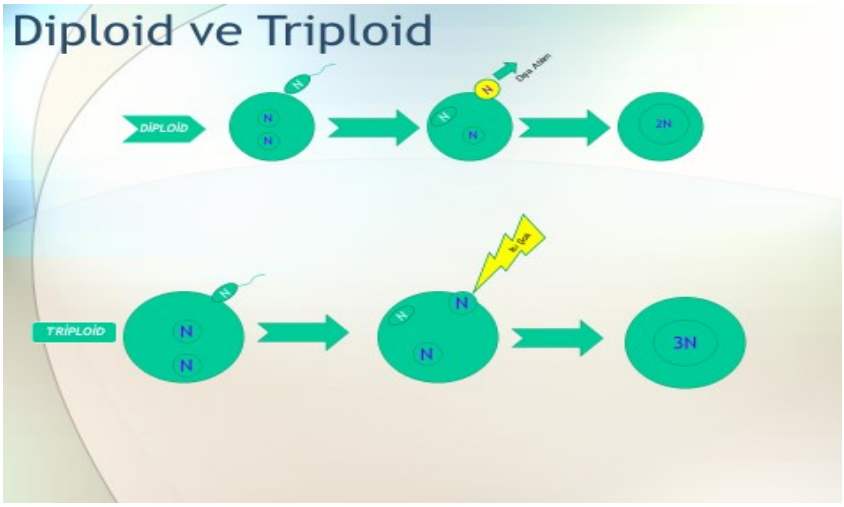

Figure 4. Diploid (at the top) and Triploidi (below) shematics (Original)

Discussion

In the study of Arslan et al. (2010), a suitable hormone protocol was investigated for the indirect hormonal sex conversion method of masculinized female broodstocks used to raise whole female rainbow trout (*O. mykiss*). For this purpose, the larvae to be used as rootstock were fed with feeds containing 1,2 and 3 mg 17α-methyltestosterone (MT) per kilogram for 600 days from the first feeding stage. As a result, the study showed that oral MT applications gave more successful and stable results in obtaining masculinized female brootstocks in rainbow trout. It has been demonstrated that high-dose MT may adversely affect both sperm duct development and gonad development. They also pointed out that the actual dose of MT to which the larvae are exposed at different water temperatures may differ due to the changing metabolic rate.

In another study, Afonso et. al. (2000) reported that estrogen levels in male bony fish were examined in very low amounts or could be said to be absent. However, male Koho salmon (*Oncorhynchus kisutch*) examined the effects of estrogen production after elimination. The aromatose inhibitor fadrozole was used in a group of fish in the sexual maturation period, 1 mg or 10 mg AI/kg was injected approximately 1.5 months before natural spawning, and they observed that estradiol inhibited as a result (Malison, 1986; Bhandari et al., 2004; Tovo-Neto et al., 2018).

Before testosterone administration in order to obtain masculinized female individuals that the business needs, the amounts of individuals desired to be obtained is determined first (Yavuz, 2012). Calculation was performed on how much feed can be consumed during this period, and the hormone dose to be applied is determined by calculating 750 days (eg, 75 days of application should be made in a business with a water temperature of 10 degrees) (Takatsu et. al., 2013).

The hormone whose dose has been determined is weighed on a balance with an accuracy of 0.0001. After weighing, the stock solution is prepared. Then it is taken from the stock solution to be sprayed on the feed, diluted again in 250-300 ml of industrial alcohol (ethanol) and sprayed on the feed. While the hormone mixed with alcohol is sprayed onto the feed, it should be mixed with the feed and penetrated into the whole feed as much as possible (Mikolajczyk et al., 2007).

After the hormone spraying process is completed, it is laid in a thin layer and dried. Mixing is done from time to time within a few hours, allowing the alcohol in the feed to evaporate and the feed to dry completely, shown in picture 6. The bait whose alcohol has been evaporated and completely dried is stored to be given to the fish (Malison, 1986; Yavuz, 2013).

The fish to be applied should have just started to withdraw the food sac and to take food from outside. After this application has started, these fish should not be given any other feed until the end of the program (Eroldoğan et al., 2015; Schroeder et al., 2017).

In order to achieve a successful result from sex change, great care must be taken to keep the water temperature constant. For this reason, it is recommended to carry out similar applications in spring waters with constant water temperature throughout the year (Güner et al., 2016).

Picture 2. Hormone and feed mixed, dry (Original)

Considering the aquaculture sector, which is developing day by day, market demands are of great importance. It is known that businesses that make the same product more efficient are always one step ahead of others. For this reason, biotechnological studies have gained great importance in hatcheries that produce trout eggs and fry in recent years. However, while carrying out these studies, it is necessary to pay attention to whether the preferred method and application methods are both suitable for human health and commercially low-cost. Currently, many trout farms in our country are able to grow market size trout ranging from 250-330 gr in a period of 10-12 months (Yeşilayer et al., 2008). Marketing problems arise as the male individuals in the population reach sexual maturity during this period. For this reason, the issue of producing monosex female individuals has gained importance (Sezgi, 2008). Although there are many methods for gender manipulation, the production of monosex individuals by hormone triggering is an economical method that does not require much expertise when compared to methods such as androgenesis, gynogenesis, and hybrid production (Bhandari et al., 2004; Arslan et al., 2010; Arslan et al., 2012).

A manipulation can be done by administering anabolic steroids to the larvae after the marsupial period makes sex control possible (Afonso et al., 2000; Budd et al., 2015). In this context, it is aimed to produce XX genotype male breeders with indirect hormonal application in determining the sex by hormone method. With the indirect hormonal method, the use of hormones in every production is prevented, and an advantage is provided in terms of both material and human health. Single-sex production methods have been developed that show the desired characteristics according to the grown species and will provide commercial profit. In

trout breeding, male individuals reach sexual maturity one year earlier than female individuals. They spend some of the energy they receive with food on gonadal development as well as growth. This is an economic loss for producers. The meat of male trout is juicy and especially tasteless during breeding periods. In terms of appearance, female fish are more preferred by the consumer. In terms of processing facilities, it is known that female individuals leave 3-5% more profit in terms of carcass weight compared to male individuals of the same age. Considering all these reasons, some trout producers import eggs with all female characteristics and use them in their production. This both increases foreign dependency and is an economic loss for the country. The spread of these studies in our country is important in terms of ensuring sustainability by preventing foreign dependency of production, reducing foreign exchange flow abroad, and preventing the transfer of zoonotic diseases from abroad (Biçer et al., 2017).

Ethical approval

The author declares that no ethical approval needed for this review study.

Data availability statement

The authors declare that data are available from authors upon reasonable request.

Funding organizations

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