Reproductive performances of triploid male and female Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) at different ages

ODANG CARMAN1,2,*, AKHMAD TAUFIQ MUKTI3,*, MUHAMMAD ZAIRIN JR1, ALIMUDDIN1

1Department of Aquaculture, Faculty of Fisheries and Marine Science, Institut Pertanian Bogor, Jl. Agatis, Kampus IPB Dramaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8622909, *email: odangca@apps.ipb.ac.id
2Department of Aquaculture, Faculty of Fisheries and Marine Science, Universitas Airlangga, Jl. Mulyorejo, Kampus C Unair, Surabaya 60115, East Java, Indonesia. Tel.: +62-31-5911451, *email: akhmad-t-m@fpk.unair.ac.id

Abstract. Carman O, Mukti AT, Zairin JR M, Alimuddin. 2023. Reproductive performances of triploid male and female Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) at different ages. Biodiversitas 24: 4235-4242. Triploidization has been known as a chromosome set manipulation strategy to produce sterile fish. This study aimed to examine the reproductive and sterility performances of triploid male and female Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) at different ages. The triploidization process using heat shock of 41°C for four minutes at Four Minutes After Fertilization (MAF) of the zygote old made the population of triploid tilapia. Two steps of fish rearing were conducted, namely indoor fish rearing and outdoor fish grow-out. Fish were reared in a controlled laboratory (indoors) at the Reproduction and Genetics Laboratory of Aquatic Organisms, Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University for four months, and then fish grow-out was performed in a controlled pond for two months. Reproductive performances such as Hepatosomatic Index (HSI), Gonadosomatic Index ( GSI), gonadal development, and sterility of triploid and diploid for both male and female tilapias were analyzed. These results showed that triploid tilapia generally indicated late gonadal development and growth compared to diploids before and during maturation. Triploid female tilapia showed a lower HSI than diploid female tilapia (*P*<0.05). Otherwise, triploid and diploid males showed the same HSI. GSI of triploid males and females were lower than diploid males and females (*P*<0.05) at every age, namely three-, four-, five- and six-month-old fish. Based on the size and histology of the gonad, triploid male and female tilapias showed sterile characteristics with gametogenesis inhibition.

Keywords: Nile tilapia species, reproductive performances, sex group, sterility, triploidy

INTRODUCTION

Triploidization is a standard method to produce sterile individuals to control the animal population (Thresher et al. 2014) and stimulate growth. Triploid induction has been applied commercially to produce sterile fish, which may influence consumer acceptance of the produced fish (Cnaani et al. 2013). The production of sterile fish has great potential in developing the aquaculture industry. The artificial induction of triploid fish is mainly used to improve the quality associated with sexual maturation, such as higher growth rates, stronger disease resistance, and better organoleptic properties (Larsen et al. 2014; Wong and Zohar 2015). Triploid fish production has been developed in the salmon farming industry to control the reproductive cycle, accelerate fish growth, and reduce migration (Glover et al. 2016). Several studies have been conducted to compare triploid and diploid, including early development (Sambraus et al. 2020; Bazaz et al. 2021), growth (Fraser et al. 2014b, 2021, 2022b; Taylor et al. 2019; Benhaim et al. 2020; Bortoletti et al. 2022), internal organ morphology (Martínez-Llorens et al. 2021), vertebral malformation (Smedley et al. 2016; Vera et al. 2019), skeletal development (Salbuvik 2018; Smedley et al. 2018), and lens morphology (Olsvik et al. 2020).

Triploid fish are found spontaneously in wild and cultured populations. Generally, physical and chemical treatments successfully generate triploid in many fishes. Triploid induction has also been successfully performed using temperature shock (Alcántar-Vázquez et al. 2016; Karami et al. 2016a; García et al. 2017; Karbalaei et al. 2017; Shimada et al. 2017; Iqbal et al. 2020; Oliver et al. 2020; Sato et al. 2020), pressure shock (Chalmers et al. 2018), electric shock (Hassan et al. 2018; Okomoda et al. 2020b), electroproportion (Okomoda et al. 2020a), and chemical shock (Qin et al. 2018). According to traditional concepts, triploid fish usually have disordered meiosis, leading to low fertility or complete infertility (Zhang et al. 2021, 2022). The sterility of triploid fish causes energy that is supposed for gonadal development and gametogenesis used for somatic growth. When gonadal development, triploid fish cannot hold the pairs of chromosomes, and gonads eventually, triploid fish cannot develop properly to become sterile. Generally, the testes and the ovaries of triploid fish are more petite than diploid fish, and the gonadal development is abnormal. Among triploid induction, most visible chromosomes are damaged or missing. Triploid fish become sterile because of an odd number of chromosome sets that inhibit or interfere with the process of meiosis and subsequent failure in gonadal development or production of aneuploid gametes; aneuploidy is likely the cause of sterility (Sellars 2013).

The histology profiles by do Nascimento et al. (2017) on yellowtail tetra found little vitellogenic oocytes in
ovaries and spermatids in the testis. Gomelsky et al. (2015) also produce a triploid female loach with a gonad that is not mature and has no eggs. Ovaries in triploid females never progressed past the first phase and never produced the female sex hormones. Gametogenesis disorders that affect gonadal development are two fundamental influences of triploidy on the basic physiology of fish. Both are related to changes in the size and number of cells. The volume of the nucleus increased to accommodate the extra genetic material and cellular volume. An essential consequence of the increased volume of the nucleus and cellular resulted in a decrease in the volume ratio of the surface area (Jackson et al. 2018).

On the other hand, some researchers stated that the sterility of triploid fish could not be predicted because the testes and ovaries indicate the development of gametogenesis. Fully motile spermatozoa also produced some triploid loaches (Zhou et al. 2023b). A triploid with three complex genomes is fertile (Fjelldal et al. 2014). In contrast, triploid male salmon found that testes were developed but unable to produce fertile sperms. In the triploid male salmon, gonads grow more significantly than triploid females and produce functional sperm. Still, their spermatozoa are aneuploidy (Fraser et al. 2014a), so they cannot produce offspring that live after the egg fertilization from the diploid female. Tilapia is the best aquaculture commodity in tropical and subtropical areas (El-Sayed 2006). Based on these facts, this study aimed to examine the reproductive and sterility performances of triploid males and females of Nile tilapia at different ages.

MATERIALS AND METHODS

This study was conducted at the Reproduction and Genetics Laboratory of Aquatic Organisms and Field Stations and Health Laboratory of Aquatic Organisms, Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor, Indonesia in 2015 to 2017. In this study, the experimental protocols and care of animals were followed by international and national standards and ethical guidelines and approved by the Scientific Committee, IPB University.

Fish preparation

The triploid and diploid populations of Nile tilapia strain Wanayasa (NIRWANA) originated from Purwakarta, West Java, Indonesia. According to Mukti (2016), methods and procedures of artificial fertilization and zygote incubation were carried out. The triploidization process using a heat shock of 41°C for four minutes at four Minutes After Fertilization (MAF) of zygote age produced the population of triploid tilapia. This treatment produced 91-100% triploid NIRWANA fish as identified using chromosome counting prepared according to Kligerman and Bloom (1977) and Mukti et al. (2016).

Indoor fish rearing

Five to six Days Post-Hatching (DPH) and the end of the yolk sac, fish larvae and triploid and diploid populations were reared in ten of 50 L aquaria at a density of one fish L⁻¹, respectively. The fish were fed a live diet once a day, i.e., Moina sp. as much as 1 g day⁻¹ for three days, followed by Tubifex sp. of 7.5 g day⁻¹ for ten days and a commercial pellet feed (crude protein content of 32%), at-satiation, three times a day for 15 days. Furthermore, the fish, both triploid and diploid populations, were reared in ten of 180 L aquaria at a density of four fish L⁻¹. The fish were fed 0.5-1.0 mm diameter of commercial pellets, which contained a crude protein of 40%, at satiation, three times a day until the fish was two months old.

Then, two-month-old fish were reared in the 200 L-volumed aquaria at a density of 20 fish per aquaria, the three separate aquaria between populations of triploid and diploid, respectively. The fish was fed a commercial pellet containing a crude protein of 40% at satiation until the three-month-old fish. Furthermore, the fish was fed commercial pellet feed containing a crude protein of 32% at satiation until the five-month-old fish. The feeding frequency was three times a day. The males and females of three-, four-, and five-month-old fish were collected from as many as 10-15 fish randomized to the observation of gonad. Then, the Hepatosomatic Index (HSI) and Gonadosomatic Index (GSI) were measured, and the gonad histology was prepared.

Outdoor fish grow-out

The two-months-old fish was also reared in a 2.0 m × 1.0 m × 0.7 m-sized hapa net (mesh size of 10 mm) at a 20.0 m × 10.0 m × 1.5 m-sized concrete pond according to Mukti et al. (2020a). Pisciculture in the hapa net was also conducted separately between male and female sexes, diploid and triploid fish. Each treatment was repeated three times at a density of 10 fish m⁻². Fish was reared in a hapa net until the 6-month-old fish. The fish was fed a commercial pellet feed containing a crude protein of 40% in the first month, and the feed had a crude protein of 32% in the next three months, at satiation, three times a day. At the end of the grow-out, triploid and diploid fish were randomly collected as many as 10-15, male and female, to observe the gonad, measurement of HSI and GSI, and histology of the gonad.

Measurement of HSI, GSI, and gonads histology

HSI and GSI were surgically measured according to Mukti et al. (2020b), both triploid and diploid fish on three-, four-, five-, and six-month-olds, respectively. Fish samples were anesthetized using an MS-222 solution of 10 mg L⁻¹ for 3-5 minutes. The fish’s Total Length (TL) and Body Weight (BW) were measured; then, an abdominal section of the fish was dissected. All internal organs in the fish body were removed, and the BW of the fish again without internal organs was weighed.

Furthermore, fish liver and gonads were weighed separately. Fish gonads of males and females, both triploid and diploid, were photographed for data documentation overview of fish gonads. Most gonads of three fish samples for each three-, four-, and five-month-old fish, triploid and diploid, were fixed by immersion in a Buffer Neutral Formalin (BNF) solution of 30-50 mL. Then, the gonad
sample was readily prepared histologically in the laboratory according to McCann (2015). The gonad histological was observed under the BH2-RFCA Olympus microscope completed by the camera.

**Data analysis**

HSI and GSI data were analyzed statistically using Analysis of Variance (ANOVA). They proceeded to Duncan’s multiple range test using Excel 2018 software program with a confidence interval of 95% for any significant differences among treatments. At the same time, the gonad histology of triploid and diploid fish for both males and females was analyzed descriptively according to Genten et al. (2009).

**RESULTS AND DISCUSSION**

Reproductive performances of triploid and diploid Nile tilapias in different age periods were presented in Table 1 and Figures 1, 2, and 3. The BW of triploid fish, both males and females, was higher and significantly different (P<0.05) than diploid fish in all age periods of fish (3-6 months). Triploid female tilapia have lower HSI than diploid females. In contrast, triploid and diploid male tilapias’ HSI are not significantly different (P>0.05). The GSI average of triploid fish is lower compared with diploid fish and showed a significant difference (P<0.05) than diploid tilapia, both male and female, during periods of five- and six-month-old fish (Table 1).

**Table 1.** Body weight, hepatosomatic index, and gonadosomatic index of triploid and diploid, both male and female of Nile tilapia and egg diameter at different ages (n=10)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Fish group</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW (g)</td>
<td>Triploid</td>
<td>60.5±9.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.0±9.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>119.3±11.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>388.0±52.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diploid</td>
<td>44.8±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.2±6.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>106.8±10.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>264.7±45.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>HSI (%)</td>
<td>Triploid</td>
<td>34.1±3.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60.4±9.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.6±6.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>272.6±59.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diploid</td>
<td>33.5±3.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.2±6.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.1±9.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>244.4±61.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>GSI (%)</td>
<td>Triploid</td>
<td>2.2±0.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.4±0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.9±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1±0.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diploid</td>
<td>2.6±0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6±0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8±0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.0±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ED (mm)</td>
<td>Triploid</td>
<td>0.2±0.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4±0.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.3±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.1±0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Diploid</td>
<td>0.3±0.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6±1.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.5±1.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.2±1.3&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Note: * Male sex, ♀ Female sex. BW: Body Weight, HSI: Hepatosomatic Index, GSI: Gonadosomatic Index, ED: Egg Diameter. Egg samples to measure diameters are 25 eggs of the fish individual. Different superscripts in the same row indicate significant differences (P<0.05)

**Figure 1.** Morphological male (testes) and female (ovary) gonads, both triploid and diploid of Nile tilapia at different ages (n=10) (bar scale=10 mm)

**Figure 2.** Histological female (ovary) gonad, triploid and diploid of Nile tilapia at different ages (n=3). imo: immature oocyte, do: degradative oocyte, df: degradative follicle, pvo: previtellogen oocyte, og: oogonia, vo: vitellogenic oocyte, mo: mature oocyte (bar scale=50 µm)
Fish age (month) | Group | Triplet | Diploid
--- | --- | --- | ---
3 | | | |
4 | | | |
5 | | | |

**Figure 3.** Histological male (testes) gonad, triploid and diploid of Nile tilapia at different ages (n=3). ls: lobul seminiferus, spt: Spermaticid, spz: Spermatozoa (bar scale=10 µm)

Figure 1 shows that the gonad size of triploid tilapia was much smaller compared to diploid male and female fish. In gonads histology, it appears that gonads of triploid female fish (ovary) had obstacles in both the development and the growth marked by the number of damage or degradation of oocytes and vacuoles (Figure 2). In the histology of triploid male fish, gonads (testes) observed different gametogenesis as gonads of diploid male fish. Testicular triploid tilapia showed much damage and emptied lobular seminiferous. They indicated that the number of spermatids was fewer than in testicular diploid males and found no spermatozoa in the testes as diploid males tilapia (Figure 3).

**Discussion**

This study showed that triploid female tilapia have a lower HSI than diploid female tilapia, which was similar to the study result of Felip et al. (2009) in the European sea bass *Dicentrarchus labrax* (Linnaeus, 1758), Gomes et al. (2020) in the rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792), and Karami et al. (2016b) in the African catfish *Clarias gariepinus* (Burchell, 1822). Triploid female European sea bass has a low HSI that may reduce Vitellogenesis (Vtg) synthesis in the liver mediated by estradiol. The low HSI in triploid tilapia is associated with the low weight of the fish liver. This matter is likely related to decreasing liver Vtg activity (synthesized Vtg); on the contrary, increased content of Vtg causes an increase in liver weight. Reverse liver weight loss indicated the decline of Vtg content in the liver. Inhibition of vitellogenesis in triploid fish is caused by low levels of the steroid hormone of estradiol in the body, which is a mediator of Vtg synthesis in the liver. Koedprang and Na-Nakorn (2000) also proved that the triploid female silver barb does not occur in vitellogenesis. In salmon, triploid females do not produce mature oocytes because the production of steroid hormones is insufficient to support the Vtg synthesis and grow comparable oocytes, including in juveniles (Felip et al. 1999). Furthermore, it does not find vitellogenic oocytes in triploid fish during sexual maturation in fish diploid (Benfey et al. 1989; Phelps 2018). Phelps (2018) also confirmed that estradiol-17β (E2) levels had higher diploid females during spawning.

Vtg is a major blood-borne protein that serves as a primary precursor of the yolk sac in oviparous vertebrates and invertebrate animals (Tyler and Sumpter 1990). During vitellogenesis, oviparous vertebrates, including Teleostei, synthesize and secrete Vtg from the liver to respond to E2 follicles (Mommsen and Walsh 1988). Vtg synthesis and secretion also occurred in mature and male Teleostei and hepatocytes cultured by exogen estradiol treatment (Parks et al. 1999; Phelps 2018). After Vtg is secreted into the blood, Vtg is transported into mature oocytes through receptor-mediated endocytosis. Next, Vtg is stored as yolk sac protein, lipovitellin, and fosvitin. Further serves as a source of nutrients for developing embryos and larvae. Gomes et al. (2020) proved that the Vtg process does not occur in triploid female rainbow trout *O. mykiss*.

Vtg is also closely related to the gonadal development of fish, especially females. Vtg also plays an essential role in increasing the GSI in fish. Vtg content increased in plasma also increases the gonadal development of fish, so the GSI has also increased, along with the development and growth of gametes. Vtg is the main blood protein in mature females and is absorbed to accelerate growth, and many oocytes reach the final oocyte size of 80% (Mommsen and Walsh 1988).

As HSI, the GSI of triploid tilapia, especially females, is lower than diploid tilapia (Table 1). Gonad size of triploid tilapia, both males and females, is much smaller than diploid tilapia (Figure 1), similarly according to Soltan et al. (2017) in tilapia, Derayat et al. (2013) in the Atlantic cod *Gadus morhua* (Linnaeus, 1758), Pushpa Geetha and Jayaprakas (2014) in blue gourami *Trichogaster trichopterus* (Pallas, 1770), and Lahnsteiner et al. (2020) in Salmonidae. The study by Phelps (2018) found that triploid fish had lower plasma Vtg than diploid fish, either before the beginning or at the end of vitellogenesis, included in GSI mean and oocyte diameter. The study Neal (2014) conducted on largemouth bass *Micropterus salmoides* (Lacepède, 1802), Chatthaiphan et al. (2016) in bighead catfish *Clarias microcephalus* (Güntner, 1864), and Felip et al. (2009) in the European sea bass showed that triploid fish has the lowest GSI compared to diploid fish, both males and females. In this study, the GSI of triploid females had no significant difference from triploid males. In contrast, the diploid female had higher compared to the diploid male, similarly according to Park et al. (2016) found in the marine medaka *Oryzias latipes* (Hamilton, 1822). This study showed that the GSI of triploid females was very low during maturation season (Table 1), and the ovaries were rudimentary (Figures 1 and 2), similarly according to Xu et
Based on the histology of gonads, the triploid tilapia, especially females (oocytes), indicated disruption or obstruction of gametogenesis in gonads (Figure 2). Many oocytes and follicles are degraded or damaged in ovarian triploid tilapia, and very few do not reach mature oocytes (immature oocytes). The development of oocytes is facing obstacles significantly on triploid tilapia, striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) (Ibrahim et al. 2017; Carman et al. 2022), Atlantic salmon *Salmo salar* (Linnaeus, 1758) (Ibrahim et al. 2017; Carman et al. 2022), and yellowtail tetra *Astoynax altiparanae* (Garutti & Britski, 2000) (do Nascimento et al. 2017). Conversely, triploid salmon specifically demonstrate the delay of oocyte growth, and the degree of oocyte development differs. In contrast, the other species of salmon, triploid have oocytes that develop (Sheehan et al. 1999) but do not complete (Krisfalusi et al. 2000). Triploidization of the mud loach fish *Misgurnus anguillicaudatus* (Cantor, 1842) cause stunted gonad development of fish (Nam et al. 2004). Cherfas et al. (1994) found that 1-year-old males and females carp had poorly developed gonads and were sterile, similarly according to Mori et al. (2006) in the barfin flounder and Park et al. (2016) in the marine medaka. Kizak et al. (2013) conducted a different study on the brown trout *Salmo trutta fario* (Linnaeus, 1758), and Murray et al. (2018) in the Atlantic salmon *S. salar* showed that gonadal development in females was reduced. In contrast, gonadal development in males usually seems, as the study conducted by Fjelldal et al. (2014). Zhou et al. (2018) also found that both male and female triploid loaches *M. anguillicaudatus*, indicate gonadal development.

Triploidization can increase ovarian cellular senescence and apoptosis, leading to abnormal gonadal morphology and fibrosis. Downregulation of genes responsible for the proper meiosis regulation and chromosome segregations during meiosis probably influences meiotic maturation through disordered division of meiotic in chromosomes (Nynca et al. 2022). Benfey et al. (1989) explain that triploid female fish showed gametogenesis disorder and could not produce live offspring. The plasma E2, testosterone, and Vg in triploid brook trout fish are generally lower than in diploid fish. Triploid fish developed oocytes in ovaries, only half the total triploid fish. In three-year-old fish, 13 of the 19 fish did not form gametes, including perinucleolar, yolk vesicle, and yolk globule, while three produced 72 mature-staged oocytes. It is made in abnormal chromosome pairs during meiosis and the inability of most of the germ cells of triploid fish to complete gametogenesis and produce euploid gametes. The configuration of the chromosome pairs is not usually univalent, and bivalent may occur (Carrasco et al. 1998). The triploid showed disruption of gonadal development and became sterile, such as triploid salmon (Taylor et al. 2013; Benfey 2015), yellowtail tetra *A. altiparanae* LIRP (do Nascimento et al. 2017), Arctic char *Salvelinus alpinus* (Linnaeus, 1758) (Fraser et al. 2022), and Pacific oyster *Crassostrea gigas* (Thunberg, 1793) (Zhou et al. 2023a). Sterility causes inhibition of gonadal development, which can be observed through gonad size as shown in Figure 1 and gonadal histology of fish as shown in Figures 2 and 3. These are also found in sterile tilapia (Pandit et al. 2015; Tao et al. 2020), common carp *Cyprinus carpio* (Linnaeus, 1758) (Majhi et al. 2017), and frog *Pelophylax esculentus* (Linnaeus, 1758) (Chmielewska et al. 2022).

On the other hand, several studies showed that a few triploid males and females of species had developed testes and ovaries, respectively. Still, males did not render milt, females had low fecundity and fertilized eggs did not hatch. Biswas et al. (2007) stated that one of the causes of sterility in the triploid fish represented lower E2 binding capacity and receptors of triploid fish.

Similarly, in the testes histology of triploid male tilapia (Figure 3), although the testes have evolved to the spermatids stage, the amount is microscopic compared with diploid tilapia. Testicular histology of triploid tilapia seems a lot of damage and lobule seminiferous empty. There are no spermatogonia, spermatocytes, or spermatids. Although gametogenesis is impaired, triploid males have circulating steroid hormone levels sufficient to trigger testicular development partially or wholly; they can produce few spermatozoa and typical secondary sexual characteristics. These properties have been reported on the triploid of several salmon or tilapia and other species, such as loaches (Zhou et al. 2018, 2023b) and bighead catfish *Clarias macrocephalus* (Günther, 1864) (Wachirachaikarn et al. 2017). Since male germ cells do not undergo meiosis to sexual maturation, testicular development, in general, is not impaired in triploid males (Huang et al. 2021; Xu et al. 2023). Although triploid males show maturity sexually, it remains sterile because it produce aneuploid spermatozoa and do not produce life offspring, such as in common carp (Majhi et al. 2017), tilapia (Pandit et al. 2015), grass carp *Ctenopharyngodon idella* (Valenciennes, 1844) (Clemens et al. 2016), and *Carassius auratus* (Linnaeus, 1758) (Wang et al. 2021).

Although the cycle decreased about one month, the triploid male shows a typical secondary sexual characteristic and standard hormonal profile. Triploid females showed no signal of hormonal maturation, especially at the pituitary level. Although triploid males and females are genetically sterile, females do not undergo physiological maturity (Benfey 2015; Delomas and Dabrowski 2018). Triploid fish had genetically sterile because the cells could not undergo normal meiosis to produce euploid gametes. A tiny number of triploid cells that undergo meiosis ideally and complete maturation only reach males with functional and aneuploid spermatozoa (Huang et al. 2021).

Most triploid male testes develop because some spermatogonia are produced before meiosis and primary spermatocytes. The fish gonad of triploid males is more significant than triploid females, and males can occur in maturation. This fact occurred in salmon fish (Fraser et al. 2014b). This study also shows that the gonads of triploid male tilapia are bigger than triploid female tilapia. However, both are much smaller than diploid tilapia. The main characteristic of triploid tilapia was delayed gonadal development compared to diploid tilapia. In addition, both
male and female triploid tilapia had sterile features, which indicated delayed gametogenesis in the gonad.

Triploidization is one of the best solutions to overcome the problems of tilapia cultivation to date. Triploid tilapia provides many advantages such as accelerated growth and increased flesh quantity (dressing and edible carcass), and flesh quality (Mukti et al. 2020a). In the future, triploidy will become an effective model for tilapia aquaculture management.

ACKNOWLEDGEMENTS

The authors would like to thank Lembaga Pengelola Dana Pendidikan (LPDP), Kementerian Keuangan, the Republic of Indonesia, who partially supported this study. The authors wish to thank the Head and Staff of Tilapia and the Common Carp Aquaculture Development Agency at Purwakarta, West Java, Indonesia, for providing Nile tilapia (NIRWANA) broodstocks and Muhamad Amin, Ph.D. who helped with English editing of this article. We would also like to thank the proofreader, editor, and reviewers for suggestions and corrections to improve this article.

REFERENCES


Fraser TWK, Hansen TJ, Rema SC, Olsen RE, Fjelldal PG. 2022b. Triploid Atlantic salmon × brown trout hybrids have similar seawater growth and welfare issues as triploid Atlantic salmon, but both were heavier at harvest than their diploid counterparts. Aquaculture 552: 10.1016/j.aquaculture.2022.737975.


