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Growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia (*Oreochromis niloticus*)

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Abstract: This study aimed to compare the growth performance, survival rate, flesh, and proximate composition of sex-grouped triploid and diploid Nile tilapia. The triploid population was obtained through heat shock at 41 °C for 4 min, 4 min after fertilization. Before sexing, 50 fish were reared in aquaria at a density of 1 fish L^{-1} for 2 months. After sexing, both triploid and diploid fish were grouped into all-male, all-female, and mixed-sex groups and reared in hapas at a density of 10 fish m^{-2} for 4 months. Each group was replicated three times. The highest body weight, body length, and growth rate were observed in all-male triploids, while the lowest of those parameters were obtained in all-female diploids. The highest survival rate was achieved in both all-male and mixed-sex triploids, and it did not significantly differ from the mixed-sex diploid ($P > 0.05$). The triploid fish had a higher edible carcass percentage than diploids. Proximate analysis indicated that the crude protein content of triploids was higher than that of diploids, while the crude lipid and ash contents were lower than those of diploids ($P < 0.05$). Triploid Nile tilapia had the best growth performances, including flesh quantity and quality, compared to diploids.

Key words: Growth performance, triploid production, monosex, mixed-sex, Nile tilapia

1. Introduction

Sterile fish are beneficial in aquaculture as the fish will reduce or even prevent the use of energy for reproduction in sterile metabolism processes. As a result, most of the anabolic energy will be transferred to somatic growth. Sterile fish also have the potential for a better survival rate compared to diploid fish. Devlin et al. [1] stated that the increase in the growth of fish brings substantial benefits in shortening culture period, improving the efficiency of feed utilization and the efficiency of production, and ensuring product availability. Correspondingly, culturing sterile fish is one of the best farming management approaches in aquaculture practices, as it enables the use of the metabolism pathway to obtain somatic tissue quickly instead of producing either sperm or eggs in the spawning season [2].

The high ability (uncontrolled) of tilapia reproduction causes unexpected density in the pond with varied size and slow growth, making it less commercially profitable in aquaculture. Sterilization is the best possible solution to solve the problems in tilapia culture [3]. Lutz [4] mentioned that among the future's aquaculture commodities, tilapia is a candidate fish to produce functionally sterile seeds on a large scale. The induction of triploidy is one of the methods of producing sterile fish. The culture of triploid fish could provide benefits such as increased growth, carcass production, survival rate, and flesh quality [5–7].

The production of triploid tilapia has been developed for more than four decades, and triploidy will be an effective management tool in tilapia farming in the future [8]. Triploid tilapia has small testes or ovaries, low gonad weight, and high body weight, protein utilization, and protein efficiency ratio compared to diploid tilapia. Thus, its farming is conceivably beneficial [9]. In some cases, the growth performances of triploid tilapia were reported to be superior or equal to those of diploid tilapia [10–12].

On the other hand, some studies indicated that male tilapia has faster growth compared to female tilapia [13–15]. The production level of monosex male tilapia farming was 10% higher compared to the mixed-sex population [16,17]. Associated with the presence of sexual dimorphism in terms of growth, many efforts were made to produce all-male seed populations for the purpose of monosex culture, which generally can be obtained through four common

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methods, namely manual sexing [18] at body size of 5–7 cm, hybridization [7,19], hormonal treatments [15,20–27], or chromosome set manipulations, such as androgenesis [18,28], to produce YY supermale parent stocks [29–31].

So far, the combined effects of triploidy and growth-related sexual dimorphism superiorities in tilapia are still unknown. The strain of fish, including tilapia, also possibly influences growth performance during the culture period. Therefore, the present study tries to clarify the effect of those superiorities on growth, survival rate, flesh percentage, and proximate composition of Nile tilapia during the grow-out period.

2. Materials and methods

2.1. Experimental fish preparation

In this study, the fish used were of the Wanayasa strain of Nile tilapia known as NIRWANA, produced through a family selection program between genetic improvement for farmed tilapia and genetically enhanced tilapia in Indonesia. The broodstocks were obtained from the Tilapia and Common Carp Aquaculture Development Agency in Purwakarta, West Java, Indonesia. Artificially fertilized eggs (4 min after insemination) were subjected to heat shock treatment at 41 °C for 4 min to produce triploid fish. This treatment produced triploid Nile tilapia of 91%–100%, as identified using the chromosome counting method according to Kligerman and Bloom [32] and Mukti et al. [33]. Embryos were incubated in glass funnels in a recirculating system, and diploid fish were produced using a similar procedure.

Larvae of both triploid and diploid were separately reared in 50-L aquaria at a density of 1 fish L^{-1}. A total of 10 aquaria were used for triploid and diploid fish, respectively. The 2-day-old fish were fed on *Moina* sp. for 3 days, followed by tubificid worms for 10 days, and then commercial diet (33% crude protein content) for 15 days. Following, fish were transferred into 180-L aquaria, reared at a density of 4 fish L^{-1}, and fed on a commercial diet (40% crude protein content) for 30 days. Sexing was conducted morphologically by observing the genital openings at the average fish weight of 6.5–10 g to separate males and females of both triploid and diploid fish. The sexing was also confirmed by gonad preparation and observation using the squash method with acetocarmine stain. Twenty fish from different groups, namely all-male triploid, all-female triploid, mixed-sex triploid, all-male diploid, all-female diploid, and mixed-sex diploid, respectively, were prepared for performance evaluation.

2.2. Performances evaluation

Previously prepared all-male, all-female, and mixed-sex groups of both triploids and diploids were separately transferred and reared in floating nets of 2.0 m ´ 1.0 m ´ 0.7 m (mesh size of 10 mm) placed in concrete ponds of 20 m ´ 10 m ´ 1.5 m at a density of 10 fish m^{-2} with water exchange rate of 1 L s^{-1}. Water quality parameters, such as temperature, dissolved oxygen, and pH, were measured every week with ranges of 27–29 °C, 3.4–4.4 mg L^{-1}, and 6.7–7.3, respectively. Three floating nets were used as replication for each group. First, fish were fed on a 1-mm-diameter commercial diet (40% crude protein content) to satiation for 30 days, then they were fed on a 3-mm-diameter commercial diet (33% crude protein content) to satiation during the last 3 months (90 days), three times a day.

In general, the maturation period of tilapia begins for 90-day-old fish. In this study, the maturation period was also observed at the 90th day of fish rearing. The sex of the fish was checked monthly. Body weight (BW), body length (BL), mortality, and feed intake data were measured every month. Biomass gain; the relative percentages of biomass, BW, and BL gains of triploids compared to diploids; BW and BL gains; absolute growth rate (AGR); feed conversion ratio (FCR); and survival rate (SR) were analyzed based on data of initial and final grow-outs, while specific growth rate (SGR) was analyzed every month during 4 months of grow-out of fish. Dressing, edible carcass, and proximate data of male and female triploid and diploid fish were analyzed at the end of the experimental period.

The growth performances were calculated according to Hariati [34]. The formulas were used to calculate biomass gain (D), the relative percentage of triploid:diploid biomass gain, BW gain, the relative percentage of triploid:diploid BW gain, BL gain, the relative percentage of triploid:diploid BL gain, AGR, FCR, SR, and SGR, respectively, as follows:

$$\Delta \text{ Biomass (g)} = \text{Final biomass (g)} - \text{initial biomass (g)}$$

$$\Delta \text{ B 3N:2N (\%)} = \frac{\Delta \text{ biomass of triploid (g)} - \Delta \text{ biomass of diploid (g)}}{\Delta \text{ biomass of diploid (g)}} \times 100$$

$$\Delta \text{ BW (g)} = \text{Final body weight (g)} - \text{initial body weight (g)}$$

$$\Delta \text{ BW 3N:2N (\%)} = \frac{\Delta \text{ BW of triploid (g)} - \Delta \text{ BW of diploid (g)}}{\Delta \text{ BW of diploid (g)}} \times 100$$

$$\Delta \text{ BL (mm)} = \text{Final body length (mm)} - \text{initial body length (mm)}$$

$$\Delta \text{ BL 3N:2N (\%)} = \frac{\Delta \text{ BL of triploid (mm)} - \Delta \text{ BL of diploid (mm)}}{\Delta \text{ BL of diploid (mm)}} \times 100$$

$$\text{AGR (g day}^{-1}\text{)} = \frac{\text{Final body weight (g)} - \text{initial body weight (g)}}{\text{Length of rearing (days)}}$$

$$\text{FCR} = \frac{\text{Feed consumed by fish (g)}}{\Delta \text{ body weight of fish (g)}}$$

$$\text{SR (\%)} = \frac{\text{Live fish number at the final of rearing}}{\text{Live fish number at the initial of rearing}} \times 100$$

$$\text{SGR (\% day}^{-1}\text{)} = \frac{\text{Ln final body weight} - \text{Ln initial body weight}}{\text{Length of rearing (days)}} \times 100$$

The dressing is the fish's body without head, fins, scales, and internal organs, while the edible carcass is a cut of the right and the left sides of the fish's body. The dressing and edible carcass data were determined according to Buchtova et al. [35] based on ten samples from males and females of both triploids and diploids, respectively. The dressing and the edible carcass percentages were calculated by the following formulas, respectively:

$$\text{Dressing (\%)} = \frac{\text{Dressing weight of fish}}{\text{Body weight of fish}} \times 100$$

$$\text{Edible carcass (\%)} = \frac{\text{Edible carcass weight of fish}}{\text{Body weight of fish}} \times 100$$

Increase of triploid dressing percentage (DP) and edible carcass percentage (ECP) compared to diploid was calculated using the relative percentages of triploid:diploid dressing and edible carcass formulas, respectively, as follows:

$$\Delta\text{Dressing 3N:2N (\%)} = \frac{\text{DP of triploid (\%)} - \text{DP of diploid (\%)}}{\text{DP of diploid (\%)}} \times 100$$

$$\Delta\text{Edible carcass3N:2N (\%)} = \frac{\text{ECP of triploid (\%)} - \text{ECP of diploid (\%)}}{\text{ECP of diploid (\%)}} \times 100$$

In addition, flesh proximate analysis of fish (crude protein, crude lipid, ash, and carbohydrate contents) was evaluated according to AOAC protocol [36] based on ten samples from both male and female triploids and diploids, respectively.

2.3. Statistical analysis

Data on growth performances (biomass gain, body weight and body length gains, AGR, and SGR); FCR, SR, and flesh percentages (dressing and edible carcass percentages); and proximate content were statistically analyzed using analysis of variance (ANOVA) with SPSS 10 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was followed by the ANOVA test with a confidence level of 95%.

3. Results

3.1. Growth performance, survival rate, and feed conversion ratio

The growth performances of the tested fish groups are shown in Table 1. The results showed that the growth of triploid fish was significantly higher ($P < 0.05$) compared to that of diploid. The biomass gains (D B 3N:2N) of

Table 1. The growth, survival rate, and feed conversion ratio performances of sex-grouped triploid and diploid Nile tilapia fish during 4-month grow-out period (n = 20).

Parameter	Fish groups					
	Triploid			Diploid		
	All-male	All-female	Mixed-sex	All-male	All-female	Mixed-sex
Initial biomass (g)	278.6 ± 5.2	190.0 ± 8.3	236.2 ± 6.0	205.0 ± 8.9	136.0 ± 8.8	183.4 ± 5.8
Final biomass (g)	8056.7 ± 405.5	5193.3 ± 445.6	7013.3 ± 551.4	6130.0 ± 366.6	4626.7 ± 277.6	5676.7 ± 465.0
DBiomass (g)	7778.1 ± 404.3^{a}	5003.0 ± 437.9^{e}	6777.1 ± 548.9^{b}	5925.0 ± 363.5^{c}	4490.7 ± 284.9^{f}	5493.2 ± 462.9^{d}
DB3N:2N (%)	31.3	11.4	23.4	-	-	-
Initial BW (g)	13.9 ± 0.3	9.5 ± 0.4	11.8 ± 0.3	10.3 ± 0.4	6.8 ± 0.4	9.2 ± 0.3
Final BW (g)	402.8 ± 20.3	278.5 ± 23.2	350.7 ± 27.6	317.0 ± 13.5	252.3 ± 10.2	288.3 ± 15.5
DBW (g)	388.9 ± 20.2^{a}	269.0 ± 22.8^{d}	338.9 ± 27.4^{b}	306.7 ± 13.6^{c}	245.5 ± 10.7^{e}	279.2 ± 15.3^{d}
DBW3N:2N (%)	26.8	9.6	21.4	-	-	-
Initial BL (mm)	99.2 ± 0.0	93.3 ± 0.0	92.5 ± 0.0	96.3 ± 0.0	92.8 ± 0.0	91.2 ± 0.0
Final BL (mm)	274.5 ± 2.1	241.3 ± 6.7	266.5 ± 5.6	250.0 ± 2.4	232.2 ± 1.9	243.4 ± 4.6
DBL (mm)	175.7 ± 2.1^{a}	147.9 ± 6.7^{d}	174.0 ± 5.6^{b}	153.7 ± 2.4^{c}	139.3 ± 1.9^{e}	152.2 ± 4.6^{c}
DBL3N:2N (%)	14.3	6.2	14.3	-	-	-
AGR (g day^{-1})	3.2 ± 0.2^{a}	2.2 ± 0.2^{d}	2.8 ± 0.2^{b}	2.6 ± 0.1^{c}	2.1 ± 0.1^{e}	2.3 ± 0.1^{d}
FCR	1.2 ± 0.1^{b}	1.4 ± 0.1^{c}	1.1 ± 0.0^{a}	1.2 ± 0.1^{b}	1.4 ± 0.0^{c}	1.4 ± 0.0^{c}
SR (%)	100.0 ± 0.0^{a}	93.3 ± 5.8^{c}	100.0 ± 0.0^{a}	96.7 ± 2.9^{b}	91.7 ± 2.9^{c}	98.3 ± 2.9^{ab}

D = Gain, D B 3N:2N = relative percentage of triploid:diploid biomass gain, BW = body weight, D BW 3N:2N = relative percentage of triploid:diploid body weight gain, BL = body length, D BL 3N:2N = relative percentage of triploid:diploid body length gain, AGR = absolute growth rate, FCR = feed conversion ratio, and SR = survival rate. Different superscripts in the same row indicate significant differences ($P < 0.05$).

all-male, all-female, and mixed-sex triploid fish were 31.3%, 11.4%, and 23.4% higher than those of diploids, respectively. A similar pattern was found in body weight gain (D BW 3N:2N) and body length gain (D BL 3N:2N). The highest values of body weight and length gains (26.8% and 14.3%, respectively) were observed in all-male triploids, followed by mixed-sex triploids (21.4% and 14.3%, respectively), while the lowest values (9.6% and 6.2%, respectively) were seen in all-female triploids. Furthermore, all-female diploid fish significantly showed the most inferior growth performance compared to other groups.

All-male triploids had the highest absolute growth rate (AGR) compared to other groups, followed by mixed-sex triploids, then all-male and all-female diploids. Meanwhile, the mixed-sex triploids had the best feed conversion ratio, followed by all-male triploids and diploids. The survival rates of all-male and mixed-sex triploids and mixed-sex diploids were higher compared to other groups, as shown in Table 1.

Figure 1 shows the monthly body weight and body length recorded during the 4-month grow-out period. In general, triploids grew faster than diploids, and all-male triploids showed the highest growth rate while all-female diploids showed the lowest growth rate.

In this study, it was observed that in both triploid and diploid fish, males grew faster than females during the experiment. In triploid and diploid groups, the biomass gains of the males were 55.5% and 31.9% higher than those of females, respectively. Before the maturation period, the average body weights of triploid and diploid males were 16.6 and 10.7 g greater than those of triploid and diploid females, respectively. Meanwhile, during the maturation period, the average body weights of triploid and diploid males were 103.3 and 50.5 g greater than those of triploid and diploid females, respectively. These results showed that the role of sexual dimorphism in the growth of Nile tilapia had a similar pattern as the role of ploidy level, the effects of which were highly significant during the maturation period.

All-female and mixed-sex triploid groups showed similar growth rates at the 90th day (Figure 2). The mixed-sex triploid group had a higher specific growth rate (SGR) than other sex groups at the 120th to 180th days, while the all-female triploid group had similar SGR as the all-male diploid group at the 120th day. On the other hand, all-female triploid and all-male and mixed-sex diploid groups had similar SGR at the 150th day. Meanwhile, the all-female triploid group had similar SGR as the mixed-sex diploid group at the 180th day (Figure 2).

3.2. Flesh percentage and proximate composition

The edible carcass percentages of male and female triploids were higher than those of diploids. The highest and lowest dressing percentages were found in triploid and diploid females, respectively ($P < 0.05$). The increase in dressing and edible carcass percentages of female triploids were 8.6% and 10.5% higher than those of female diploids, respectively. Meanwhile, the increase in dressing and edible carcass percentages of male triploids were 2.1% and 5.9% higher than those of the diploids, respectively (Table 2).

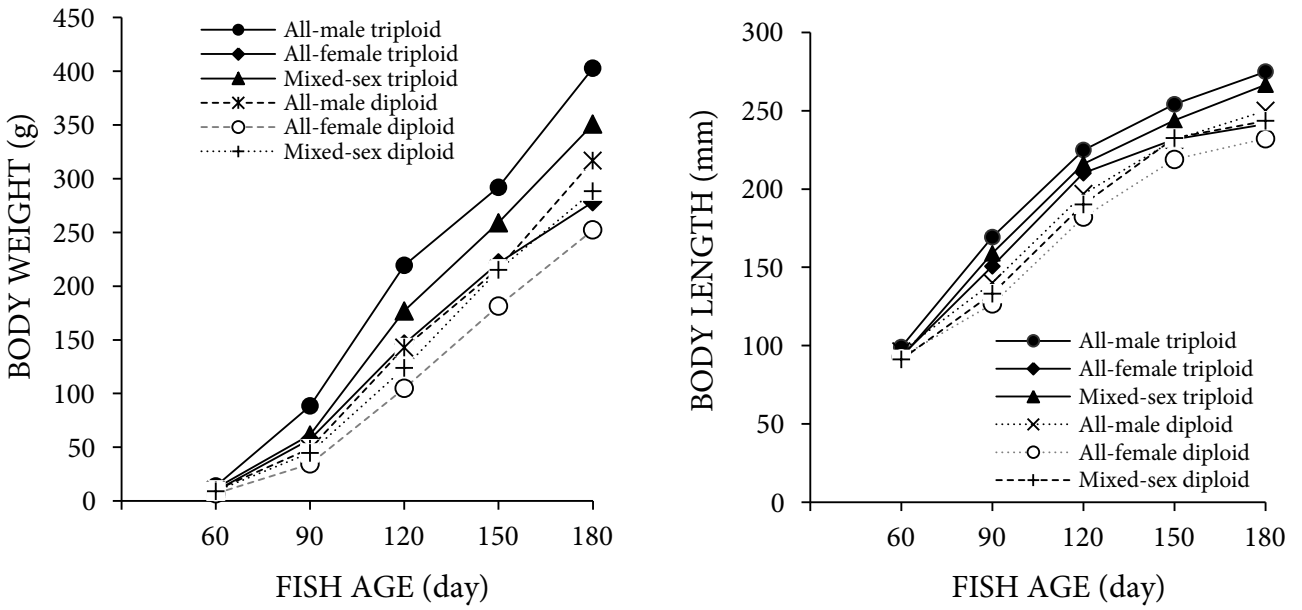

Figure 1. Body weight and body length of all-male, all-female, and mixed-sex triploid and diploid Nile tilapia fish during 4-month grow-out period.

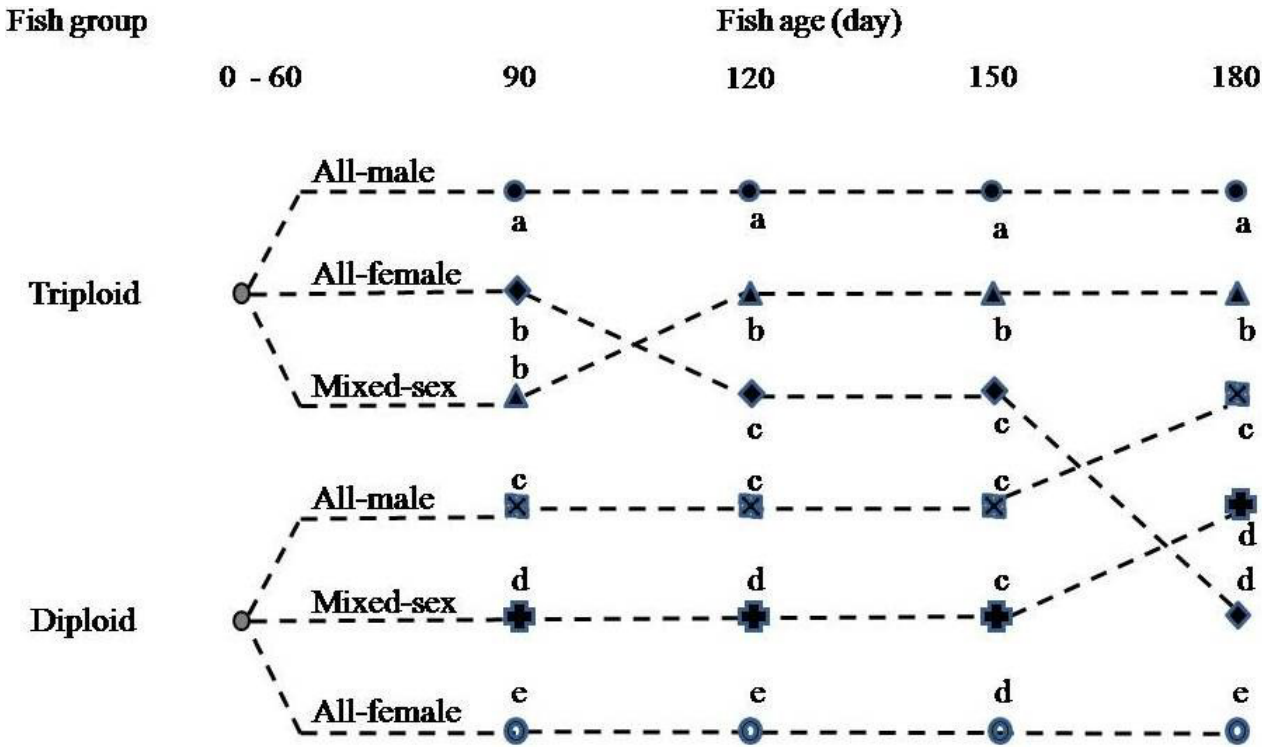

Figure 2. Schematic sequential specific growth rate (SGR) of triploid and diploid Nile tilapia fish during 4-month grow-out period. Different letters at the same fish age indicate significant differences ($P < 0.05$).

Table 2. Flesh percentages of male and female triploid and diploid Nile tilapia fish (n = 10).

Fish group		Body weight	Dressing		Edible carcass	
		(g)	Weight (g)	(%)	Weight (g)	(%)
Triploid	♂	414.1 ± 39.2^{a}	238.3 ± 19.9^{a}	57.6 ± 1.8^{b}	170.9 ± 16.0^{a}	41.3 ± 1.4^{a}
	♀	260.8 ± 24.0^{c}	154.0 ± 13.5^{c}	59.1 ± 1.6^{a}	109.4 ± 10.8^{c}	42.0 ± 1.2^{a}
Diploid	♂	332.0 ± 29.7^{b}	187.2 ± 18.4^{b}	56.4 ± 1.6^{b}	129.4 ± 12.4^{b}	39.0 ± 1.6^{b}
	♀	259.4 ± 14.1^{c}	141.0 ± 7.8^{c}	54.4 ± 1.3^{c}	98.5 ± 6.0^{d}	38.0 ± 1.4^{b}

Different superscripts in the same column indicate significant differences ($P < 0.05$).

Flesh proximate analysis of triploid and diploid fish is shown in Table 3. The crude protein content of female triploids was similar to that of male triploids; however, it was higher than that of diploid fish ($P < 0.05$). On the other hand, crude lipid and ash contents of male and female triploids were lower than those of diploids. There were no significant differences in carbohydrate contents between triploid and diploid fish.

4. Discussion

This study revealed that ploidy level and sexual dimorphism play essential roles in Nile tilapia growth performance. The high growth of male triploids and low growth of female diploids indicated that both ploidy level and sexual dimorphism significantly affected Nile tilapia growth (Table 1; Figures 1 and 2).

Tave [37] reported that triploidization leads to an increase in sterility and growth. The cell size of triploids is larger than that of diploids, and energy for gamete production is reduced or inhibited. In most cases, triploids showed heavier body size and faster growth than diploids in common carp (*Cyprinus carpio*) [38], African mud catfish (*Clarias gariepinus*) [39], Chinese catfish (*C. fuscus*) [40], and Atlantic salmon (*Salmo salar*) [41]. Besides, the performances of triploid fish were not only species- and age-dependent but also depended on the experimental

Table 3. Flesh proximate analysis of male and female triploid and diploid Nile tilapia fish (% dry weight) (n = 10).

Fish group		Crude protein	Crude lipids	Ash	Carbohydrates
Triploid	♂	85.6 ± 0.3^{ab}	5.1 ± 0.2^{b}	6.2 ± 0.2^{c}	3.2 ± 0.7^{a}
	♀	87.0 ± 1.1^{a}	5.0 ± 0.4^{b}	5.9 ± 0.0^{d}	2.2 ± 1.5^{a}
Diploid	♂	84.2 ± 1.3^{b}	5.9 ± 0.3^{a}	7.1 ± 0.0^{a}	2.8 ± 1.7^{a}
	♀	84.3 ± 1.8^{b}	5.5 ± 0.0^{a}	6.4 ± 0.3^{b}	3.8 ± 1.5^{a}

Different superscripts in the same column indicate significant differences ($P < 0.05$).

conditions and the interactions between the environment and genetics [7]. The individual body size of triploids was more significant due to the larger cell size compared to diploids [42]. However, Aliah et al. [43] reported that cell size was not correlated with organ size in sticklebacks (*Gasterosteus aculeatus*). Furthermore, in 2- to 3-month-old sunshine bass (*Morone* spp.), diploids grew faster compared to triploids [44].

The increase in triploid growth is due to the influence of sterility, diverting energy (nutrients) for somatic growth rather than gonadal development and sexual activity [14]. Most studies concluded that the significant difference in growth rate between triploid and diploid fish occurred during the maturation period in fish such as turbot (*Scophthalmus maximus*) [45] and European sea bass (*Dicentrarchus labrax*) [46]. In this study, it was found that the growth difference (30.0%) between triploid and diploid fish had already occurred before (≤90 days) and during the maturation period (90–180 days). Also, the growth of triploids showed more significant differences compared to diploids (39.3%). A similar phenomenon has been reported in fancy carp (*C. carpio*) [47].

The role of sexual dimorphism in growth in tilapia has been revealed in the last three decades. Male tilapia grew faster compared to females, so all-male monosex culturing in this species is worldwide applied. Similar cases were found in catfish (*C. gariepinus*) [48] and crucian carp (*Carassius auratus*) [49].

The comparison of the growth performance among the six groups showed that all-male triploid and all-female diploid fish grew faster and slower, respectively, than the fish in other groups during the experiment. The interaction effect between triploidy and sexual dimorphism in growth was not significant among all-female triploid, all-male diploid, and mixed-sex diploid groups at the 120th to 150th days. In the same groups, all-male diploids grew faster than the others and the interaction effect between triploidy and sexual dimorphism on growth was not significant among all-female triploids and mixed-sex diploids at the 180th day (Figure 2). This phenomenon seemed to be species-specific as found in rainbow trout (*Oncorhynchus mykiss*) by Tabata et al. [50], Mozambique tilapia (*O. mossambicus*) by Varadaraj and Pandian [51], and European sea bass by Felip et al. [52]. Those authors reported that female triploids grew faster than either male triploids, male and female diploids, and mixed-sex diploid.

The lowest growth was observed in all-female diploids, although it looked as if the female diploids went through rapid reproductive development and sexual maturity. Thus, the available energy might be allocated for gonadal development or gametogenesis instead of somatic growth. In this study, it was recorded that at the 120th day, the majority of female diploids began to spawn and incubate either fertilized or unfertilized eggs in the mouth. This generally allows the female to not feed during egg incubation for 15 days until the larvae can swim freely, as reported by Byamungu et al. [53]. In other words, the role of ploidy level in growth during the maturation period was significantly more important than that before the maturation period. These results also revealed that high body weight gain in male and female triploids during the maturation period seemed to be due to the sterility of triploid fish and the reproductive activity of diploid fish.

In this study, triploid fish had higher flesh percentages compared to diploids, and female triploids also had higher flesh percentages. Similar results were reported in gilthead sea bream (*Sparus aurata*) [54] and rainbow trout [55]. However, in common carp [56] up to the size of 400 g, the dressing weight of triploids was not significantly different from that of diploids. The results of this study indicated that female triploids had higher flesh percentages than male triploids as the females were more sterile than males, while the higher flesh percentages in triploids compared to diploids seemed correlated with normal gonadal development in diploids and reduced development in triploids.

Triploid Nile tilapia tends to be high in crude protein and low in crude lipid and ash compared to diploids. In terms of sex, both triploid and diploid male and female fish show the same crude protein, crude lipid, and carbohydrates contents, while the ash content is

significantly different. This shows that triploidy in Nile tilapia affects flesh quality, especially crude lipid and ash contents. These results are supported by the findings of other researchers [5,6,11], but further studies are needed to gather more valuable information.

The interaction effect between triploidy and sexual dimorphism, strongly related to growth, had a positive contribution to production performance, especially during the maturation period. Based on the examination of various aspects related to production, the result revealed that all-male triploid Nile tilapia cultures have the potential to be developed. Hence, in the future, an applicable method for mass all-male triploid seed production should be considered. One of the possible strategic efforts is production of supermale tetraploids as parent stock by combining the chromosome set and hormonal manipulations.

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Conflict of Interest

The authors have no conflicts of interest to disclose.

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