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Growth performance, feed utilization and gonad development of diploid and triploid Nile tilapia, *Oreochromis niloticus*

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Abstract
A 16 week experiment was conducted to evaluate growth performances, gonads development, carcass and proximate analysis of diploid and triploid Nile tilapia, *Oreochromis niloticus*. Triploid were induced by inhibiting the second polar body formation in fertilized eggs using heat chock. Newly hatched fry were transferred and reared in 5 liter aquaria for three weeks and then transferred to 80 L indoor aquaria, provided with a recirculated water for 8 weeks. After the rearing period diploid and triploid fingerlings transferred into 300 liter tanks and fed 30% protein diet to apparent satiation. At the end of feeding trial (16 weeks), triploid *O. niloticus* had significant heaviest, longest and deepest bodies compared with diploids and the same trend was also observed for weight gain and specific growth rate. Triploid *O. niloticus* showed the best feed conversion ratio and protein efficiency ratio compared to diploids. Gonado-somatic indices (GSI) of triploid males and females were significantly smaller than diploids. On the other hand, Hepato-somatic indices, (HSI) of triploid *O. niloticus* males were larger than that of diploid with significant differences between the two groups. However, HSI of triploid females were smaller than that of diploid females but the differences were insignificant. Carcasses of triploids *O. niloticus* had the highest percentages of dressing and flesh and the lowest by-products compared to diploid. Also, triploid flesh contained the highest ($P<0.05$) percentages of fat and the lowest ash. Protein content and dry matter did not significantly ($P>0.05$) affected by ploidy induction in *O. niloticus.*

Keywords: tripliody, growth, carcass, sexual maturation, Nile tilapia

1. Introduction

Tilapias (*Oreochromis, Sarotherodon* and *Tilapia* spp.) are a group of fishes of major economic importance in tropical and subtropical countries, but their uncontrolled and prolific breeding at a small size in mixed sex culture constitutes a serried constraint on their efficient production (Soltan *et al.,* 2013). Early sexual maturation resulting in unwanted reproduction and overcrowding has long been accepted as a major limitation in the culture of most tilapia species, particularly the commonly cultured *Oreochromis niloticus* and *O. mossambicus*. This unwanted reproduction generally results in suppression of growth and reduction in yields in cultured populations (Soltan *et al.,* 1999) [25]. The females continue to spawn at frequent intervals, even if the eggs are not fertilized. This status in diverting the energy from growth to egg production and consequently reduced weight gain. In a mixed population, when eggs are fertilized and develop, the females do not feed during the mouth incubation and brooding period, which is a considerable drain on body reserves (Abdel-Hakim *et al.,* 2000).

Numerous solutions to this problem (the unwanted reproduction) have been proposed including manual sexing and separation of the sexes, culture in cages, controlled use of predator species, production of monosex hybrids, and direct hormonal sex reversal (Mair and Little, 1991). Sterility by triploidy induction in commercial fish stocks has the potential to increase production yields as metabolic energy which be used for gonadal development is redirected to somatic growth (Zhang *et al.,* 2016). As well as, they do not suffer from the associated decrease in flesh quality (Campos Vargas *et al.,* 2015). Therefore, the production of triploid Oreochromis species has attracted considerable attention as an alternative to the use of hybridization and hormones as means of producing monosex male fry to avoid the excessive reproduction and improve flesh quality (Hussain *et al.,* 1991). The present study was carried to induce triploid by heat shock and evaluate the ploidy effects on growth performances, carcass, proximate analysis and gonads development of Nile tilapia, *Oreochromis niloticus*.

2. Materials and methods

Individual brood Nile tilapia fish were marked and held in glass aquaria at 28±2°C. Both male and female fish were held in separated aquaria and used as broodstock for the production of diploid and triploid fish used in this experiment.

2.1 Triploidy Induction

Females held in transparent aquaria were observed several times a day. Ready to spawn, females are identified with swollen and reddened papillae, then netted out of the aquarium and then anaesthetised using few drops of Ethyleneglycol-monophenyl-ether in a half bucket full of

water. After washing the chemical away, female was gently hand-stripped and the eggs were collected in 500 ml glass vial containing physiological saline (0.9% NaCl). An appropriate male was taken out of the aquarium and then stripped of the sperms by using suitable pipette. They were added into the glass vial containing physiological saline (0.9% NaCl). No anaesthesia was used to strip males, and the sperm obtained were microscopically examined for motility.

Triploid were induced by inhibiting the second polar body formation in fertilized eggs using heat shock. Eggs were stripped from a single female and fertilized with fresh milt from a single male and then divided into two groups, untreated (diploid), and treated where eggs of the other group were heat-shocked to induce retention of the second polar body. Heat-shock treatment was applied by immerging the fertilized eggs in warm water (41ºC) for a duration of 4.5 min, 4 min post fertilization as described by Puckhaber and Hörstgen-Schwark (1996).

Diploid and triploid eggs were incubated in separate incubators. Maximum of 150 fertilized eggs were put into glass cups of 35 cm^3 and were incubated at 28ºC for 7 days. The outflow of eggs from incubation glasses, due to the tangential movement caused by the adjusted water, was protected by fitting smooth ended cylindrical meshes on the top of the cups. Triploidization success was confirmed by chromosome preparations Puckhaber and Hörstgen-Schwark (1996) in ten embryos out of each treated batch.

2.2 Fry Rearing

Newly hatched fry were transferred into 5 liters aquaria for three weeks and then transferred to 80 L indoor aquaria, provided with a recirculated, aerated and controlled temperature (28±2°C) water supply. Triploids and diploids were kept separately at a stocking rate of 50 fry/aquarium for two months and fed commercial starter pellets (42% protein) to apparent satiation. At the end of this rearing period, the diploid (11.4 g) and triploid (11.9 g) fry were separately restocked in 300 liters tanks (in three replicates) at a density of 80 fish/aquarium (in three replicates). During the feeding trial fish kept on a 12-hour photoperiod in a recirculated water system maintained at 28°C for 16 weeks. On-growing fish were fed to apparent satiation with formulated pellets (30% protein). Ingredients, composition of the diets and their chemical analysis are presented in Table (1).

Table 1: Ingredients (g kg^{-1}) and composition of the diets.

Ingredients	Starter diet	Grow-out diet
Fish meal (72%)	220	100
Shrimp meal	180	0
Soybean meal	300	440
Yellow corn	180	320
Wheat bran	50	70
Soybean oil	40	40
Vit. & min. mixture[1]	30	30
Chemical analysis % (dry matter basis)		
Dry matter	93.16	92.67
Crude protein (CP)	42.23	30.33
Ether extract (EE)	7.64	6.03
Crude fiber (CF)	5. 21	6.33
Ash	9.12	8.43
NFE[2]	36.80	48.88
Gross energy (MJ kg^{-1}) [3]	20.20	19.02

[1]Vitamin & mineral mixture/ kg premix: vitamin D3, 0.8 million IU; A, 4.8million IU; E, 4g; K, 0.8g; BI, 0.4g; Riboflavin, 1.6g; B6,0.6g; B12, 4mg; pantothenic acid, 4g; Nicotinic acid, 8g; Folic acid, 0.4g Biotin, 20 mg, Mn, 22g; Zn, 22g; Fe, 12g; Cu, 4g; I, 0.4g; Selenium, 0.4g and Co, 4.8 mg.

[2]Nitrogen free extract (NFE) = 100 – (CP + EE + CF + Ash).

[3]Gross energy calculated using gross calorific values of 0.2363, 0.3952, 0.1715 and 0.1715 MJ/g for protein, fat, crude fiber and NFE, respectively according to Brett (1973).

2.3 Fish Samples and Measurements

Body weight, body length and depth were individually measured for each aquarium at the initiation and the termination of the feeding trail. Weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) were calculated using the following equations:

WG (g/fish) = FBW – IBW; SGR% = [lnFBW- ln IBW]/t × 100, where FBW is final body weight (g); IBW is initial body weight (g); ln= natural logarithmic; t=time in days. FCR=FI /WG, where FI is feed intake (g); PER=WG/protein intake (g). Gonado-somatic index (GSI) and hepato-somatic index (HSI) were measured according to Hussain *et al.,* (1995).

GSI = [gonad weight (g) / fish weight (g)] × 100.

HSI = [liver weight (g) /fish weight(g)] × 100.

2.4 Carcass and Proximate Analysis of Fish and Diets

At experiment termination, three fish were chosen at random from each aquarium and exposed to carcass assessment according to the methods described by Lovell (1981). Another three fish were chosen at random from each aquarium for proximate analysis of whole fish body according to the methods of AOAC (1995). Fish and diet samples were oven-

dried 105°C for 24 h, ground, and stored at -20°C for subsequent analysis. Dry matter was determined after drying fish samples in an oven (105°C) for 24 h. Ash was analyzed by incineration at 550°C for 12 hour. Crude protein was determined by micro-Kjeldhal method, N×6.25 (using Kjeltech auto analyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by Soxhlet extraction with diethyl ether (40–60°C). Crude fiber content of diets was determined using the method of Van Soest *et al.,* (1991). Nitrogen-free extract was computed by taking the sum of values for crude protein, crude lipid, crude fiber and ash then subtracting this sum from 100.

2.5 Statistical Analysis

Statistical analysis of the obtained data was analyzed according to SAS (1996). Differences between means were tested for significance according to Duncan's multiple rang test as described by Duncan (1955).

3. Results

Results of growth performance and feed utilization of triploid and diploid *O. niloticus* are shown in Table 2. The obtained results showed that triploid fish recorded the heaviest (P<0.05), longest (P>0.05) and deepest (P>0.05) fish compared with diploid *O. niloticus* at the experiment termination. In the same trend triploid *O. niloticus* recorded the highest significant (P<0.05) weight gain (130.21 g/fish) and specific growth rate (2.21%/day) compared with that recorded for diploid *O. niloticus (*114.90 g/fish and 2.15%, respectively. There were no significant (P>0.05) difference between the two ploidy groups in case of feed intake (FI). However, triploidy induction significantly improved feed conversion ratio (FCR) where triploid group showed better value (1.46) compared to diploid group (1.71). The higher (P<0.05) protein efficiency ratio, PER value (2.28) was recorded for triploid compared to PER (1.95) value of diploid group (table 2).

Gonado-somatic index (GSI) of triploid and diploid males found to be 0.61 and 1.12% (Table 3). GSI also measured 1.22 and 4.12% for triploid and diploid females, respectively. Also, hepato-somatic index (HSI) of triploid males is 3.15 compared to 2.22% for diploid males and 2.82 compared to 2.45% for triploid and diploid females, respectively.

Table 2: Growth performance, feed intake and feed utilization of diploid and triploid *O. niloticus.*

	Diploid	triploid	±SE	Probability
Initial body weight (g)	11.40	11.90	0.98	0.607
Final body weight (g)	126.30 b	142.11 a	2.20	0.017
Initial body length (cm)	9.23	9.44	0.20	0.678
Final body length (cm)	17.7	18.5	0.30	0.067
Initial condition factor (K)	1.45	1.42	0.04	0.366
Final condition factor (K)	2.28	2.24	0.03	0.239
Initial body depth (cm)	2.91 b	3.11 a	0.07	0.450
Final body depth (cm)	5.62	5.93	0.14	0.697
weight gain (g/fish)	114.90 b	130.21 a	1.24	0.047
Specific growth rate	2.15 b	2.21 a	0.03	0.029
Feed intake (g/fish)	196	190	1.29	0.034
Feed conversion ratio	1.71 a	1.46 b	0.03	0.036
Protein efficiency ratio	1.95 b	2.28 a	0.02	0.043

Means followed by different letters in each row significantly (P<0.05) different.

Carcass analysis (table 4) illustrated that, diploid fish gained the lowest (P<0.05) dressing percentage (49.44%) and flesh (34.94) compared to triploids carcasses (53.01 and 37.12%, respectively). By-products percent did not significantly differ among the two ploidy groups. Results of proximate analysis of fish flesh are illustrated in Table 4. Whole-body proximate composition was affected by ploidy induction, with triploids having higher (P<0.05) lipid and lower ash levels than diploids. Protein and dry matter contents of the two ploidy groups indicated some variation but not significant (P<0.05).

Table 3: Gonado and Hepato-somatic indices of diploid and triploid O. niloticus.

	Diploid	triploid	±SE	Probability
GSI (male)	1.12 a	0.61 b	0.06	0.003
GSI (female)	4.12 a	1.22 b	0.21	0.004
HSI (male)	2.22 b	3.15 a	0.19	0.007
HSI (Female)	2.45	2.82	0.11	0.323

Means followed by different letters in each row significantly (P<0.05) different

Table 4: Carcass traits and chemical analysis of diploid and triploid *O. niloticus*.

	Diploids	Triploids	±SE	*Probability*
Carcass traits:				
Dressing %	49.44 b	53.01 a	0.79	0.023
Flesh %	34.94 b	37.12 a	0.62	0.044
By-products %	61.46	62.36	0.90	0.547
Proximate analysis of flesh				
Dry matter%	24.23	27.68	0.56	0.049
Protein%	73.35	74.87	2.35	0.236
Fat%	12.62 b	15.36 a	1.43	0.045
Ash%	13.85 a	11.24 b	0.44	0.048

Means followed by different letters in each row significantly ($P<0.05$) different

4. Discussion

4.1 Growth performance and feed utilization

Growth parameters of *O. niloticus* in the present study indicated that, triploid fish were heavier than diploids and the differences were significant. These results agreed with studies on triploid *O. niloticus* (Soltan *et al.,* 1999, Hussain *et al.,* 1995 and Brämick *et al.,* 1995, channel catfish (Wolters, *et al.,* 1982), tench *Tinca tinca* (Flajshans *et al.,* 1993) common carp (Cherfas *et al.,* 1994) and Atlantic salmon (Galbreath and Thorgaard 1995). Flajshans *et al.,* (1993) found that triploid females of tench, *Tinca tinca* displayed 13.52% higher live weight than diploid females and triploid males displayed 23.66% higher live weight than diploids males and the differences between diploid and triploid males and females were significant. On the other hand, Puckhaber and Hörstgen-Schwark (1996) indicated that triploids *O. niloticus* showed poorer growth performance than diploids. Hussain *et al.,* (1996) reported insignificant length differences between triploids and diploids of *O. niloticus.* With other fish species, Johnson *et al.,* (1986) found that, there are no significant differences between length, body weight, gut weight and condition factor in triploid and diploid coho salmon. Galbreath and Thorgaard (1995) stated that, condition factor was greater for diploid Atlantic salmon than triploid. On the other hand, Hussain *et al.,* (1995) found that condition factor (K) of triploid *O. niloticus* female was higher compared with diploid females.

Triploids *O. niloticus* gained the highest significant ($P<0.05$) weight gain and specific growth rate compared with diploid fish (Table 2). Sterile fish may convert a greater part of the nutrients absorbed on body weight gain and therefore may attain a higher growth rate and more efficient feed conversion compared with the diploid ones. In previous studies, Hussain *et al.,* (1995), Brämick *et al.,* (1995), Galbreath and Thorgaard (1995) found insignificant differences ($P>0.05$) between diploids and triploids of *O. niloticus* in regard to weight gain and specific growth rate.

Feed intake, feed conversion ratio and protein efficiency ratio were better for triploids than diploid (Table 2). Cassani and Caton (1986) stated insignificant differences between triploids and diploids grass carp in regard to feed conversion ratio. Also, Henken *et al.,* (1987) found that diploids and triploids of African catfish, *Clarias gariepinus* converted their feed with similar efficiency. Benfey (2015) indicated that, ploidy did not affect feed conversion efficiency in Atlantic salmon (*Salmo salar*). Contradictory and variable performance results have been published for triploids of several species such as Atlantic salmon (Benfey, 2015) and Atlantic cod (Campos Vargas *et al.,* 2015) compared to their diploid siblings. The differences have been related to factors such as culture conditions (Piferrer *et al.,* 2009), gamete quality (Taylor *et al.,* 2011), stage of the life cycle and family and/or strain effects (Taylor *et al.,* 2013).

4.2 Gonadal Development

Gonadal development in triploid *O. niloticus* at the end of the experiment was retarded compared to diploid. In triploids of both sexes average gonads (expressed as GSI) were smaller than diploids with significant differences ($P<0.05$) between the two ploidy groups (Table 3). The higher values in females may be due to the higher weight of ovaries compared to spermatic system of males. Similar findings have been reported for tilapia by Brämik *et al.,*(1995), Puckhaber and Hörstgen-Schwark (1996) and for other fish species, Cyprinid loach, *Misgurnus anguillicaudatus* (Suzuki *et al.,* 1985), Coho salmon, *Oncorhynchus kisutch* (Johnson *et al.,* 1986), African catfish, *Clarias gariepinus* (Henken *et al.,* 1987), white crappies, *Pomoxis annularis* (Parsons, 1993), tench, *Tinca tinca* (Flajshans *et al.,* 1993) and Atlantic salmon (Galbreath and Thorgaard 1995).

The results of 6 different crosses between triploid males and normal diploid females show that triploid spermatozoa were unable to fertilize the eggs obtained from diploids females and the same result also obtained when the eggs from triploids female were used to be fertilized by spermatozoa normal diploid males. Therefore, this experiment had confirmed that both female and male triploid *O. niloticus* were functionally and reproductively sterile. Such reproductive sterility in mixed-sex culture of Oreochromis species would improve production by preventing precocious sexual maturation, particularly in ponds.

4.3 Hepatosomatic Index (HSI)

Hepatosomatic index (HSI) of triploid males were larger than that of diploids with significant differences between the two groups while the HSI of triploid females were smaller than that of diploids but the differences were not significant (Table 3). Similar findings have been reported for coho salmon, *Oncorhynchus kisutch* (Johnson et al., 1986) but for *O. niloticus,* Hussian *et al.,* (1995) found insignificant differences between triploids and diploids males and females *O. niloticus* for HSI.

4.4 Carcass traits and proximate analysis
Carcass traits of *O. niloticus* at the end of this experiment showed that triploids *O. niloticus* had higher percentages of dressing, viscera and by-products and lower percentage of flesh as compared with that obtained from the diploid fish (Table 4). Henken *et al.,* (1987) found that triploid African catfish *Clarias gariepinus* had higher gutted weight compared with diploids fish. Flajshans *et al.,* (1993) found that triploid females of tench, *Tinca tinca* displayed 3.49% higher dressing percentage than diploid females but triploid males displayed 1.3% lower dressing percentage than diploids males and the differences between diploid and triploid males and females were not significant. On the other hand, Galbreath and Thorgaard (1995) reported that the dress-out percentage of diploids Atlantic salmon did not significantly different from that recorded for triploid. Also, Hussian *et al.,* (1996) reported insignificant differences between diploids and triploids *O. niloticus* in the percentage of dress-out and gut weight.

Percentages of viscera and by-products were higher in bodies of triploids compared with diploids. Higher percentages were caused by accumulation of more fat around viscera of sterile triploids. Due to fish sterility, fat had not been mobilized for egg and spermatozoa production. A similar finding was observed by Hussian *et al.,* (1995) in *O. niloticus*. This suggests that lower energy diets may well be more appropriate for these fish to avoid the build-up of this wasted lipid.

Triploid carcasses contained the highest significant ($P<0.05$) fat and the lowest ash content compared with diploid carcasses while protein and dry matter contents were not significantly ($P>0.05$) affected (Table 4). Fish sterility by triploidy induction seems to induce stimulation of fat retention which is also associated with an increase in growth rate, (Recoubryatsk *et al.,* 1992). Hussain *et al.,* (1995) found no significant differences between the muscle of diploid and triploid of *O. niloticus,* within a sex, for the percentage of crude protein, crude lipids, moisture or ash, but they have a significant difference in the percentage of crude protein, crude lipid and moisture between the sexes.

5. Conclusions
In summary, we produced 100% triploid Nile tilapia *Oreochromis niloticus* by inhibiting the formation of second polar body of fertilized eggs using heat chock. Compared to the diploid, in our study, the triploid *O. niloticus* exhibited a fast growth rate. Therefore, the use of triploid tilapia may have important benefits for aquaculture and would help to avoid many of the problems associated with the precocious maturity and excessive reproduction display by tilapia in pond conditions. The problems lie with finding economic methods of producing large numbers of triploid tilapia fry particularly as the direct production of triploids using tetraploid females and diploid males, respectively which appears to impossible in *O. niloticus* at the present time.

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