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Comparison of Growth Performance, Gonadal Structure and Erythrocyte Size in Triploid and Diploid Brown Trout (*Salmo trutta fario* L, 1758)

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Abstract

The aim of this study was to compare growth, gonadal structure and red blood cell sizes of triploid and diploid brown trout (*Salmo trutta fario*). Triploidy was induced by providing a heat shock treatment to the fertilized eggs. From the 18th month post-fertilization triploid (TBT) and control diploid (CBT) brown trout groups were investigated up to 32nd month. Survival, specific growth rate, feed conversion ratio, condition factor, relative growth rate, absolute growth rate, gonadosomatic index, hepatosomatic index and carcass yield were determined in both groups. It has been determined that the triploid fish showed greater live weight than diploid fish despite the difference was not significant (P>0.05). In triploid group triploidisation success was obtained as 95% and male/female ratio was found as 44.4-55.6%. The red blood cells were significantly larger in triploids than diploid ones (P<0.05). Regarding histological section of gonads, triploidy resulted in reduced gonadal development in female, while triploid males seem to exhibit normal gonadal development. Our results suggest that since triploid females could grow more than males, the growth performance studies on triploid brown trout should be performed with all-female stocks under fish farming practices especially for the production of large size trout.

Keywords: Triploidy, brown trout, growth, gonadal development, cytometry.

Triploit ve Diploit Dere Alabalıklarında (*Salmo Trutta Fario* L, 1758) Büyüme Performansı, Gonat Yapısı ve Eritrosit Boyutunun Karşılaştırılması

Özet

Bu çalışmada, triploit ve diploit dere alabalıklarının (*Salmo trutta fario*) gelişimleri, gonat yapıları ve eritrosit büyüklükleri karşılaştırılmıştır. Döllenmiş yumurtlara sıcaklık şoku uygulamasıyla triploidizasyon yapılmıştır. Döllenmeden sonraki 18.aydan itibaren 32.aya kadar triploid (TBT) ve diploit (CBT) dere alabalığı grupları incelenmiştir. Her iki deneme grubunda yaşama oranı, spesifik büyüme oranı, yem dönüşüm oranı, kondisyon faktörü, nisbi ve mutlak büyüme oranı, gonadosomatik indeks, hepatosomatik indeks ve karkas verimi saptanmıştır. Triploit balıklar istatiksel olarak önemli olmasa da diploit balıklara göre daha fazla gelişim göstermiştir (P>0,05). Triploit grubunda triploidizasyon başarısı %95 olup erkek/dişi oranı %44,4-55,6 olarak bulunmuştur. Kırmızı kan hücreleri triploitlerde diploitlere göre önemli derecede büyüktür (P<0,05). Gonatların histolojik kesitlerine bakıldığında, triploit uygulaması dişilerin düşük gonat gelişimine sebep olurken erkeklerin normal gonat gelişimi sergilediği görülmüştür. Bu çalışmanın sonuçlarına göre, triploit dişiler erkek balıklardan daha iyi geliştiğinden, gelişme performansı ile ilgili çalışmaların özellikle kiloluk alabalık üretiminin yapıldığı çiftlik şartlarında tümü-dişi populasyonlar ile yapılması faydalı olacaktır.

Anahtar Kelimeler: Triploit, dere alabalığı, gelişme, gonat gelişimi, sitometri.

Introduction

Although many problems resulting from sexual differentiations may avoid by production of monosex populations in fish species, some degradations due to maturation will still occur if monosex fish stocks are not marketed before they become mature that especially has a negative impact on the growth (Bromage, 1992), also on the harvest yields and flesh quality (Werner *et al.*, 2008). The remedy for overcoming such problems is sterilization and the most effective way is the chromosome set manipulation. Application of chromosome set manipulation techniques can be applied to produce triploid and sterile fish. The most effective method currently avalaible for large scale sterilisation of trout

is the induction of triploidy (Crozier and Moffett, 1989; Lincoln, 1996). Inducing triploidy is the only practical means in which to sterilize large numbers of fish without using of potentially harmful chemicals or radiation (Benfey, 1988). In this method, the main aim is to produce sterile fish by using normal spermatozoa. Triploidization technique that sterilization can be achieved by administration of an environment shock shortly after post fertilization. Therefore, degradations due to sexual maturation are overcome by triploidy technique (Piferrer *et al.*, 2009). Triploidy can be induced by one of shocks such as thermal (heat or cold), mechanical (pressure) or chemical (cytochalasin B) used for the retention of the second polar body during the second meiosis phase, then three chromosome sets can be generated for every embryonic cells (Johnstone, 1992). The heat shock has extensively been used on salmonidae species. Chromosome manipulations have been implemented in salmonids such as rainbow trout, Atlantic salmon, pink salmon, coho salmon, brown trout and arctic charr, so as to optimize shock protocols for inducing triploidy (Shelton et al, 1986; Benfey, 1988; Arai and Wilkins, 1987; Crozier and Moffett, 1989; Quillet *et al.*, 1991; Johnstone, 1992; Gillet *et al.*, 2001; Guner *et al.*, 2005). Quillet *et al.* (1991) showed that the mass production of triploid brown trout is feasible by heat shocks that induce very high rates of triploidy (close to 100%) without major reduction of survival.

Culture of brown trout (*Salmo trutta fario* L, 1758) has been less well studied due to some difficulties such as poor growth rate and condition factor, high feed conversion rate and non-aggressive feeding in fry as well as on-growing stages compared to rainbow trout (Kızak *et al.*, 2010). The purpose of this study was to investigate the effect of triploidization on growth and gonadal development of brown trout and also provide information about possibility of triploid brown trout culture for rearing practices that could also minimize genetic interactions with local stocks.

Materials and Methods

Materials

This study was conducted in a private trout farm in Izmir Kemalpasa and Ege University Fisheries Faculty laboratories. Brown trout (*Salmo trutta fario* L, 1758) eggs and sperm were collected from brood fish for triploidy induction. Fry were firstly reared in hatchery throughs (40 cm × 42 cm × 180 cm) then transferred into concrete raceways (100 cm × 125 cm × 840 cm) and raised until harvesting. Water temperature and dissolved oxygen were measured with an oxygenmeter (wtw-oxi 315i).

Fish were fed by restricted feeding on a commercial diet with trout granules and pellets after first feeding in order of 300 - 400 µm trout starter feed (55% crude protein and 10% crude lipid), 1 – 2.5 mm fry feed (50-55% crude protein and 12-15% crude lipid) and 3-4 mm extruder (45% crude protein and 19-20% crude lipid).

Methods

Eggs and sperm were stripped from mature brown trout breeders and dry fertilization was carried out. Triploidy was induced by applying a heat shock shortly after fertilization. Fertilized eggs were heat shocked 15 min post-fertilization at approximately 26.5°C for 20 min duration. The eggs without heat shock treatment were kept as control.

Comparisons between triploid and diploid fish were conducted in 6 replicate groups, each one containing 200 fish. Trials were established on the 18th month after fertilization and carried out until the 32nd month. Three replicates were used for each treatment. Approximately 250 ml·s^{-1}, 1 L·s^{-1} and 2 L·s^{-1} of freshwater were supplied for larvae, fry and growing stages, respectively. Initial mean weights of triploid brown trout (TBT) and diploid brown trout (CBT)(Control) were 119.59 ± 12.92 g and 122.67 ± 15.01 g, respectively. Fish were weighed using an electronic balance (precision 0.01 g) and measured using a measuring scale (total length to 1 mm) within periods ranging between 1 - 3 months to determine growth performance. Fish were anaesthetized with 30 ppm clove oil (Sigma) before sampling.

In the present study, specific growth rate (SGR), condition factor (CF), relative growth rate (RGR), absolute growth rate (AGR), gonadosomatic index (GSI), visceral somatic index (VSI), hepatosomatic index (HSI), carcass yield (CY), survival and feed conversion rate (FCR) were calculated as stated below (Bagenal and Tesch, 1978; Jackson, 1988; Busacker *et al.* 1990; Razak *et al.*, 1999; Korkut *et al.*, 2007).

$SGR\ (\%) = [\ln (W_2) - \ln (W_1) / t\ ] \times 100$
$CF = (W / L^3) \times 100$
$RGR\ (\%) = [(W_2 - W_1) / W_1] \times 100$
$AGR = (W_2 - W_1) / t$
GSI (%) = [gonadal weight (g) / W (g)] × 100
VSI (%) = [visceral weight (g) / W (g)] × 100
HSI (%) = [weight of liver (g) / W (g)] ×100
CY (%) = [(W - visceral weight) (g) / W (g)] × 100
Survival = [number of survived fish / initial number of fish] × 100
FCR = consumed feed (kg) / weight gain (kg) ,

where W is weight of body, W_2 is final weight, W_1 is initial weight, t is the period of trial and L is total length.

Determination of Triploidy

Erythrocyte method, i.e. red blood cell size measurement, was used to determine ploidy levels. Blood samples were drawn from anterior ventral of

body using the heart puncture technique by 2.5 ml syringe through hearts of triploid and diploids (n=400). Samples were kept in ice until analyses. Air-dried blood smears were fixed in ethanol (96%) and stained with giemsa (5 ml 5% giemsa + 95 ml PBS, pH 6.8). Major and minor axes of erythrocytes on stained smears were measured under a light microscope at ×40 magnification by using an ocular micrometer with an each unit 2.5 µm and then photographed. Sizes of erythrocytes were calculated according to Kankaya (1998).

Assesment of Gonad Histology

Ovaries and testis of fish were examined both morphologically and histologically. Gonad samples were taken from all trial groups randomly on the 19th month after fertilization for histological examination of spermatogonium and oocytes. Fish were dissected 2nd and 14th months in summer periods after established the trial groups. Testis and ovaries were fixed in buffered formaldehyde (100 ml formaldehyde – 40 %, 4 g $NaH_2PO_4 \cdot H_2O$, 6 g $NaHPO_4$, 900 ml distilled water). After 4 hours fixation, samples were washed out with 50% ethanol and kept in 70% ethanol. Sections of fixed samples (5 µm) were cut and stained with Haematoxylin & Eosin (Hinton, 1990) for light microscopic investigation of spermatogonium and oocyte under 4× and 20× magnifications. Histological sections were taken pictures.

Statistical Analyses

Data were expressed as mean ± SE and evaluated by variance analyse method using the statistical package SPSS 14.0. Means were analyzed by the LSD test. Differences between groups were accepted as significant when $P<0.05$. Chi squared test (χ^2) was carried out as a non-parametric test for SGR, FCR, RGR, AGR and CF. Differences in survival between groups were analyzed using Fischer's exact χ^2 test with 95% confidence interval.

Results

Dissolved Oxygen and Water Temperature

Water quality values were similar in all trials and remained within acceptable ranges throughout the research. Some water parameters of study media were given in Table 1.

The mean temperature and dissolved oxygen of source water were 11.4±1.7°C and 7.9±1.9 ppm, respectively. Water temperatures were measured between 9.7-13.1°C, while dissolved oxygen between 6.9-9.8 ppm in all of trial groups.

Growth Performances and Survival

At the end of 32nd month survival rates of TBT and CBT were recorded as 79% ± 5.0 and 90% ± 5.3, respectively. No significant difference was observed in survival in both groups ($P>0.05$), although it was higher in control group.

The final mean live weight and total length of TBT and CBT were measured as 740.50 ± 101.01 g - 37.83 cm and 688.75 ± 104.85 g - 37.06 cm, respectively. Overall, there were no differences in growth between TBT and CBT ($P>0.05$). The growth parameters, SGR, CF, RGR, AGR and FCR are given in Table 2. Similarly, growth parameters of triploid and diploid brown trout were not statistically different ($P>0.05$).

Somatic Indices

The somatic indices VSI, HSI and CY of both groups are given in Table 3. Indices did not differ between triploid and diploid brown trouts ($P>0.05$). At the end, gutted carcass weight of triploids were insignificantly greater than diploids.

The final live weight and indices of triploid and

Table 1. Physico-chemical conditions of water media

	$CaCO_3$	Ca	Total hardness	Mg	SBV	HCO_3^-	NO_2	NH_4
Fresh water	80	32.1	180	24.3	2.9	176.9	0.01	0.0017

Note: $CO_3^=$, NO_3 (ppm), PO_4 (ppm), Silis (ppm) are absent

Table 2. The growth performances of triploid and control brown trouts at the end of trials (mean ± SE). (SGR: specific growth rate, CF: condition factor, RGR: relative growth rate, AGR: absolute growth rate, FCR: feed conversion rate)

Parameters	TBT	CBT
SGR (%)	0.39 ± 0.04	0.39 ± 0.03
CF	1.37 ± 0.05	1.36 ± 0.08
RGR (%)	0.011 ± 0.003	0.011 ± 0.002
AGR	1.35 ± 0.24	1.26 ± 0.23
FCR	0.48 ± 0.10	0.52 ± 0.10

Values are presented as mean ± SE. Values in the same row having no superscript letter indicates insignificant differences between groups ($P>0.05$).

Table 3. Somatic indices of triploid ve control brown trout (VSI: visceral somatic index, HSI: hepatosomatic index, CY: carcass yield)

INDEX	TBT	CBT
VSI (%)	12.9 ± 1.77	14.2 ± 2.01
HSI (%)	1.79 ± 0.30	1.57 ± 0.20
CY (%)	82.0 ± 1.77	81.2 ± 2.01

Values are presented as mean ± SE. Values in the same row having no superscript letter indicates insignificant differences between groups (P>0.05).

Table 4. Final live weight and indices of triploid ve control brown trout in both sexes (W_2: final live weight, GSI: gonadosomatic index, VSI: visceral somatic index, HSI: hepatosomatic index, CY: carcass yield)

	Triploid male	Diploid male	Triploid female	Diploid female
W_2 (g)	696.11	597.95	784.89	779.55
GSI (%)	1.93	2.73	4.70^a	6.50^b
VSI (%)	10.91	15.9	14.90	12.50
HSI (%)	1.42	1.75	2.16	1.39
CY (%)	84.00	81.39	80.00	81.08

Values are presented as mean ± SE. Values in the same row having different superscript letters are significantly different (P<0.05).

control brown trout in both sexes were given in Table 4. Female fish gained more weight than males.

GSI of female brown trout in TBT and CBT groups were 4.70% and 6.50%, respectively (Table 4). Ovaries examined from triploids were smaller and less developed (Figure 1). The GSI were significantly higher (P<0.05) in diploid females than triploid females. Indices were recorded low due to lower gonadal development in summer period.

GSI of male brown trout in TBT and CBT groups were 1.93% and 2.73%, respectively (Table 4). The GSI of triploid and diploid male fish did not differ significantly (P>0.05). Males had almost normal gonadal development. The appearance of testes in diploids was elongated whereas asymmetrically shaped in triploid males (Figure 4).

External Morphology and Histology of Gonads

Morphological and histological analyses shows that the fish of TBT group consist of 44.4% female and 55.6% male. The ratio of female and male fish within CBT group were 45% and 55%, respectively. The proportion of females and males in triploid and diploid was similar.

Triploid females had rudimentary ovaries (Figure 1) containing perinucleolar oocytes (Figure 2), while vitellogenic oocytes, oil droplets and egg protein particles were observed in ovaries of diploid females within CBT (Figure 3). Regarding of histologic sections, triploid females were understood to be sterile.

Triploid males seem to exhibit normal gonadal development when examined histologically. Seminifer tubules, spermatagonia, primer spermatocyte, seconder spermatocyte, spermatids, spermatazoa and leyding cells were identified in testis of triploid males (Figure 4-5).

Seminifer tubules spermatagonia, primer spermatocyte, sekonder spermatocyte, spermatid, spermatazoa and leyding cells were identified in testis of diploid males (Figure 6).

Determination of Triploidy

Triploidy was controlled by analysis of red blood cells and verified by determining the size of erythrocytes. The mean size of erythrocytes in triploids were greater than those of diploids. Mean erythrocyte size was 14.6±0.34 µm in triploid and 11.3±0.26 µm in diploid brown trouts. The difference between erythrocyte sizes was found significant as a result of variance analysis (P<0.05). The erythrocytes of triploid and diploid fish under light microscopy has shown in Figure 7. Triploidy rate was determined as 95% by applying heat shock in TBT group.

Discussion

In present study, despite survival rate was lower in triploids than in diploids at the end of trial, difference between groups was insignificant (P>0.05). Survival rates were in accordance with results of some studies that were carried out salmonidae species. It was cited that survival of triploid rainbow trout, obtained by heat shock triploidization, was lower than control (Solar *et al.*, 1987). Ojolick *et al.* (1995) reported that survival of triploid and diploid rainbow trout were 68.5% and 39%, respectively. Bonnet *et al.* (1999) indicated that mortality rate was around 20.4% for triploid and diploid rainbow trout until market size. Sutterlin *et al.* (1987) reported that triploid hybrid Atlantic salmon showed higher survival than triploid Atlantic salmon, while Oppedal

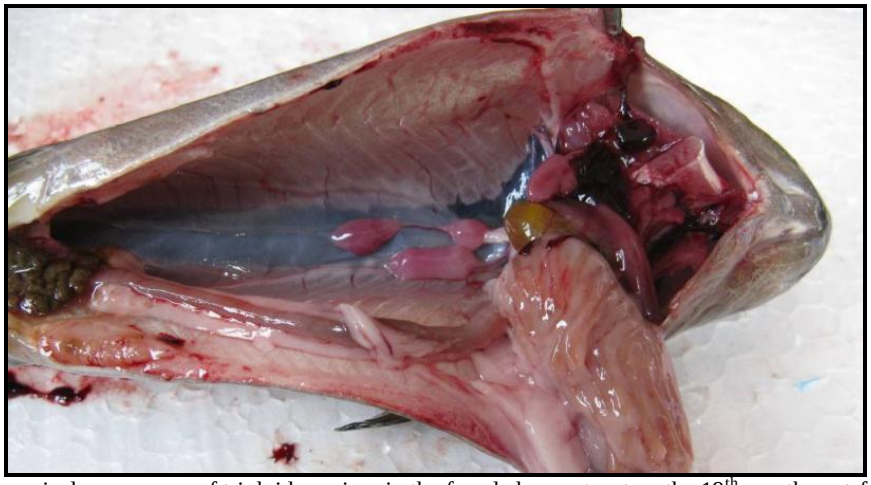

Figure 1. Macroscopical appearance of triploid ovarium in the female brown trout on the 19th month post-fertilization.

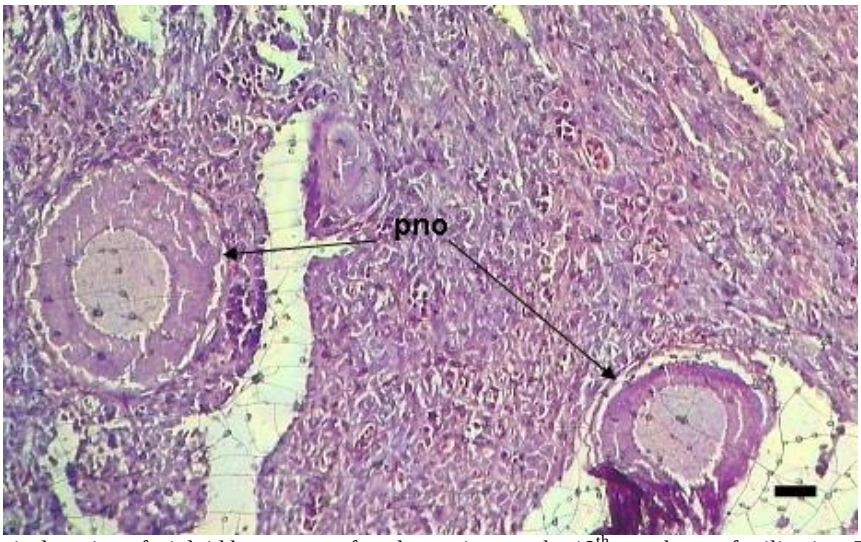

Figure 2. Histological section of triploid brown trout female ovarium on the 19th month post-fertilization. Bar = 40 µm. Abbreviation: pno, perinucleolar oocytes.

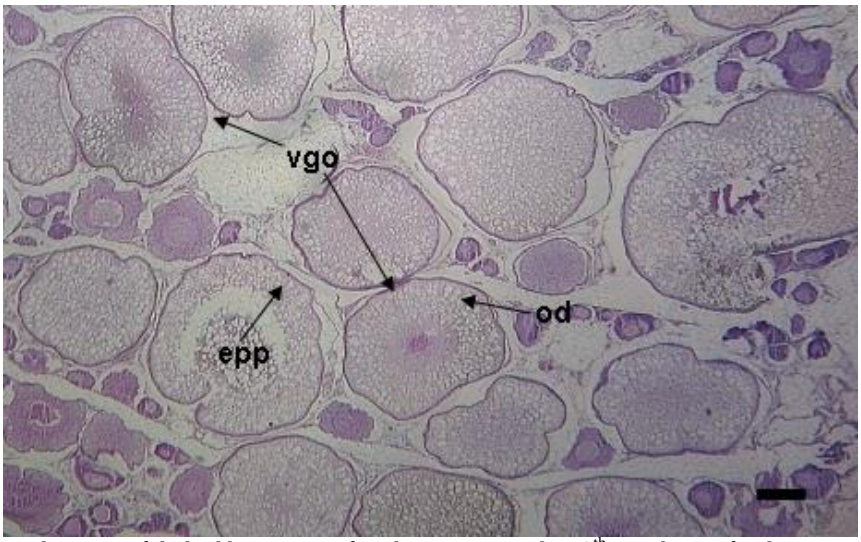

Figure 3. Histological section of diploid brown trout female ovarium on the 19th month post-fertilization. Bar = 250 µm. Abbreviations: vgo, vitellogenic oocytes; od, oil droplets; epp, egg protein particles.

Figure 4. Macroscopical appearance of triploid testis in the male brown trout on the 19th month post-fertilization.

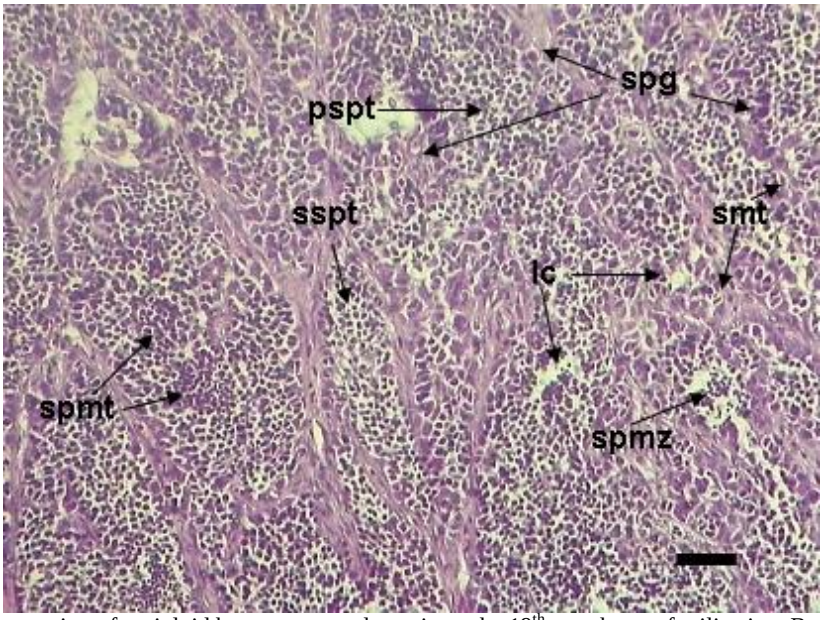

Figure 5. Transverse section of a triploid brown trout male testis on the 19th month post-fertilization. Bar = 20 µm. Abbreviations: smt, seminifer tubules; spg, spermatagonia; pspt, primer spermatocyte; sspt, sekonder spermatocyte; spmt, spermatid; spmz, spermatazoa; lc, leyding cells.

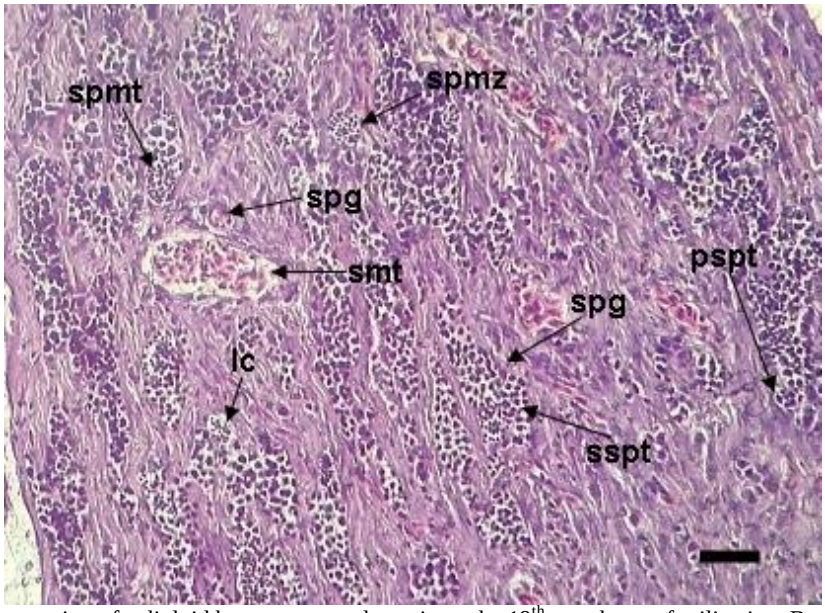

Figure 6. Transverse section of a diploid brown trout male testis on the 19th month post-fertilization. Bar = 20 µm. Abbreviations: smt, seminifer tubules; spg, spermatagonia; pspt, primer spermatocyte; sspt, sekonder spermatocyte; spmt, spermatid; spmz, spermatazoa; lc, leyding cells.

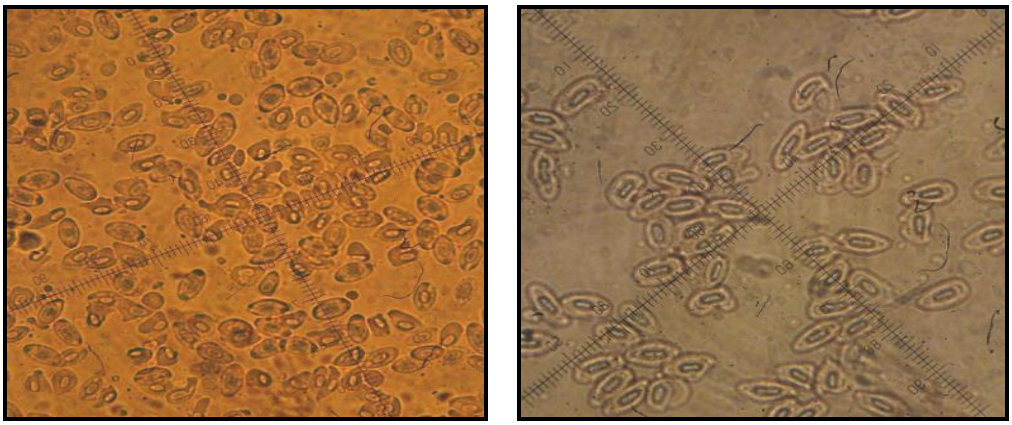

Figure 7. Erythrocytes of diploid (left) and triploid (right) brown trouts (×40 magnification, each unit 2.5 µm).

et al. (2003) indicated that the survival rate of triploid Atlantic salmon seems to be similar or even higher than those of diploids. In another study, it was observed that there is no difference between triploid and diploid Atlantic salmon in terms of survivals (McGeachy *et al.*, 1995).

Growth differences were not observed between TBT and CBT groups (P>0.05), so triploids showed no superiority in growth over diploids. At the end of trials, the fish in TBT and CBT groups reached to 740.50 g and 689.75 g live weight, respectively. Although there was no statistical difference in weight between triploid and diploid at 32 months of age, it can be said that triploid fish in TBT shows better growth than diploid fish in control group. Generally, growth advantage of triploid fish is expected after sexual maturation, when somatic growth of diploid fish is usually suppressed by the reproductive process (Purdom, 1983; Koedprang and Na-Nakorn, 2000). Oppedal *et al.* (2003) reported that triploid Atlantic salmon has a similar or better growth rate comparing with diploid Atlantic salmon. Except for Salmonidae species some studies reported that triploid chinese catfish (*Clarias fuscus*) had better growth rate than diploids (Qin *et al.*, 1998), while diploid hybrid stripped bass grew faster than triploids (Kerby *et al.*, 2002). On the other hand, there was no difference between triploid and diploid on seabass in terms of weight gain (Felip *et al.*, 1997).

There was also no difference between groups in terms of growth parameters (P>0.05). FCR was lower for TBT than for control (P>0.05), which suggests that the triploids were more efficient at converting feed into live weight. On the other hand, FCRs recorded very low in both groups because of considerably lower specific growth rates (Table 2). Bhat *et al.* (2011) demonstrated that the length of restricted feeding and subsequent appetite feeding periods influenced growth performance and feed efficiency. In the present study, feeding regime was applied as restricted that caused very low SGR and FCR throughout trials.

Female and male ratio were 44.4% and 55.6% respectively in TBT, while 45% female and 55% male were recorded for CBT. When the growths are compared in terms of same sexes, it was revealed that final mean live weight was higher in triploid male and female than in diploid ones (Table 4), but difference between same sexes was not significant (P>0.05). Any growth advantage of triploids is generally realized only for females, and only during the spawning period of the diploids, when growth of the latter decreases or stops entirely (Benfey, 1988). In the present study female triploids were considerably larger than diploid and triploid males.

There are three different variables that should be taken into account in triploidy studies; the time after fertilization when the shock was applied, the temperature of the shock and the duration of the shock (Felip *et al.*, 1997). Crozier and Moffett (1989) indicated that heat shock applied to brown trout eggs after insemination is capable of producing triploids. In the present study, triploidization was done succesfully and heat shocking at 26.5°C resulted in high percentage of triploid rate obtained as 95%. Quillet *et al.* (1991) optimized production of triploid brown trout by applying heat shock at 28°C that induced high rates of triploidy (nearly 100%) without causing much mortality. Okada (1985), Loopstra and Hansen (2008) reported 86% and 100% triploidy, respectively in rainbow trout by hydrostatic pressure. 77-91% triploidy rate was obtained in *Salmo trutta* species by heat shock at 29°C for different durations (Arai and Wilkins, 1987). Crozier and Moffett (1989) stated that heat shock of 28°C of 10 min duration initiated 5-15 min post-fertilization produced high rates of triploidy in brown trout. Brydges and Benfey (1991) found that the optimum hydrostatic pressure was in the range of 5.5 to 6.5 min at 9.5-10.5 kpsi, applied 25-30 min post-fertilization for triploid brown trout. Guner *et al.* (2005) achieved 85% and 100% triploidy in rainbow trout applying two different heat shock. McGeachy *et al.* (1995) obtained 86% triploidy in Atlantic salmon by pressure shock. 100% triploidy was achieved on seabass (*Dicentrarchus labrax*) and turbot (*Scophthalmus maximus*) by cold shock with 0°C (Felip *et al.*, 1997; Piferrer *et al.*, 2003). Pradeep *et al.* (2012) reported 94.4% and 98.8% triploidy on hybrid

red tilapia by heat and cold shocks. In another study, 72.5% triploidy was recorded by cold shock on *Puntius gonionotus* (Koedprang and Na-Nakorn, 2000). According to the aforementioned studies, the success of triploidy may vary depending on species and inducing methods.

There are different methods for determining of triploids (Purdom, 1993; Pandian and Koteeswaran, 1998) and one of them is measuring the size of erythrocytes. Triploid cells have 50% more DNA than diploid cells, their nuclei and the cells themselves are significantly larger than diploid nuclei and cells (Benfey, 1988). Red blood cell shows significantly differences in terms of size among ploidies (Benfey, 1999). Crozier and Moffett (1989) reported that measurement of the major axis of eryhtrocytes were applied to brown trout. Piferrer *et al.* (2003) determined the triploidy rate on turbot by measuring the major axis of red blood cells. Measurement of red blood cells is an alternative way to understand the ploidy levels of fish without sacrificing them. Moreover, it is more practical, rapid and inexpensive assay for triploidy determination when comparing with karyotype analyses (Koedprang and Na-Nakorn, 2000; Gao *et al.*, 2007). Microscopic observation of erythrocyte cells showed that erythrocyte sizes in TBT and CBT were determined as 14.6 µm and 11.3 µm, respectively (Figure 7). The mean size of triploids were greater than those of diploids ($P<0.05$). These results confirmed that controls were diploid, while heat-shocked fish were triploid. The erythrocyte sizes of triploid *Puntius gonionotus* were reported approximately 1.63 times larger because of having extra chromosome set than diploid erythrocytes (Koedprang and Na-Nakorn, 2000). The results of present study revealed an increase in erythrocyte size in agreement with previously reported on triploid fish species such as turbot, catfish, sea trout, hybrid red tilapia, sea bass and rainbow trout (Crozier and Moffett, 1989; Felip *et al.*, 1997; Kankaya, 1998; Qin *et al.*, 1998; Piferrer *et al.*, 2003; Cal *et al.*, 2006; Pradeep *et al.*, 2012).

Kerby *et al.* (2002) confirmed the sterility of triploids by examining gonads that remained reduced and dysfunctional. Although there is no significant difference between TBT and CBT groups in terms of GSI ($P>0.05$), it was observed that GSI of triploid females was lower than diploid females and also difference between them is statistically significant ($P<0.05$) (Table 4). Our results support of Lincoln and Scott (1984) in which GSI differences was observed between diploid and triploid rainbow trouts and diploid females have well developed ovaries. Felip *et al.* (2001) stated that diploid females reached to sexual maturity while triploid females did not, so that their GSI remained a low level. The gonadal development of triploids is retarded to a much greater extent in females than in males (Benfey, 1988). In present study, GSI of triploid males were found to be lower than diploid males whereas no significant difference was found in the weight of testes between them ($P>0.05$). Comparison of GSI with growing shows that the highest growth rate was observed in TBT group that have lowest GSI level (Table 4). From this point, it can be considered that somatic growth of fish could increased by decreased gonadal development. The growth advantage of triploid females over diploid females in salmonidae generally occurs during the spawning period when growth of diploids decreases or stops in association with development of ovaries (Benfey *et al.*, 1989; Bonnet *et al.*, 1999).

Benfey (1988) reported that only one late perinucleolar stage was found in a single triploid pink salmon which appears to be a typical triploid fish and all the triploid females had ovaries apparently devoid of oocytes. In present study, perinucleolar oocytes were observed in ovaries of triploid brown trout females which were understood to be sterile (Figure 2). Histological analyses showed that diploid female brown trouts had ovaries containing oocytes in different vitellogenic stages (Figure 3). Ovarium of triploid rainbow trout females were reported as less developed gonads that contain oogonia and but no primary oocytes, so these fish were indicated as sterile (Lincoln and Scott, 1984). According to Okada (1985), none of triploid rainbow trout females reached to sexual maturity and the reproductive cells remained stable in oogonia stage until 37^{th} month. At an age when diploid females have ovaries full of oocytes, triploid females have string-like gonads lacking significant numbers of developing oocytes, whereas triploid males tend to develop testes similar in size and appearance as diploid males (Krisfalusi *et al.*, 2000). Triploid males are generally not considered of benefit of aquaculture because of their progression through spermatogenesis (Felip *et al.*, 1999). Seminifer tubules, spermatagonia, primer spermatocyte, seconder spermatocyte, spermatids, spermatazoa and leyding cells were identified in testis of triploid brown trout males (Figure 5). Lincoln and Scott (1984) reported that triploid rainbow trout males have well-developed testis which contained mostly spermatocyte rather than spermatozoa, unlike in diploid testis. Similar results were also reported for diploid and triploid turbot (Cal *et al.*, 2006).

VSI is generally used for determining the effect of diets on visceral organs (Korkut *et al.*, 2007). There is no significant difference between TBT and CBT in terms of VSI ($P>0.05$) (Table 3). Gillet *et al.* (2001) cited that VSI of *Salvelinus alpinus* is lower in triploid males. It can be considered that sterilization causes somatic growth instead of developing reproductive traits, because of that VSI being lower in triploids. Induction of triploidy positively influences body and fillet development mainly by muscle fibre hypertrophy (Werner *et al.*, 2008). Carcass yield occured higher in male of TBT than CBT males ($P>0.05$). Gillet *et al.* (2001) found that carcass somatic index of diploid Arctic charr females were

significantly reduced that of immature triploids. In present study, when carcass yield, VSI and HSI are taken into account, it was obviously revealed that triploid females and males developed more than diploids. The situation of lower GSI and higher VSI in triploid brown trout females might be explained by containing significant quantities of visceral fat. The fat content was reported significantly greater for triploid *C. gariepinus* (Henken *et al*., 1987).

In conclusion, despite triploidy induction could not cause a significant increase in size, the growth performance characteristics were determined better in triploid brown trout comparing with diploids. Induced triploidy is widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management, whereas the only clear differences relate to the effects of impaired gametogenesis on the reproductive physiology and behaviour of triploids, especially in females which are useful for practical fish culture to avoid the economically detrimental effects of maturation (Benfey, 1988; 1999). In order to make more reliable analysis on growth performance and rantability, further studies are encouraged to work with all-female population in details. On the other hand, triploidy production in hatchery programs would potentially minimize genetic interactions with local stocks and encourage such studies on triploid fish.

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