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Effect of Hormonal Masculinization in Tilapia (oreocromis niloticusj with some aspects of Growth performance and production

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EFFECT OF HORMONAL MASCULINIZATION IN TILAPIA (*OREOCROMIS NILOTICUS*) WITH SOME ASPECTS OF GROWTH PERFORMANCE AND PRODUCTION

THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF PHILOSOPHY
IN THE
DEPARTMENT OF FISHERIES
FACULTY OF AGRICULTURE
UNIVERSITY OF RAJSHAHI
BANGLADESH

BY

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ROLL NO. M-1911067513
SESSION: 2018-2019

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Dedicated

To

My Beloved Parents

&

Honorable Teachers

DECLARATION

I hereby declare that the research work embodied in this thesis entitled **"EFFECT OF HORMONAL MASCULINIZATION IN TILAPIA (*OREOCROMIS NILOTICUS*) WITH SOME ASPECTS OF GROWTH PERFORMANCE AND PRODUCTION"** has been carried out by me for the degree of Master of Philosophy under the guidance of supervision of **Professor Dr. Md. Delwer Hossain,** Department of Fisheries, University of Rajshahi, Rajshahi-6205, Bangladesh.

I also declare that the result presented in this dissertation is my own investigation and any part of this thesis work has not been submitted to elsewhere for any degree/diploma or for similar purpose.

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June 2021

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CERTIFICATE

This is to certify that **Hasanuzzaman** worked under our supervision as a M. Phil. Fellow, Roll No. M-1911067513, Session: 2018-2019, Fisheries, University of Rajshahi, Rajshahi-6205, Bangladesh. It is our great pleasure to forward his thesis entitled **"EFFECT OF HORMONAL MASCULINIZATION IN TILAPIA (*OREOCROMIS NILOTICUS*) WITH SOME ASPECTS OF GROWTH PERFORMANCE AND PRODUCTION"** which is a genuine record of research carried out at Faculty of Agriculture, Department of Fisheries and its experimental field and in the Faculty and Agriculture, Department of Fisheries, University of Rajshahi, Rajshahi-6205, Bangladesh.

This work is original and has not been submitted so far in part or in full, for the award of any degree or diploma by any other institute in home or abroad. Hasanuzzaman has fulfilled all the requirements for submission of the thesis for the award of the degree of **Master of Philosophy**.

Supervisor

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-The author

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LIST OF ABBREVIATIONS

The following abbreviations have been used through the text:

%	:	Percent
ADB	:	Asian Development Bank
ANOVA	:	Analysis of Variance
CF	:	Condition Factor
cm	:	Centimeter
CP	:	Crude Protein
DO	:	Dissolved Oxygen
DoF	:	Department of Fisheries
FAO	:	Food and Agriculture Organization
FCR	:	Food Conversion Ratio
g	:	Gram
GDP	:	Gross Domestic Product
GIS	:	Geographic Information System
GPS	:	Global Positioning System
Ha	:	Hectare
Ing.	:	Ingredients
Kg	:	Kilogram
Km	:	Kilometer
L	:	Liter

mg	:	Milligram
MMT	:	Million Metric Ton
MT	:	Metric Ton
MT	:	Methyltestosterone
oC	:	Degree Celcius
ppm	:	Parts per million
SGR	:	Specific Growth Rate
SIS	:	Small Indigenous Species
SPSS	:	Statistical Package for the Social Sciences
SRT	:	Sex Reverse Tank
USD	:	United States Dollar

ABSTRACT

The present study was conducted to evaluate the effects of 17 α-methyltestosterone hormone on the growth performance of Nile Tilapia (*Orechromis niloticus*). The study was conducted in the Meridian hatchery, Sonagazi, Feni from February, 2019 to January, 2020. Brood fish was collected from NAMSAI farm Thailand and BFRI, Bangladesh. Eggs were collected from the fish mouth and then disinfected and finally placed to the hatching tray and jar. Hatched larvae were placed to the 12 SRT tank and applied different dose of 17 α-methyltestosterone hormone treatments. The experimental treatments were T_0 (Control), T_1 (50 mg/kg), T_2 (60 mg/kg), T_3 (80 mg/Kg) and each treatment had three replications. Study showed significant higher growth performance in T_2 treatment. The results showed that mean weight of fish was significantly higher under T_2 (533.5±1.249 g), mean length was under T_2 (29.97±0.153 cm) and SGR was under T_2 (4.259±0.0013) in comparison with other three treatments. The highest survival rate 87.6% was obtained in T_2. Highest value of FCR (1.8) was observed in T_1 and lowest (1.2) was in both T_2, T_3, respectively. Highest sex reverse percentage was found in T_2 (98%) followed by T_3 (97.883%) and T_1 (77.833%), respectively. There was a strong interaction between 17 α-methyltestosterone hormone and growth performance and sex reversal of Nile Tilapia ($p < 0.05$, $n = 4$). This study suggests that use of 17 α-methyltestosterone hormone can improve growth performance of Nile Tilapia.

Keywords: Nile Tilapia (*Oreochromis niloticus*), Growth Performances, Sex Reverse, 17 α-methyltestosterone.

CHAPTER - I

INTRODUCTION

INTRODUCTION

1.1 Introduction

Bangladesh is regarded as one of the world's best-suited fisheries regions (Shamsuzzaman *et al.,* 2017). There are 289 freshwater fish species, 475 marine fish species, 24 prawn species, 36 shrimp species, and 12 exotic fish species available in the diversified aquatic ecosystem of Bangladesh (Khan *et al.,* 2013). Fish is the second most valuable agricultural crop of Bangladesh, and its production contributes to the livelihood and employment of millions of people. The culture and consumption of fish contribute significantly to Bangladesh's people's national income and food security (Ghose, 2014). Fish regarded as highly nutritious food source due to presence of protein and many essential nutrients in Bangladesh (Thilsted *et al.,* 1997).

Inland aquaculture has great potential in Bangladesh because it generally experienced faster growth, establishment, and adoption of new technologies, species, and intensification and improvement of farming systems, particularly in pond aquaculture, across the country (DoF. 2018). Inland aquaculture has two familiar production systems in Bangladesh such as extensive and semi-intensive pond polyculture (Indian major carps and exotic carps) which contributes 80% of the total inland culture fisheries. Catfish (*Pangasianodon hypophthalmus*), tilapia, small indigenous fish species and rice-fish farming (ADB, 2005a) contributes remaining 20%. At present production of capture fisheries is decreasing drastically (Toufique, 1997) which create opportunity of natural source utilization means aquaculture expansion to meet the demand of nutritional requirement of Bangladesh people (Lewis and Gregory; 1991).

Bangladesh exists in the major fish producing countries of Asia (FAO, 2020). The total fisheries production was 4. 27 million MT with a 3.57% contribution to national GDP, 25.30% to agricultural GDP, and 11% of the population fully or partly engaged in the fisheries sector. Inland culture fisheries contribute 56.24% of total fish production, and the rest comes from inland and marine capture (DoF, 2018). According to FAO (2018), Bangladesh ranked third in open water inland capture production, fifth in aquaculture production, third in the tilapia culture development in Asia.

Aquaculture is the farming of fish and other commercially important aquatic organisms. Freshwater aquaculture contributes to ensure food security, poverty alleviation and employment generations for millions of people in worldwide. World aquaculture production exceeded 114.5 million tons in 2018, with a total value of USD 263.6 billion at farm level and 59.5 million people directly or indirectly engaged in aquaculture for their livelihoods (FAO, 2020).

Tilapia (*Oreochromis niloticus*) farming can bring enormous potential in Bangladesh tomeet the demand of nutrition. Tilapia has major specialties such as rapid growth rate, high market demand and increasing consumer acceptance making tilapia farming worldwide such as Asian countries including China, Indonesia, Philippines, Thailand and Vietnam (ADB, 2005b). With increasing acceptance and popularity among consumers, tilapia has become the world's second most important cultured fish after carps and easily adapt in tropical and sub-tropical regions of the world (Shelton, 2002). It is regarded as the most significant fish species reducing the gap of increasing worldwide demand for protein sources from fish (Ng and Romano, 2013). In per 100g Tilapia contain protein (20.1 g), phosphorus (170 mg), potassium(302 mg), selenium (41.8 mg), niacin (3.90 mg), vitamin B-12 (1.58 mcg) and is low in fat and saturated fat (0.77 g), omega-3 fatty acids, energies (96 kcal) and sodium (52 mg) (Mjoun *et al.,* 2010). In 2002, Tilapia standing on top 10 sea foods list for the first time in America's, but it became the 5th favorite seafood after shrimp, tuna, salmon, and pollock in 2008 (NFI, 2010).

Tilapia is an exotic species in Bangladesh and it is omnivore which feeds on both plankton and aquatic plants. Introduction of tilapia culture in Bangladesh has long history act as a miracle fish in aquaculture was first introduced to Bangladesh from Thailand in 1954 (Ahmed *et al.,* 1996). The Bangladesh Fisheries Research Institute (BFRI) initiated the second introduction of tilapia to Bangladesh, also from Thailand, in 1987 (Hussain, 2004) and developed low input and low cost technologies (Das *et al.,* 2010).Until tilapia culture is not well developed and familiar in Bangladesh due to its socioeconomic, technological, institutional and marketing constraints (Bart *et al.,* 2004; Ganesh and Majumder, 2004).

Tilapia has become a potential commercial fish species throughout South and Southeast Asia, Africa, and South America (Hussain *et al.,* 2004. It is now a globally framed trade species recommended by the United Nations' Food and Agriculture Organization (FAO, 2018). These species contribute to livelihood opportunities as well as nutrition and food security. There have been significant improvements in tilapia farming from 1999 to 2015 in Bangladesh (Hussain *et al.,* 2004). Tilapia farming has a high potential in Bangladesh for food insecurities and unemployment issues (Hussain, 2009). Tilapia is ready for the market in four months and can resist insufficient quality water and different disease (Ahmed *et al.,* 2012). Additionally, market demand for tilapia is higher compared to other fish.

Nile tilapia is a native fish species of Egypt that has become popular worldwide mainly as a valuable fish, easy to breed and grows in various aquaculture systems (El-Sayed, 2006). In Bangladesh, UNICEF introduced tilapia (*Oreochromis niloticus*) at 1974, but this is not flourishing due to a lack of fruitful research. Then it was further introduced in Bangladesh at 1987 by BFRI from Thailand (Gupta *et al.,* 1992). Since then, development agencies and scientists have developed technologies for better cultural techniques at the farm level.

Tilapia is likely to be the most important of all aquaculture fish in the 21st century. Tilapia has specific favorable characteristics, like tolerant to adverse environmental conditions, can survive at low dissolved O_2, euryhaline, relatively fast growth, and efficient food conversion. These characteristics make tilapia one of the best choices for farmers (Yi *et al.,* 1996; Penna-Mendoza *et al.,* 2005).

Despite having many good characteristics, one of the main impediments in tilapia production at a commercial scale is its precocious reproduction. It attains sexual maturity early and reproduces after every 4-6 weeks in the pond. The mono-sex culture technique can be used to control this unwanted reproduction of tilapia by culturing all-male tilapia in the pond. Tilapia has sexual growth dimorphism in which males grow faster and have more standard size than females (Muir and Little, 1991). To overcome these negative aspects, mono-sex populations have been introduced through steroid hormone (Hunter & Donaldson 1983).

All-male populations are used in tilapia aquaculture because the culture of mixed-sex populations often results in precocious maturation and early reproduction (Mires, 1995). Early development shunts energy to gonadal rather than somatic growth. Additionally, reproduction in ponds may lead to the harvest of many unmarketable fries. Individuals in mono-sex populations have an increased somatic growth rate due to the avoidance of energy losses associated with gonadal development and reproduction. Furthermore, all-male tilapia populations are desirable because males achieve a larger final size than females (Macintosh and Little, 1995)

There are four strategies for mono-sex male culture i.e., the manual process by visual examination; hybridization; gene manipulation, and masculinization via steroid hormone. At the time of hatching, tilapia fry is sexually undeveloped. Hence, during the early period of gonadal differentiation, changes in sex hormone level can affect the final sex independently of the genetic sex (Andersen *et al.,* 2003). One of the most common techniques for producing mono-sex populations is steroid-induced sex inversion (Hunter and Donaldson, 1983). This involves the administration of synthetic androgens or estrogens to differentiating fry.

17 alpha methyltestosterone (MT) is a synthetically produced anabolic and androgenic steroid hormone that promotes both muscle growth and the development of sexual characters. The production of mono-sex tilapia with 17 alpha methyltestosterone (MT) is well established incorporated into starter feed with a dose of 60 mg/kg feed (Popma and Green, 1990). The dietary administration of MT to improve body weight increased residual testosterone, whereas decreased the lipids required for human consumption, which might be hazardous for humans (Schardein, 1980).

Tilapia is an excellent fish for growing in the shallow and seasonal ponds in a country like Bangladesh as it enjoys suitable climate and ecological conditions for culture of this warm water species. Thus, mono-sex tilapia culture might prove practical to induce a positive approach towards tilapia culture in Bangladesh.

1.1 Objectives of the study

1. To evaluate the effects of different dose of hormone 17 alpha methyl-testosterone (MT) for masculinization in Tilapia (*Oreochromis niloticus*)

2. To study the hormonal effect in Tilapia growth performance and production in the cultural period

1.2 Scope of the study

This research work adds a new dimension to improve production of tilapia fish in Bangladesh. It will help in the production of good quality tilapia seed in hatchery and contribute in the cost minimization in the tilapia hatchery operation.

CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

It is essential to look at previous research activities related to science or experiments before performing a study or experimental procedure. 17 alpha methyltestosterone is a worldwide widely recognized method for the sex reversal of tilapia fish. The following is a close review of published and relevant research literature related to sex reversal of tilapia (*Oreochromis niloticus*) and its effects on growth performances:

2.1 Aquaculture in Bangladesh

According to FAO (1988), aquaculture is the cultivation of fish and other commercially important aquatic organisms in coastal and inland water bodies and farming required some intervention to increase production, such as regular stocking, predator protection, and some of the stock's private rights intervention.

According to FAO (2016), Bangladesh is one of the world's leading fish producing countries with a total production of 41.34 lakh MT, where aquaculture contributes 56.44 percent to total production. Average growth performance of this sector is almost 5.43 percent in last 10 years. Bangladesh is ranked 5th in world aquaculture production and it is a great achievement of the country.

Aquaculture involves farming of fish and other aquatic organisms both coastal and inland water bodies, with 'farming' implying some form of intervention to increase productions such as regular stocking, protection from predators and some form of private rights of the stock under intervention (Beveridge and Little, 2002).

According to Jahan *et al.,* (2009). Aquaculture is also considered to have the potential of food security in Bangladesh.

The entire area of inland closed (culture) water fisheries bodies is 0.84 million ha with shrimp farms constituting overall pond area (384700 ha) and ox-bow lakes (5,488 ha) (DoF, 2017).

Among fisheries sub-sector, the inland aquaculture has great potentials in Bangladesh due to its generally experienced fastest growth, establishment and adoption of new technologies, species, and intensification and improvement of farming systems, particularly in pond aquaculture, entirely over the country (Planning Commission, 2016).

According to Mazid (1999), 73% of rural households are directly engaged in freshwater aquaculture systems on the floodplains throughout the country.

Besides capture fisheries, aquaculture can acquaint with new employment opportunities in rural unemployed people by increasing both self-employment and demand for hired labor (Karim *et al.,* 2006).

Fish production from aquaculture is gradually increased and contributes to the foreign exchange earnings of the fisheries sector of Bangladesh like other Asian countries (Dey *et al.,* 2005).

2.2 Existing aquaculture system in Bangladesh

According to Edward (1993), aquaculture system can be classified into i) extensive system relying on natural food produced in the water body without additional inputs, ii) semi-intensive systems relying mostly on the natural feed but supplemented with feed and fertilizer and iii) intensive systems relying on nutritionally complete concentrate feed and fertilizers.

Thompson *et al.,* (2002) reported that most of the freshwater pond in Bangladesh are practicing either extensive or semi-intensive and intensive in very few cases.

Jahan *et al.,* (2016) reported 14 different commercial and subsistence aquaculture systems were practiced across the country. Bangladesh utilizing a variety of aquaculture techniques such as pangas, tilapia, koi and carp monoculture, polyculture of different fish species, polyculture with SIS, rice-fish integrated culture, shrimp and prawn monoculture, shrimp and prawn-rice integrated culture.

Sakib and Afrad (2014) reported adopting acceptable modern aquaculture technologies, suitable domesticated species, capitalization, efficient methods, and adequate marketing facilities are required for optimum production and commercial success in aquaculture.

According to DoF (2017), with the expansion of different developed technologies, the pen and cage culture is becoming popular day by day in Bangladesh.

Dey *et al.,* (2008) categorized freshwater fish-culture systems in Bangladesh into four groups: polyculture of carp, mixed culture, monoculture, and integrated fish culture. Polyculture of Indian major carps and a few other exotic species is most practiced culture system in Bangladesh.

Farming of exotic carp species, tilapia, pangas is massively expanded in Bangladesh (Belton *et al.,* 2014).

2.3 Nile tilpaia

Nile tilapia *(Oreochromis niloticus)* has major specialties such as rapid growth rate, high market demand and increasing consumer acceptance making tilapia farming worldwide (ADB, 2005b).

Trewavas (1983) stated its native distribution which includes tropical and subtropical Africa, Middle East. Widely distributed in Nile and Niger River basins and in lakes Tanganyika, Albert, Edward, and George, as well as in many smaller drainages and lakes in western and eastern Africa; also in Middle East in Yarkon River, Israel

According to Khallaf and Alne-na-ei (1987), Nile Tilapia is mainly herbivorous with a diet of >90% of its aquatic macro-phytes, algae and diatoms, and the rest including aquatic insects and crustaceans and fish eggs.

Classifications of the Nile tilapia as follows:

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Cichliformes

Family: Cichlidae

Genus: Oreochromis

Species: O. niloticus

Binomial name: *Oreochromis niloticus* (Linnaeus, 1758)

2.4 Culture potentiality of tilapia in Bangladesh

Tilapia *(Oreochromis niloticus)* is now commercially important food fishes in the world (Lim and Webster, 2006) and grown in almost 100 countries.

Global production of farmed Nile tilapia was 1.66 million metric ton (MMT) and 2.54 MMT in 2005 and 2009, respectively. Including other cichlids the production was 3.1 MMT out of global aquaculture production of 55.1 MMT (FAO, 2010).

Thus tilapia and other cichlids totally contribute about 5.6% of total aquaculture production. So it appears that tilapias are likely to be higher rank in global aquaculture production next to carp production (Alam *et al.,* 2012).

It has significant contribution in Bangladesh both poverty alleviation and livelihood support. Tilapia has major attributes which make tilapia as an ideal candidate for aquaculture (El-Sayed, 2006). Those attributes are:

- Rapid growth
- Omnivorous fish, can use high proportion of inexpensive plant sources in their feeds
- Stands well in wide range of environmental conditions (Such as temperature, salinity, low dissolve oxygen, etc.)
- Resistance against stress and diseases
- Short generation interval and
- Low supplementary feed require in natural environment and can take the commercial feed immediately after yolk-sac absorption.

Tilapia farming has huge potential in Bangladesh. Farming of tilapia involves in a wide range of culture systems, including small-scale, low-input, rural ponds, semi-intensive, intensive and commercial operations (Chowdhury *et al.,* 2007).

Unused water bodies are utilized by carry out tilapia culture such as countless abandon ponds and backyard ditches are used for tilapia production. Small-scale tilapia farming requires supplementary feeds comprise as mixture of rice bran, wheat bran and mustard oil cake, those are readily available in local markets (Karim and Ahmed, 2009). It has distinguished value of export market. Increased production of tilapia indicates that diverse blessings such as providing superfluous nutrition, additional income for the farmers and lastly more foreign exchange earnings through export.

According to Dey, 2000, number of constraints were reported for tilapia farming including difficult production system, lack of technical support, limited availability and poor quality of seed, high price of seed and feed, low price of tilapia in markets, poor marketing facilities and less economic return compared to carp polyculture. Although a considerable number of poor farmers involve in tilapia farming due to its high growth rate, adaptability, and higher market demand and consumer acceptance.

2.5 Problems associated with mixed-sex tilapia culture

According to Tsadik and Bart (2007), Nile tilapia grows and reproduces in a wide range of environmental conditions and tolerates stress induced by handling, and asynchronous reproductive behavior makes overcrowding pond during culture.

Siraj *et al.,* (1983) stated tilapia is a paradox in reproduction. The relative fecundity of the Oreochromis species is low, 6,000–13,000 eggs/kg/spawn.

According to Phelps and Popma (2000), there are some difficulties for culturing tilapia. These are (a) this is offset by the excellent survival of fry because to their big size at hatching, huge yolk reserve, and mouth brooding maternal care provided until hatchlings are 10 mm or larger. (b) The low fecundity of these asynchronous species is compensated for by their frequent spawning, where the low fecundity per spawn can be translated into a yearly amount of eggs/kg comparable to several group synchronous spawning species. (c) Generally, a fish species used in aquaculture should not reproduce in the culture environment before reaching market size. From this standpoint, tilapia pose certain difficulties for fish farmers. Under ideal conditions, most tilapia species achieve maturity within 6–8 months of hatching, with a weight of less than 100 grams. They will continue to reproduce in favorable conditions, with progeny fighting for food with the original population, resulting in stunted growth and unmarketable fish.

Swingle (1960) found that in 169–196 d culture cycles of mixed sex Mosambique tilapia, Oreochromis mossambicus, production exceeded 3,000 kg/ha but >90% of the harvest was composed of fish predatory fish (Lovshin 1975) and monosex culture (Shell, 1968) have been described to control tilapia overpopulation.

Phelps and Popma (2000) stated the use of a predator does not prevent reproduction but can prevent recruitment because Tilapia yields are often low, reduced by the slower growing females, and often because the predator has lower tolerance to poor water quality than tilapia, forcing the producer to limit nutrient input in order to maintain adequate water quality for the predator species.

Phelps (2010) identified that they would continue to reproduce under favorable conditions, the offspring competing with the initial stock for food, resulting in stunted growth and unmarketable fish.

According to Suresh and Bhujel (2012), early maturation and prolific breeding of *Oreochromis niloticus* in culture systems, especially earthen ponds, is a significant problem tilapia farming.

De Graaf *et al.,* (1996) stated that the large numbers of fingerlings produced through reproduction during grow-out consume the feeds and dissolved oxygen intended for the stocked tilapia. Consequently, the growth rate decreases with fewer marketable-size fish

Barras and Melard (1997) also concluded that early sexual maturity in tilapia is a well-known issue, resulting in overstocked ponds, decreased production, and poorly cultivated stocks. Fisheries scientists, economists, and commercial fish farmers collaborated to resolve the difficulties, establish a better breeding stock for tilapia, and make mono-sex, and ideally, all the male tilapia population tilapia males grow faster than females.

Angienda *et al.,* (2011) stated that the disparity in growth in favor of men makes male monosex tilapia cultures more likely than females. The metabolic energy in males is transmitted to development. They benefit from androgens anabolism.

Celik *et al.,* (2011) observed a more significant reallocation of metabolic energy for reproduction in females. While the mono-sex male population can be obtained directly or indirectly, *Oreochromis niloticus* has been identified as the preferred oral administration method in commercial uses.

According to Ng and Wang (2011), mono-sex fish can tolerate severe environmental conditions including temperature, salinity, and low dissolved oxygen; more excellent uniformity of size is achieved at harvest because none of the fish is wasting energy gonadal development.

2.6 Techniques for producing monosex tilapia

All male culture of tilapia is preferred because of their faster growth (Guerrero, 1975; Shelton *et al.,* 1978).

Rima *et al.,* (2017) identified four basic methods has practiced all over the world to maintain or produce mono-sex population in aquaculture. Identified techniques are; 1) Periodic harvesting of fry and fingerlings; 2) Monosex culture of which single-sex fish are obtained through the manual separation of sexes, environmental manipulation for sex determination, hybridization, hormone augmentation, and genetic manipulation methods (e.g., androgenesis, gynogenesis, polyploidy, and transgenesis); 3) Biological control and (4) Sterilization.

Mair and Little (1991) stated that manual sexing means sex can be separated before getting sexual maturity could produce all-male tilapia populations in the culture pond, but it has several disadvantages, including high cost and labor-intensive etc.

Hickling (1963) also stated that manual sexing, which entails the elimination of females based on sexual dimorphism observed in the urogenital papilla, is simple but is time-consuming, requires qualified personnel, and usually results in 3-10% errors.

Popma *et al.,* (1984) developed an efficient system to produce hand-sexed fingerlings but 30% of a farm's acreage had to be devoted to brood fish and fingerling production to support the remaining 70% in food fish production. Fingerlings were reared to 20–30 g for sexing, producing the equivalent of 4,000 kg/ha/y of females, most of which were discarded.

Selective crosses of tilapia using a homogametic male of one species crossed with a homogametic female of another has resulted in all male hybrids (Lovshin, 1982). This approach, first reported by Hickling in 1960, became a common method to produce males, but was largely replaced by the mid1980's. Difficulties in maintaining 2 pure lines of brood fish and keeping them separate, and the space required contributed to the decline. In addition apparent autosomal influences affected sex ratios often resulting in populations that were less than 100% males even if pure lines were maintained. The use of hormones to alter the sex ratios of fish was first demonstrated in species other than tilapia.

Yamamato (1951) concluded that sex hormones, in addition to modification of secondary sex characteristics, also affect the gonads. Androgen induced masculinization and estrogen resulted in feminization. He produced 100% female medaka (Oryzai latipus) with an estrogen in 1951 and a nearly all male population with an androgen in 1954.

The general technique has successfully altered the sex ratio of rainbow trout Salmo gairdneri, goldfish Carassius auratus, Zebra fario (Yamazaki, 1976), grass carps Ctenopharengodon idella (Stanley 1976) and tilapia (Clemens and Inslee, 1968; Nakamura and Takahashi, 1973).

According to Phelps and Popma (2000), hormone treatment does not change the fish's genotype, but it does control how the phenotypic is expressed because a treated population of fish may be phenotypically monosex, but its genetic makeup will have stayed unchanged since fertilization. So hormone treatment can result in phenotypically male fish that are genetically female or phenotypically female fish that are genetically male.

Bartley *et al.,* (2000) recognized hybridization as the process of combining different varieties of the organism to create a hybrid the crossing of interspecific strains to produce hybrids and the mating of genetically differentiated individuals or groups which may involve crosses within a species (also known as line crossing or strain crossing) or crosses between separate species.

Rosenstein and Hulata (1994) stated that hybridization between some species of tilapias (Cichlidae) such as Nile tilapia and the blue tilapia results in the production of predominantly male offspring and reduces unwanted natural reproduction in grow-out ponds.

Megbowon *et al.,* (2013) discovered that the use of 17 alpha methyltestosterone is the most efficient and least expensive method is sex reversal of tilapia.

2.7 Masculinization of tilapia through hormone

El-Sayed (2006) stated extensive attention was given to tilapia's mono-sex culture during the past two decades. Oral administration of 17α-methyltestosterone (MT) hormone is the most common and successful method used for producing all-male tilapia.

Megbowon *et al.,* (2009) stated that in tilapia, sex reversal involves the treatment administration of male steroid to recently hatched fry so that the undifferentiated gonadal tissue of generic female develops testicular tissue, thus functioning reproductively as males.

According to Guerrero and Guerrero (1988), production of the all-male population through the administration of androgen (17-alpha methyltestosterone) hormone is the most effective and economically feasible method for obtaining all-male tilapia populations.

Chambers (1984) found that this technique is applicable to fish hatcheries or hapas in which a natural diet (plankton) necessary to correct fry nutrition is available, without any damage to sex reversal.

According to Macintosh and Little (1995), 17a- methyltestosterone (17a-MT) is a synthetic male hormone that closely mimics the naturally-produced hormone testosterone. The most common sex-reversal treatment involves giving a powdered fish feed to the first-feeding (and still sexually undifferentiated) tilapia fry. This diet contains 30–60 mg 17a-MT/kg of feed until the 25–60th days post-hatching.

El-Sayed *et al.,* (2012) stated that the hormone is generally incorporated into starter larval feed at 30–60 mg MT/kg feed, during the critical period of sex differentiation.

El-gassy *et al.,* (2012) studied to solve some practical problems concerning the complete masculinization of *Oreochromis niloticus* by oral administration of 17a-methyltestosterone (17a-MT) concerning the dose of the hormone used, different doses of 17a-MT (40, 60 and 80 mg MT/kg of feed) were orally administered to sexually undifferentiated fries from the 7th to the 28th-day post-hatching to produce all-male tilapia population.

Abucay and Mair (1997) found that testosterone (MT) used to produce all-male tilapia vary wildly. The dosage rates range from 10-100 mg kg-1 MT of diet for tilapia.

2.8 Effects of hormone on sex reversal of tilapia

Guerreeo *et al.,* (1988) researched sex reversal of Nile tilapia (*Oreochromis niloticus*) by using 30 ppm 17 alpha methyltestosterone for 21 days in 50-100 sq. meter outdoor tank and found 99% male population in maturation stage.

Ridha and lone (1990) researched the effect of 17 alpha methyltestosterone hormone on masculinization, growth, survival rate of *Oreochromis spilurus* (Gunther) in a brackish water environment. Thirty-eight-day hormonal treatment phase followed by 205 days rearing with a regular diet resulted in 90.3% males at 70mg/kg of 17 alpha methyl-testosterone. No significant effects were observed in growth and survival.

Vera-Cruz and Mair (1994) treated tilapia at 30mg of MT/kg of diet fed at 20% body weight for 25 days and obtained 98.4% males in the tank and 95.4% in Hapa. He got 99% males with 60 mg MT/Kg in similar conditions. He also observed the comparative effects of natural androgen (11 Beta Hydroxyandrostenedione) and synthetic androgen (17 alpha methyltestosterone). They showed that sex reversal could be possible with both types of androgen. The study found 17 alpha-methyltestosterone more effective with 100% male production than the 11 Beta Hydroxyandrostenedione with 88.9% male production.

Abacay and Mair (1997) proved that with research on 17 alpha-methyltestosterone effects on sex-reversal of tilapia. The study found 100% male population with 40 mg MT/Kg treatment in the closed water system.

Romerio *et al.,* (2000) researched sex reversal techniques with different doses of 17 alpha methyltestosterone for 45 days of MT treatment. They found 60mg MT/Kg more efficient with 98% male population.

Kohinoor *et al.,* (2003) studied breeding biology and mono-sex male production of Nile tilapia in hatchery under different conditions. Different dosages of MT 100, 80, 60 and 40 mg/Kg were used as a treatment for 28 days, and the mean percentage of male populations obtained 98, 97, 95 and 68, respectively.

2.9 Growth performance of sex reversed tilapia

According to Mocintosh *et al.,* (1985), Sex reversed tilapia showed a better growth rate than usual because the administration of androgen has both an androgenic and anabolic effect. Several studies are comparing the growth of sex-reversed, near all-male populations, to that of a mixed-sex population after hormone treatment showed the improved growth of sex-reversed fish than non-treated because the presence of females reduce the growth rate due to their slower growth rate or reproduction.

Several studies have been conducted to increase tilapia production and it has been observed that androgen treated, all-male tilapia populations grow faster than untreated, mixed-sex tilapia populations (Guerrero and Guerrero, 1975; Hanson *et al.,* 1983; Jae-Yoon Jo *et al.,* 1988; Pandian and Varadaraj, 1988).

The increased growth performance of the androgen-treated sex reversed tilapia may be attributed to the phenomenon of muscular hypertrophy by increased muscle protein synthesis through testosterone treatment (Bhasin *et al.,* 2001).

Numerous papers have reported that 60 mg/ kg was found to induce monosex male populations (Macintosh *et al.,* 1988; Killian and Kohler, 1991; Green and Teichert-Coddington, 1994).

The increase in growth by hormone may be due to the fact that MT induced the feed digestion and absorption rate causing increase in body weight (El-Greisy and El-Gamal, 2012).

Similarly Sparks *et al.,* (2003) found that *Oreochromis mossambicus* fry fed with MT added to their feed grew significantly larger than their respective controls.

Significant increase in growth in 17α-methyltestosterone treated group of tilapia was also recorded by Ridha and Lone (2008).

A few studies have demonstrated the enhanced yield of monosex male Nile tilapia populations under experimental conditions (Mair *et al.,* 1995).

In *O. mossambicus,* 17α-MT treated fish is reported to show higher growth compared to the untreated fish reared under similar conditions (Macintosh *et al.,* 1985).

Several studies are in agreement that testosterone produces muscle hypertrophy by increasing muscle protein synthesis (Bhasin *et al.,* 2001).

Bhandari Nakamura *et al.,* (2006) found that sex reversal may also affect tilapia's meat quality.

Hanson *et al.,* (1984) reported the 10-60 ppm MT Treated male tilapia population showed better growth than the control treatment in culture conditions.

Dan and Little (2000) compared the growth performance of the mono-sex male population through MT and the mixed population of Nile tilapia, and found a percentage of 10.7 more growth in the mono-sex male population than the mixed population culture periods. He also observed that the effect of different doses (50, 75, and 100 mg/Kg) of MT hormone on sex reversal and growth performance of Tilapia (Ore*ochromis mossambicus*) and found 75 mg/kg as useful for growth performance. It showed 1.2 times greater weight gain than the other treatments.

CHAPTER - III

MATERIALS AND METHODS

MATERIALS AND METHODS

The experiment was conducted for 270 days to determine the effect of "Hormonal masculinization in tilapia (*Oreochromis niloticus*) with some aspect of growth performance and production." The experiment was carried out from February, 2019 to January, 2020.

3.1 Study area

The experiment was conducted in the Meridian hatchery which was located at Sonagazi upazila in Feni district. The study area's coordinates were obtained using 'Google Maps' software, and a map was generated by 'Arc-GIS' software Plate 1.

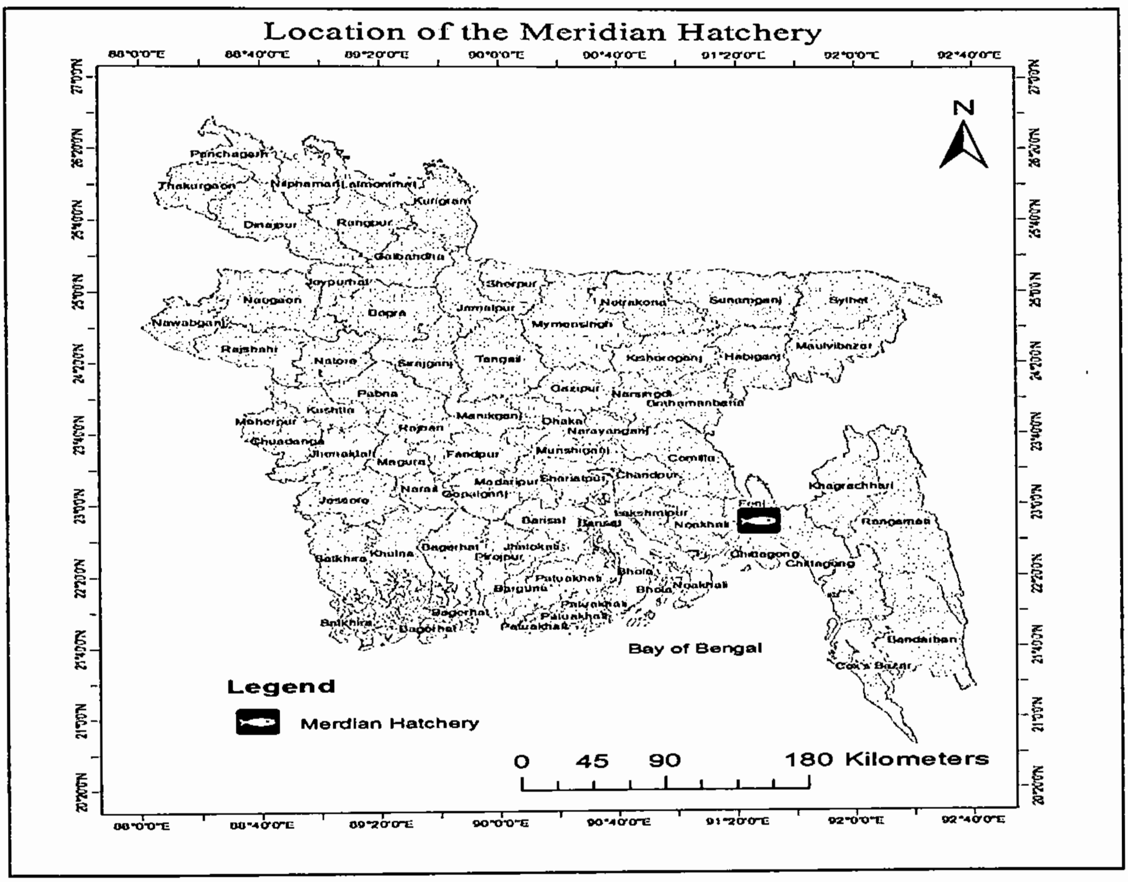

Plate 1: Maps of study area Sonagazi upazila in Feni district.

3.2 Experiment layout

Four different treatment were used to conduct this experiment which were T_0 (Control treatment without MT hormone), T_1 (50 mg MT hormone), T_2 (60 mg MT hormone), T_3 (80 mg MT hormone). Each treatment includes three replications. All the treatment were applied according to research design in the SRT hapa and sex-reverse percentage was calculated. Then fries were transferred to the nursery and then grow-out pond to evaluate effects of the MT treated feed in the growth performance and compared to the control treatment. Experimental layout is described below.

Table 1: Experiment layout.

Dietary treatment groups	Treatment × Replication (T_n× R_n)
Without MT mixed feed (Normal fish feed)	T_0R_1
	T_0R_2
	T_0R_3
50 mg MT mixed feed	T_1R_1
	T_1R_2
	T_1R_3
60 mg MT mixed feed	T_2R_1
	T_2R_2
	T_2R_3
80 mg MT mixed feed	T_3R_1
	T_3R_2
	T_3R_3

3.3 Collection of brood fish

To conduct the experiment brood fishes were collected from Bangladesh Fisheries Research Institute (BFRI), Maymensigh and NAMSAI farm, Thailand.

3.4 Preparation hapa and stocking of brood fish

Collected brood fishes was allowed to breed naturally in hapa with an area of 32 sq. meter (8 meter x 4 meter) each. A total 10 hapa was used for the breeding purpose and water height was maintained 1 meter for each hapa. A total 1600 brood fishes were used in the 10 hapas and stocking density was maintained 5 fishes/ sq. m and sex ration was maintained 2 : 1 (120 females: 40 male).

3.5 Feeding in the brood hapa

Feeding was done in relation the 1% per day of the body weight of the brood fishes and feeding frequency was twice/day. Protein content of the feed was maintained 30% in the brood fish feed.

3.6 Egg collection and disinfection

After 3 weeks of stocking, eggs were collected from the mouths of brood fish and classified into various categories. The eggs were collected and washed in clean water to remove any foreign materials. The eggs were formalin-fixed (2ml formalin/liter solution) for 2-3 minutes before being washed with clean water. Finally, the eggs were disinfected by keeping them in a 0.9 percent NaCl solution. For the experiment, 1 kg eggs were obtained from the mouths of brood fish at the same time.

3.7 Egg incubation

The eggs were incubated using both tray and jar methods. After the disinfection, eggs were moved to the tray and jar, and sufficient water flow was maintained for hatching. During the incubation period of the eggs in the tray and jar, continuous aeration was ensured for proper hatching. The collected eggs were incubated for 5-7 days.

3.8 Transfer of swim up fry to SRT (Sex reversed tilapia) hapa

Swim up fry were moved to SRT hapas for MT feed treatment after hatching. A total of 12 hapas were used, each with an area of 18 sq. m. (6 m × 3 m). Nine of the 12 hapas were used for hormonal treatment, while three were used to apply a control treatment (normal feeding). All of the hapa were placed in the 40 decimal pond, and the swim up fry stocking density was 3 thousand per hapa. 0.0117 g/ fry was the average weight of the swim-up fry.

3.9 Preparation of MT hormone treated feed

A stock solution was prepared by using 5 gm 17 alpha methyltestosterone with 10 litre 95% ethyl alcohol. The solution was preserved at 4°C temperature in refrigerator. Then, to prepared MT mixed feed for experimentation, 50 mg, 60 mg and 80 mg of MT and 120 ml solution and 120 ml additional alcohol (for rinsing and better distribution) was mixed with 1 kg feed respectively. The mixture of feed has been completely dried at room temperature or sunlight of the early morning and then sealed in air tight black container and stored in refrigerators until use to retard bacterial or fungal contamination. In the MT mixed feed following ratio of ingredients was used.

Table 2: Ingredients ration for feed preparation.

Sl. No	Ingredients Name	CP-Ing. (%)	Parts
1	Fish meal	60	3.5
2	Soy meal	44	42
3	Rice bran	10	42
4	Wheat flour	8	5.75
5	Starch	0	1
6	Palm oil	0	5
7	Vitamin mix	0	0.25
8	Min mix	0	0.5
	Total		**100**

3.10 Sex-reversed protocol

To measure sex reversal percentages and the impact of MT on growth efficiency, three separate doses of MT hormone were used as treatments (T1 = 50 mg MT hormone, T2 = 60 mg MT hormone, T3 = 80 mg MT hormone). Nine (9) of the 12 SRT hapas were used for hormone treatment (Three for each treatment). 21 days hormonal treatment (MT feed feeding protocol) was given in table-3.

Table 3: Sex reverses feeding protocol.

Day	Frequency (Times/day)	Feed quantity (gm)
Day 1-5	5	125.00
Day 6-10	5	250.00
Day 11 – 15	5	416.67
Day 16 -21	5	700.00

3.11 Fry grading and transfer to nursery hapa

Fries were graded for transfer to the nursery hapa after a 28-day rearing period in the SRT hapa. The fry were graded using a plastic net (mesh size: 4 mm). The average weight of the fries was 0.25 g/fry. The graded 2800 fry then moved to each nursery hapa. Each hapa was 40.5 sq. m (9 m × 4.5 m) and water height was maintained 1.5-2 m for each hapa. A total 12 hapas were used for the experiment; 9 for hormone treated fry and 3 for control feed trated fry. Feed was given according to the 20% body wt. and 32% protein ration was maintained during feed formulation. After a month of rearing, the fries were moved to the grow-out pond. The water quality in the nursery pond was tested on a regular basis.

3.12 Transfer of the fishes to the grow-out pond

After that, the fish were moved to the grow-out pond and reared for 150 days. There were twelve ponds (13 decimal) used, with a stocking rate of 200 fish per decimal for the rearing of the experimental fish. Ponds were marked according the experiment layout. Periodic sampling (Two sampling/ month) was done and weight, length data was taken to evaluate effects of the MT hormone treatment on the fish.

3.13 Specifying of sex rates

To specify sex rates, aceto-cramine solution was used. 1 gm carmine powder was mixed with 100 ml glacial acetic acid (45%) to prepared 1% aceto-cramine solution. After dissection of the fish, gonads was taken carefully in the glass slides and 1 drops of aceto-cramine solution was added. Then slide was placed under the microscope and gonad was observed. Web like structured gonad was female fish and finely grainy gonads were identified as male fish. Determination of the sex of was carried out in the each sampling.

3.14 Determination of water quality parameters

Water quality parameter was measured during experimentation including surface and water temperature, pH, dissolved oxygen (DO), ammonia and salinity. Protocol of water quality determination is given below.

3.14.1 Determination of surface temperature and water temperature

Surface temperature and water temperature were determined by using a Celsius thermometer.

3.14.2 Determination of dissolved oxygen (DO)

Dissolved oxygen was determined by using a dissolved oxygen meter (Instrument name: DO Meter, model number: DO200A and company name: HANNA).

3.14.3 Determination of water pH

Water pH was determined by using a pH meter (Instrument name: pH meter, model number: H198107 and company name: HANNA).

3.14.4 Determination of ammonia

Ammonia was determined by using ammonia hach-kit (Model number: NI-SA).

3.14.5 Determination of salinity

Water salinity was determined by using a Refractrometer (Instrument name: Refractrometer meter, brand name: ERMA).

3.15 Evaluation of the growth performance and survival rate

To evaluate the growth performance of tilapia under different hormone treatment following growth parameters were calculated based on the collected data.

- Weight gain (g) = Final body weight – Initial body weight
- Length gain (cm) = Final length of the fish body – Initial length of the fish body
- Survival rate (%) = $\frac{\text{Final no. of live fishes}}{\text{Initial no. of fishes}} \times 100$
- SGR= $\frac{\ln(final\ weight) - \ln(initial\ weight)}{duration\ in\ days} * 100$
- CF= $\frac{Weight\ of\ fish}{(Length\ of\ fish)^{\wedge}3} * 100$ (Kader et al, 2018)

3.16 Statistical analysis

In the present study, all the data were analyzed by statistical methods. The one way analysis of variance (One way ANOVA) was performed using SPSS (Statistic Package for social science) version IBM SPSS Statistics 23 software to determine the significant differences among means. For all tests, a criterion of $P<0.05$ was used to determine statistical significance.

3.17 Photo gallery of research activities

Plate 2: Breeding hapa

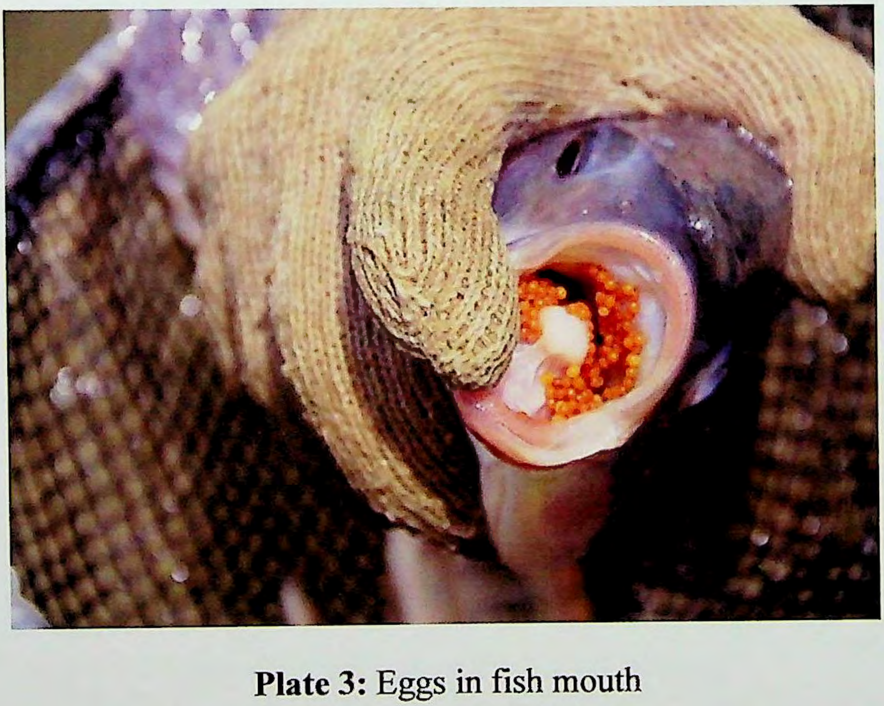

Plate 3: Eggs in fish mouth

Plate 4: Egg collection.

Plate 5: Weighing of the collected eggs.

Plate 6: Hatching of the collected eggs.

Plate 7: Feed formulation.

Plate 8: SRT hapa.

Plate 9: Monitoring in the nursey hapa.

Plate 10: Grow-out pond

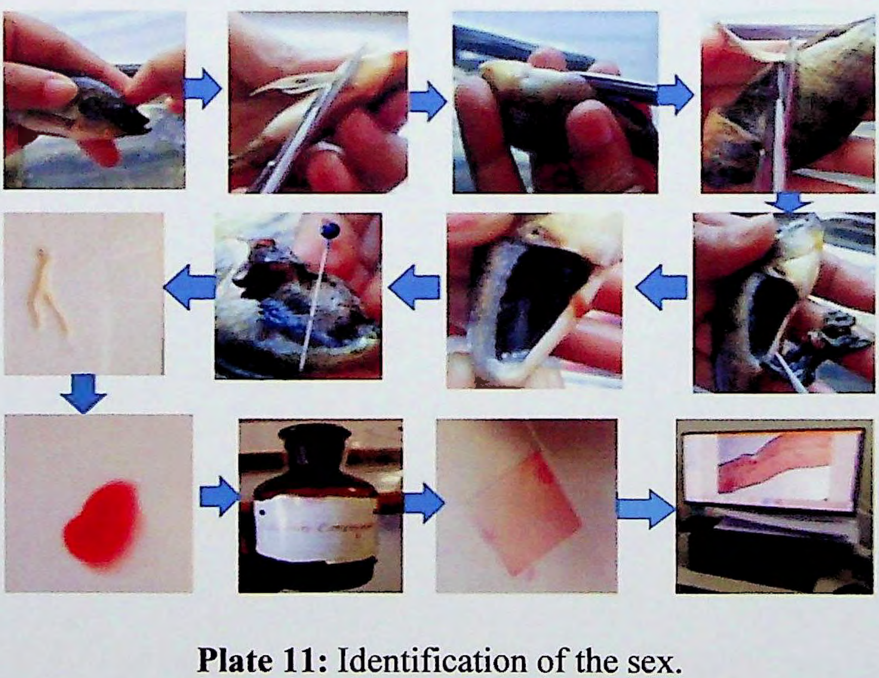

Plate 11: Identification of the sex.

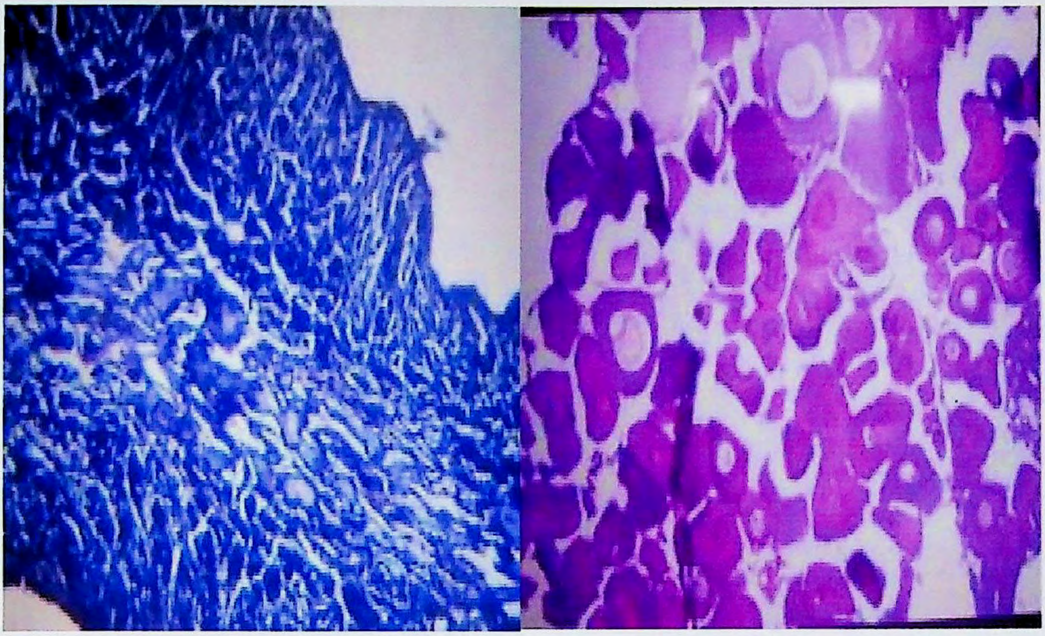

Plate 12: Male and Female gonad.

CHAPTER - IV

RESULTS

RESULTS

The effect of different hormonal masculinization on tilapia growth and production have been investigated and the findings have been presented below.

4.1 Effects of 17-alpha methyltestosterone hormone on fish weight

Fish have been sampled for 12 times in the whole study period (Appendix 1, 3). At the stocking of fish, the average weight of fry was 0.25 g. In final sampling, it showed that the average weight of each treatment such as T_0, T_1, T_2 and T_3 were 481.567, 505, 533.5, 530.367 g, respectively. The data showed that the fishes provided with 'Treatment-2' have the highest growth in terms of weight when comparing with other treatments (Figure 1).

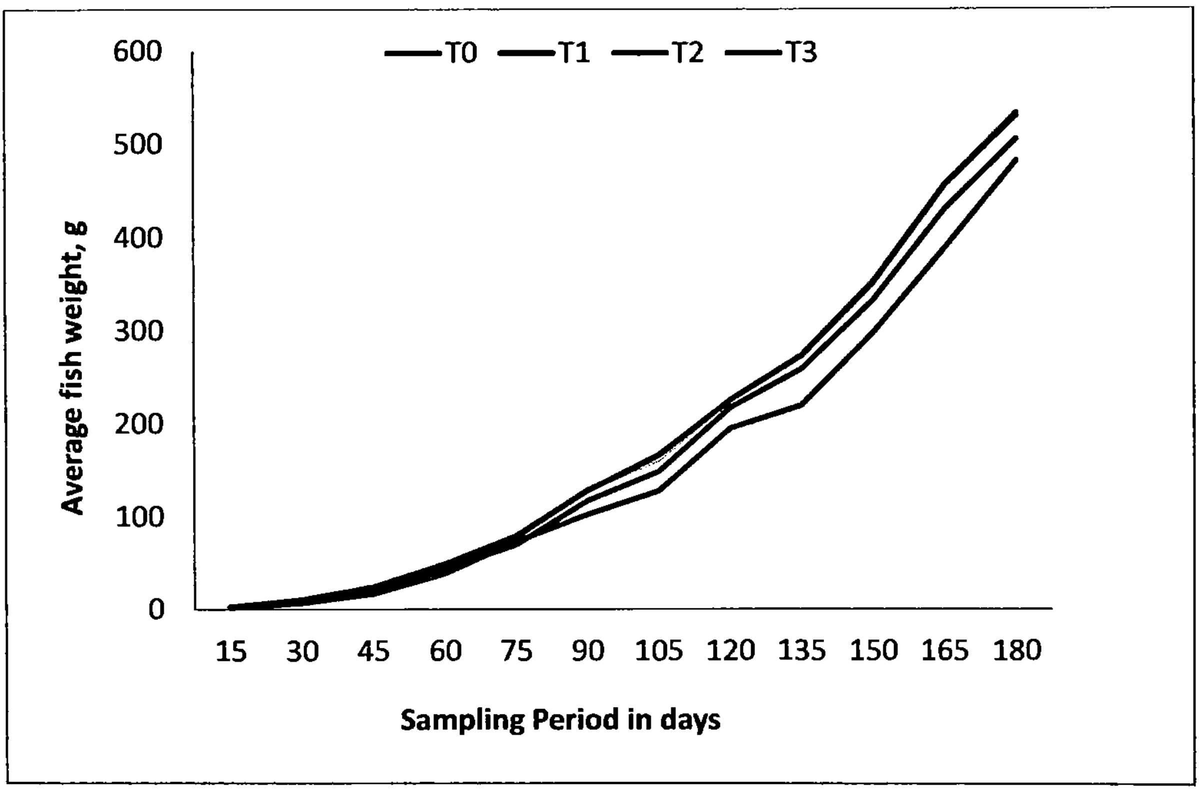

Figure 1: Average growth by weight (g).

4.2 Effects of 17-alpha methyltestosterone hormone on fish length

The initial length of fish was 0.25 cm. In final sampling, it showed that the average length of each treatment such as T_0, T_1, T_2 and T_3 were 29.03, 29.3, 29.967, 29.67 cm respectively. The data (Figure 2 and Appendix 2, 4) showed that the fishes provided with 'Treatment-2' have higher growth in terms of length when comparing with other treatments.

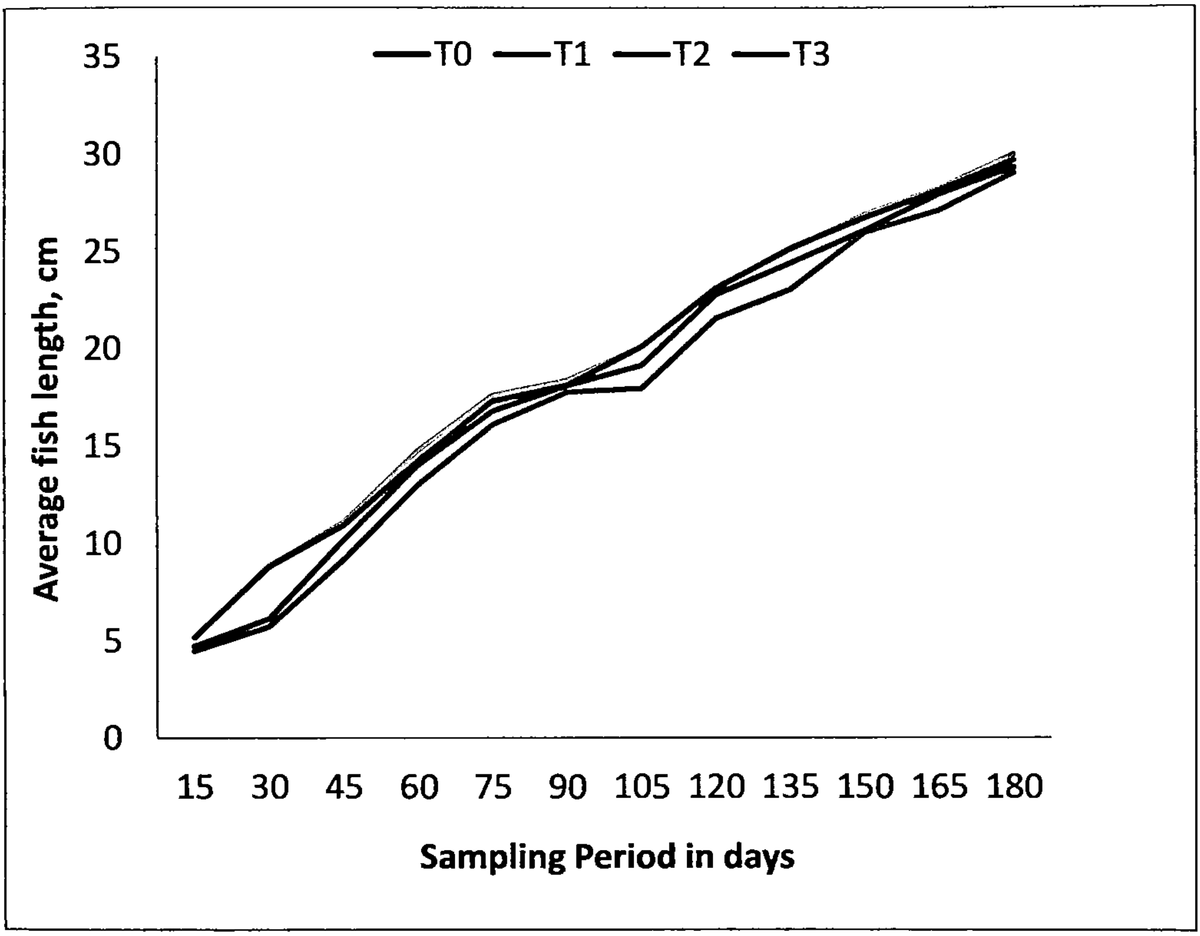

Figure 2: Average growth by length (cm).

4.3 Effects of 17-alpha methyltestosterone hormone on fish length gain

Length gain of fishes was calculated fortnightly by subtracting of final sampling fish length from initial previous sampling fish length and showed in Figure 3. Highest length gain was found in T_2R_3 (29.03 cm) treatment and lowest was T_0 R_3 (27.93 cm) treatment.

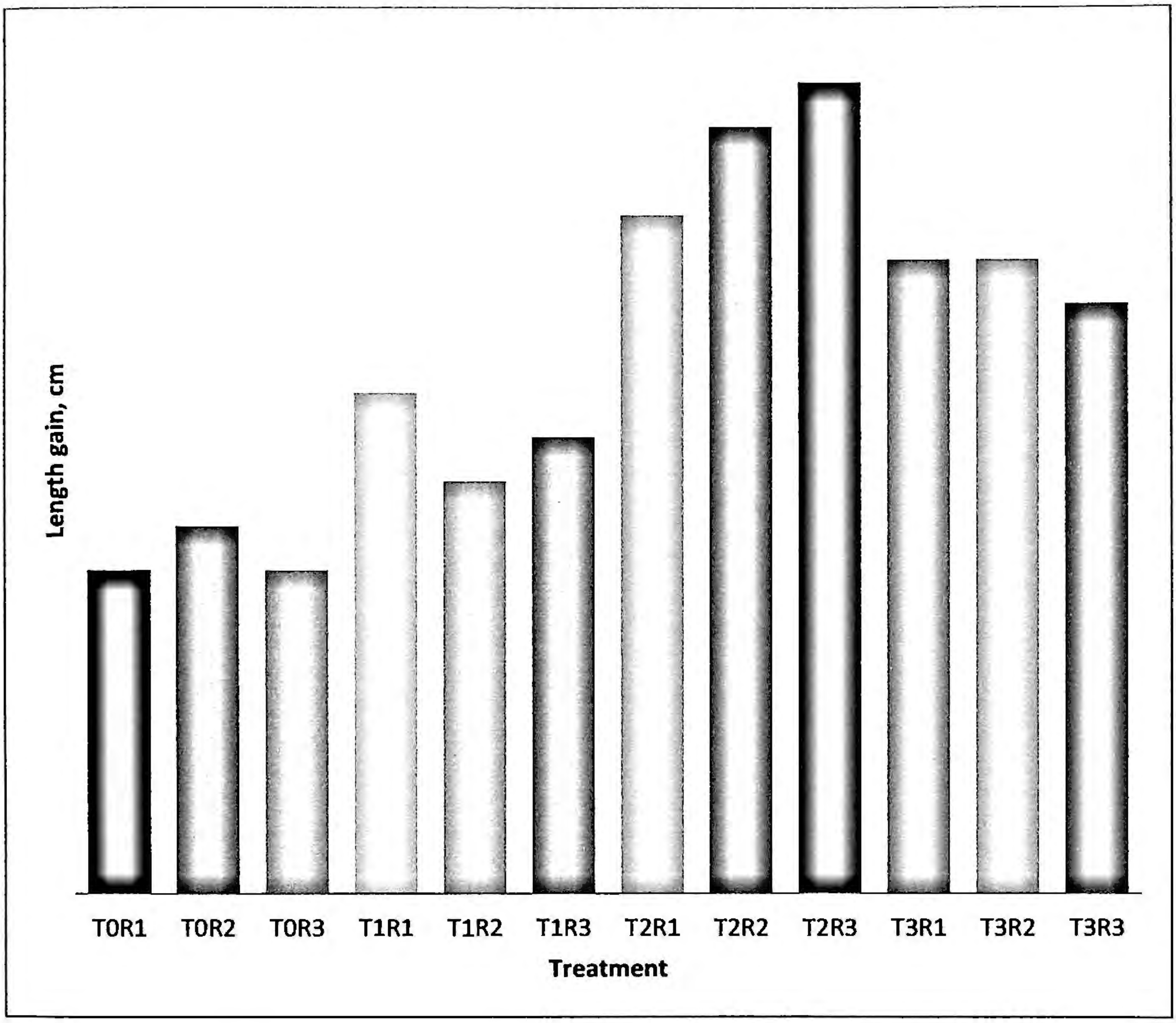

Figure 3: Fish length gain (cm).

4.4 Growth performance

Growth parameters were significantly higher in T_2 treatment that were shown in Table 4. Among hormonal masculinization treatments, significant differences ($p<0.05$) were observed for final weight, weight gain, final length, SGR and CF.

Mean weight of each treatment such as T_0, T_1, T_2 and T_3 were 481.567 ± 1.193^c, 505.0 ± 1.5875^b, 533.5 ± 1.249^a and 530.367 ± 1.097^a g and mean weight gain were 481.3167 ± 1.193^c, 504.75 ± 1.587^b, 533.25 ± 1.249^a and 530.117 ± 1.097^ag respectively.

It showed that the mean length of each treatment such as T_0, T_1, T_2 and T_3 were 29.03 ± 0.058^d, 29.3 ± 0.1528^c, 29.97 ± 0.153^a and 29.67 ± 0.056^b cm respectively.

Mean SGR of fish of T_0, T_1, T_2 and T_3 fish were 4.202 ± 0.0014^c, 4.228 ± 0.0017^b, 4.259 ± 0.0013^a and 4.255 ± 0.0011^a.

Mean CF of T_1, T_2 and T_3 were 2.008 ± 0.0147^{ab}, 1.983 ± 0.0287^b and 2.031 ± 0.0095^a respectively compared to the controlled group (T_1).

Treatment-T_0 was not considered as they were not fed by sex hormone. Values accompanied by different letters are statistically significantly different ($p < 0.05$, $n = 4$).

Table 4: Growth performance of tilapia

	T_0	T_1	T_2	T_3	Level of Significance
Mean weight	481.567±1.193^c (478.61-484.53)	505.0±1.5875^b (501.06-508.94)	533.5±1.249^a (530.39-536.61)	530.367±1.097^a (527.64-533.09)	0.000
Mean weight gain	481.3167±1.193^c (478.35-484.28)	504.75±1.587^b (500.81-508.69)	533.25±1.249^a (530.15-536.35)	530.117±1.097^a (527.39-532.84)	0.000
Mean Length	29.03±0.058^d (28.89-29.18)	29.3±0.1528^c (29.05-29.55)	29.97±0.153^a (29.59-30.346)	29.67±0.056^b (29.52-29.81)	0.000
SGR	4.202±0.0014^c (4.198-4.205)	4.228±0.0017^b (4.22-4.233)	4.259±0.0013^a (4.26-4.262)	4.255±0.0011^a (4.25-4.258)	0.000
CF	1.968±0.138^b (1.933-2.002)	2.008±0.0147^{ab} (1.97-2.044)	1.983±0.0287^b (1.92-2.054)	2.031±0.0095^a (2.008-2.055)	0.012
Sex Reversing Percentage	-	77.833±0.764^b (75.936-79.731)	98.0±0.5^a (96.759-99.758)	97.833±0.764^a (95.936-99.731)	0.000

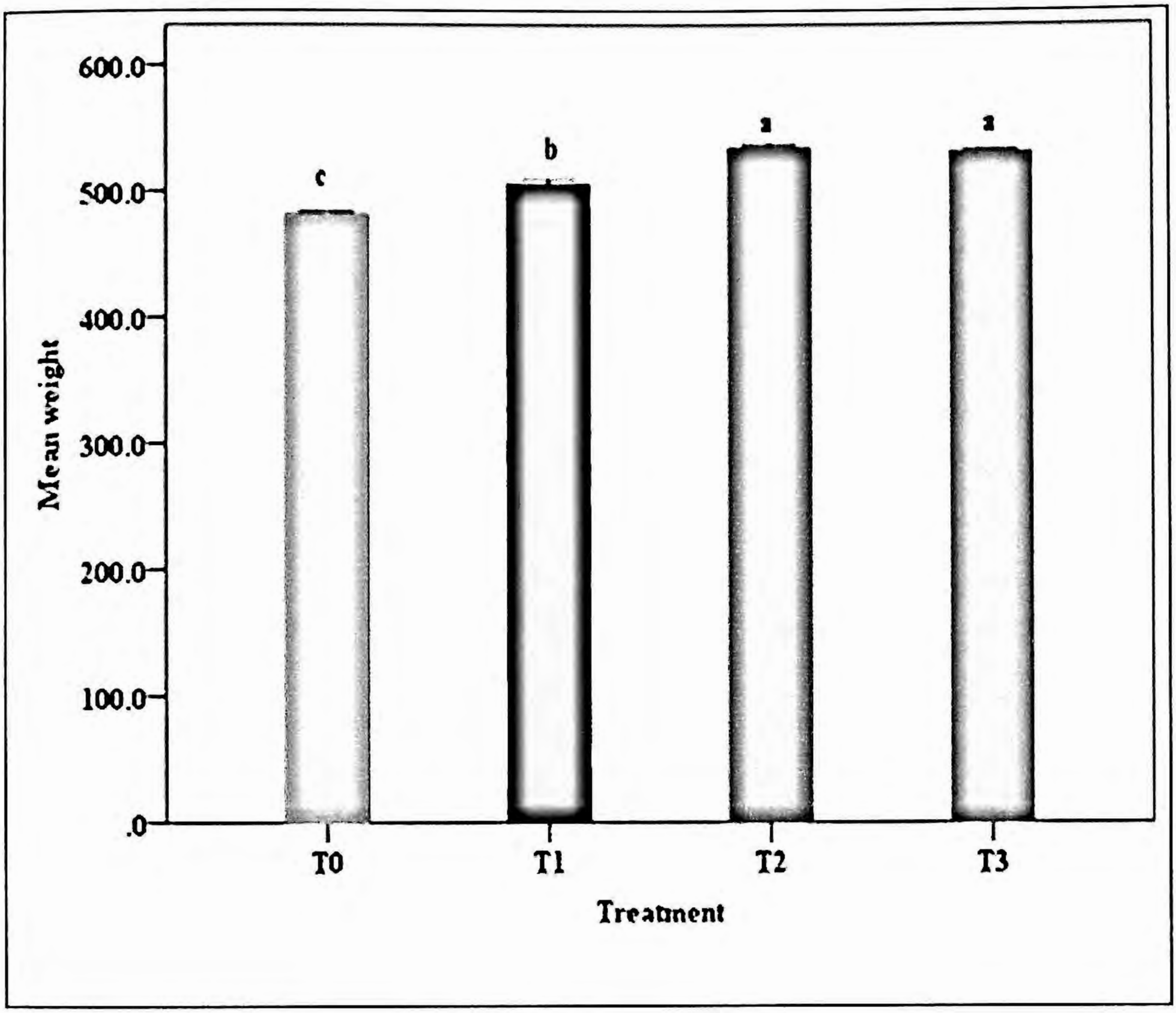

Figure 4: Effects of hormonal masculinization on fish mean weight (Mean ± SD) were shown after 6 months.

The mean weight of fish of T_1, T_2 and T_3 fish were compared to the controlled group T_0. Values accompanied by different letters are statistically significantly different ($p < 0.05$, $n = 4$) which showed that T_0 and T_1 were statistically significantly different from other treatments and there was no difference between T_2 and T_3.

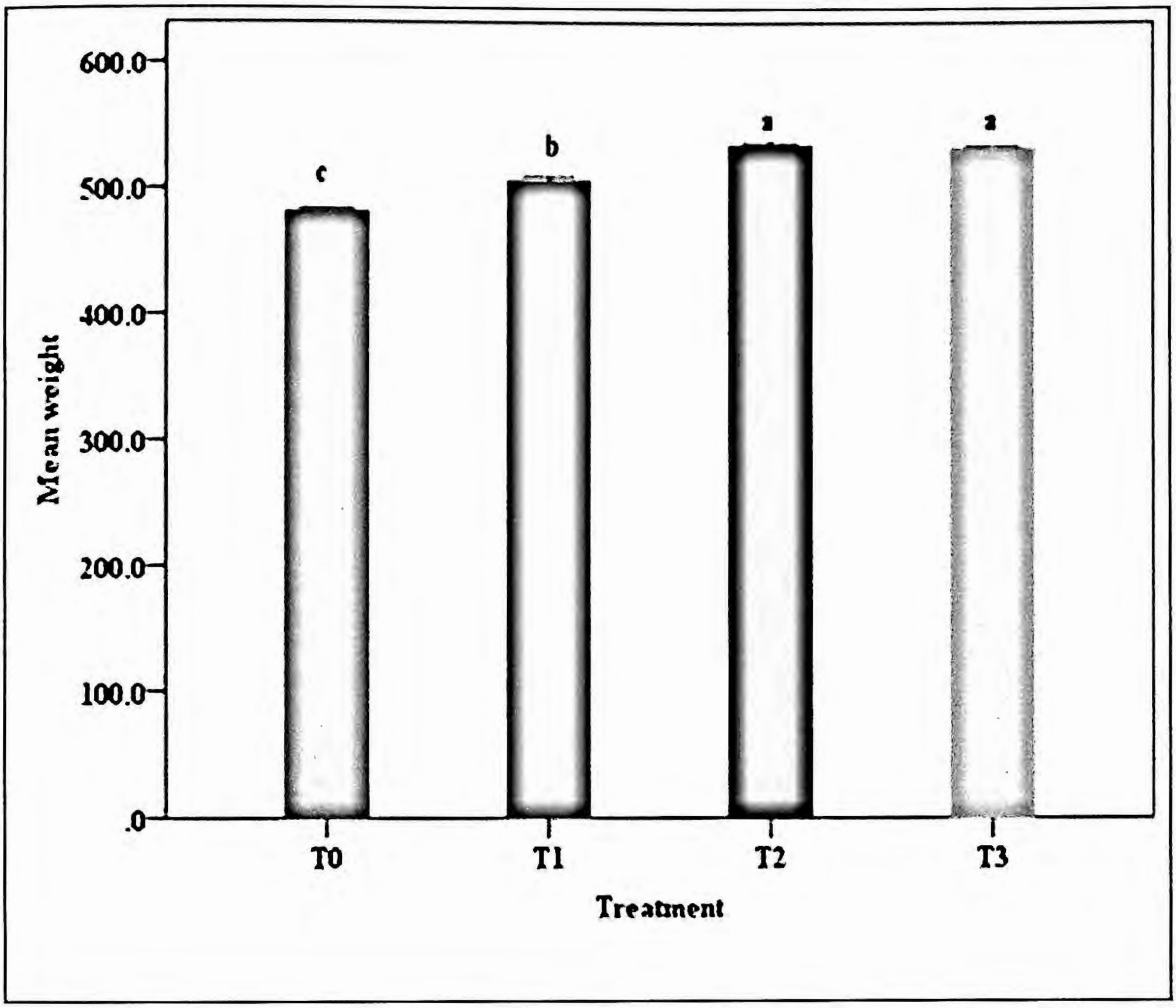

Figure 5: Effects of hormonal masculinization on fish mean weight gain (Mean ± SD) were shown after 6 months.

The mean weight gain of fish of T_1, T_2 and T_3 fish were compared to the controlled group T_0. Values accompanied by different letters are statistically significantly different ($p < 0.05$, $n = 4$) which showed that T_0 and T_1 were statistically significantly different from other treatments and there was no difference between T_2 and T_3.

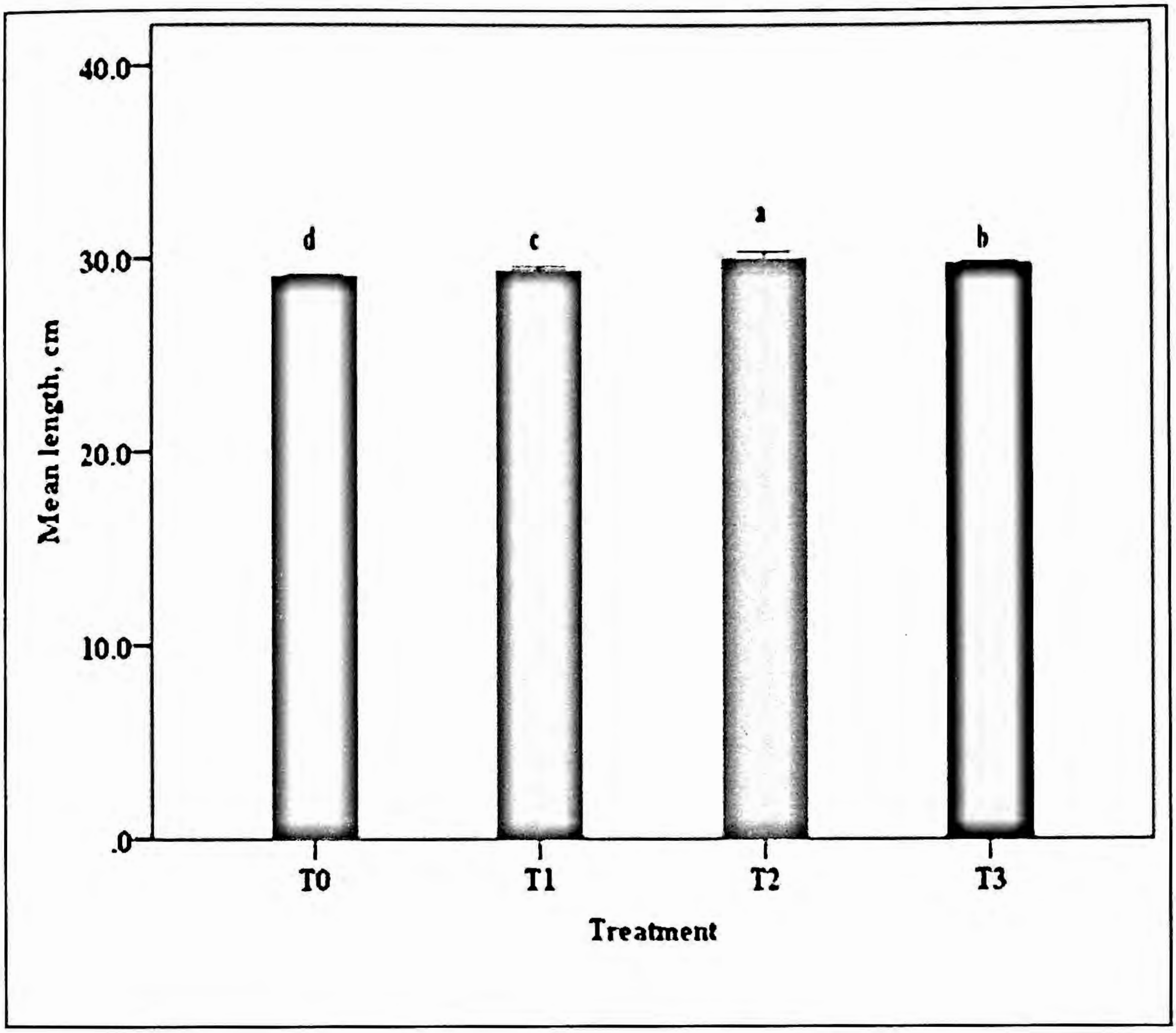

Figure 6: Effects of hormonal masculinization on fish mean length (Mean ± SD) were shown after 6 months.

The mean length of fish of T_1, T_2 and T_3 fish were compared to the controlled group T_0. Values accompanied by different letters are statistically significantly different ($p < 0.05$, $n = 4$) which showed that each treatments were statistically significantly different from each other treatments.

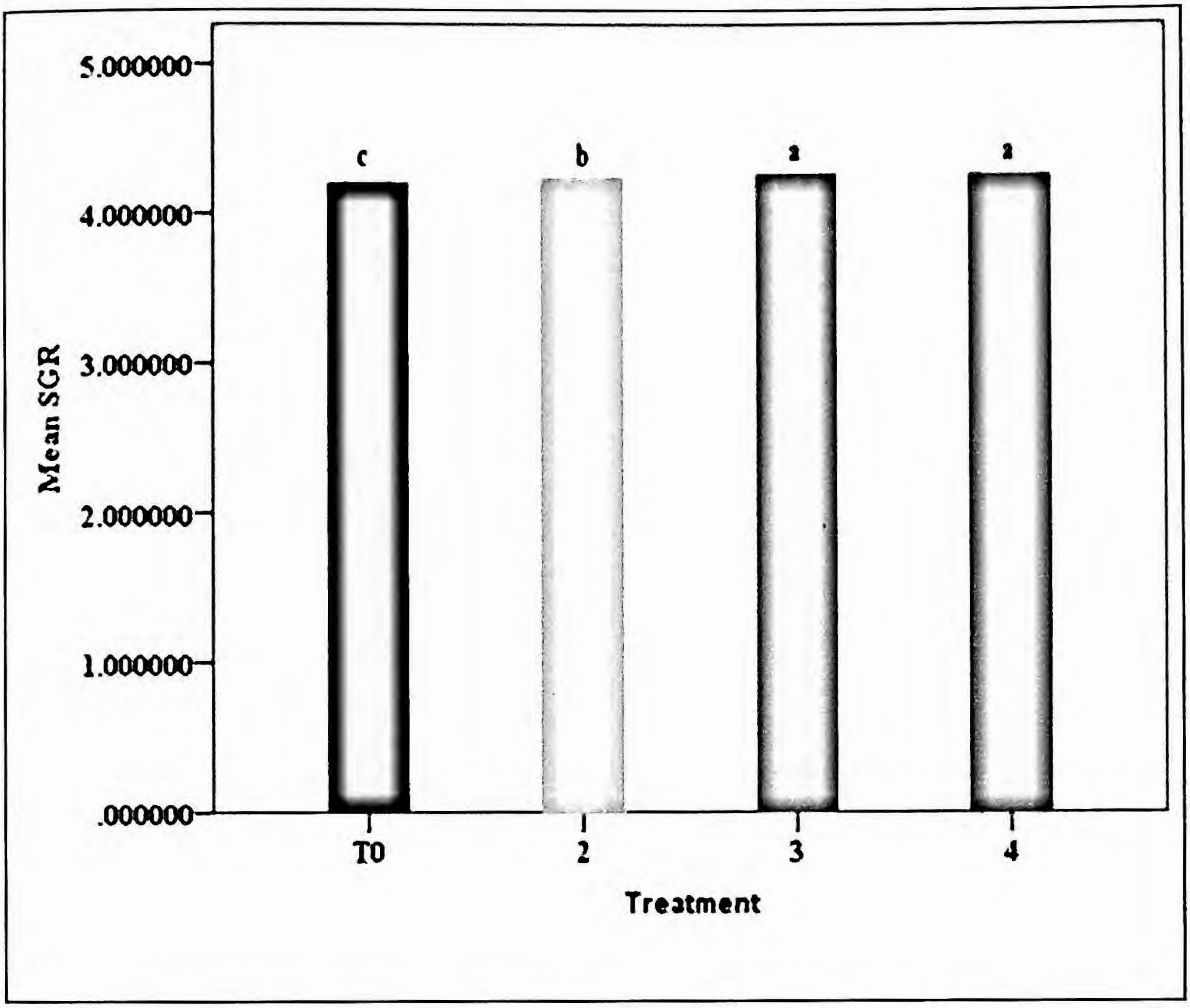

Figure 7: Effects of hormonal masculinization on fish mean SGR (Mean ± SD) were shown after 6 months.

The mean SGR of fish of T_1, T_2 and T_3 fish were compared to the controlled group T_0. Values accompanied by different letters are statistically significantly different ($p < 0.05$, $n = 4$) which showed that T_0 and T_1 were statistically significantly different from other treatments and there was no difference between T_2 and T_3.

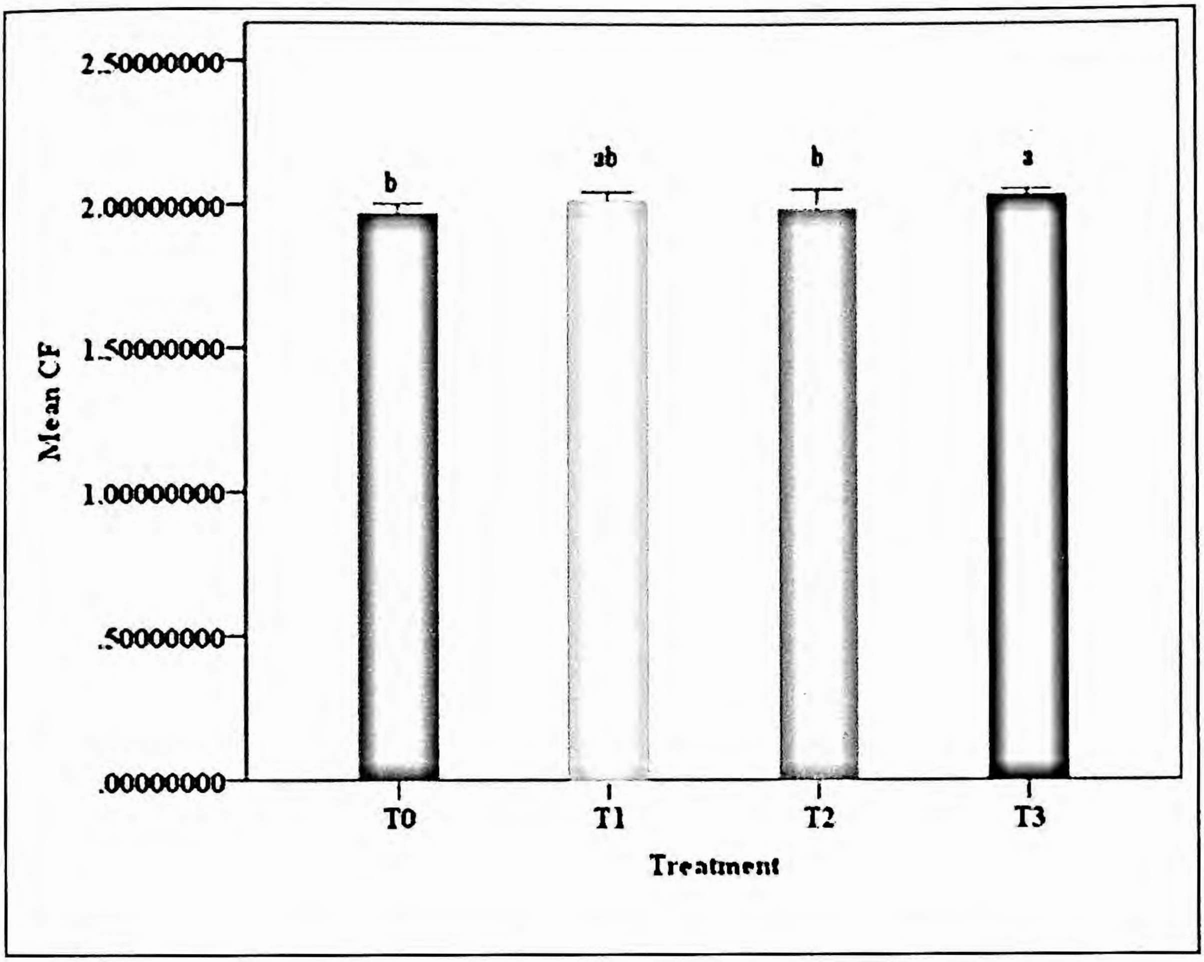

Figure 8: Effects of hormonal masculinization on fish mean CF (Mean ± SD) were shown after 6 months.

The mean CF of fish of T_1, T_2 and T_3 fish were compared to the controlled group T_0. Values accompanied by different letters are statistically significantly different ($p < 0.05$, $n = 4$) which showed that there were no difference among these four treatments.

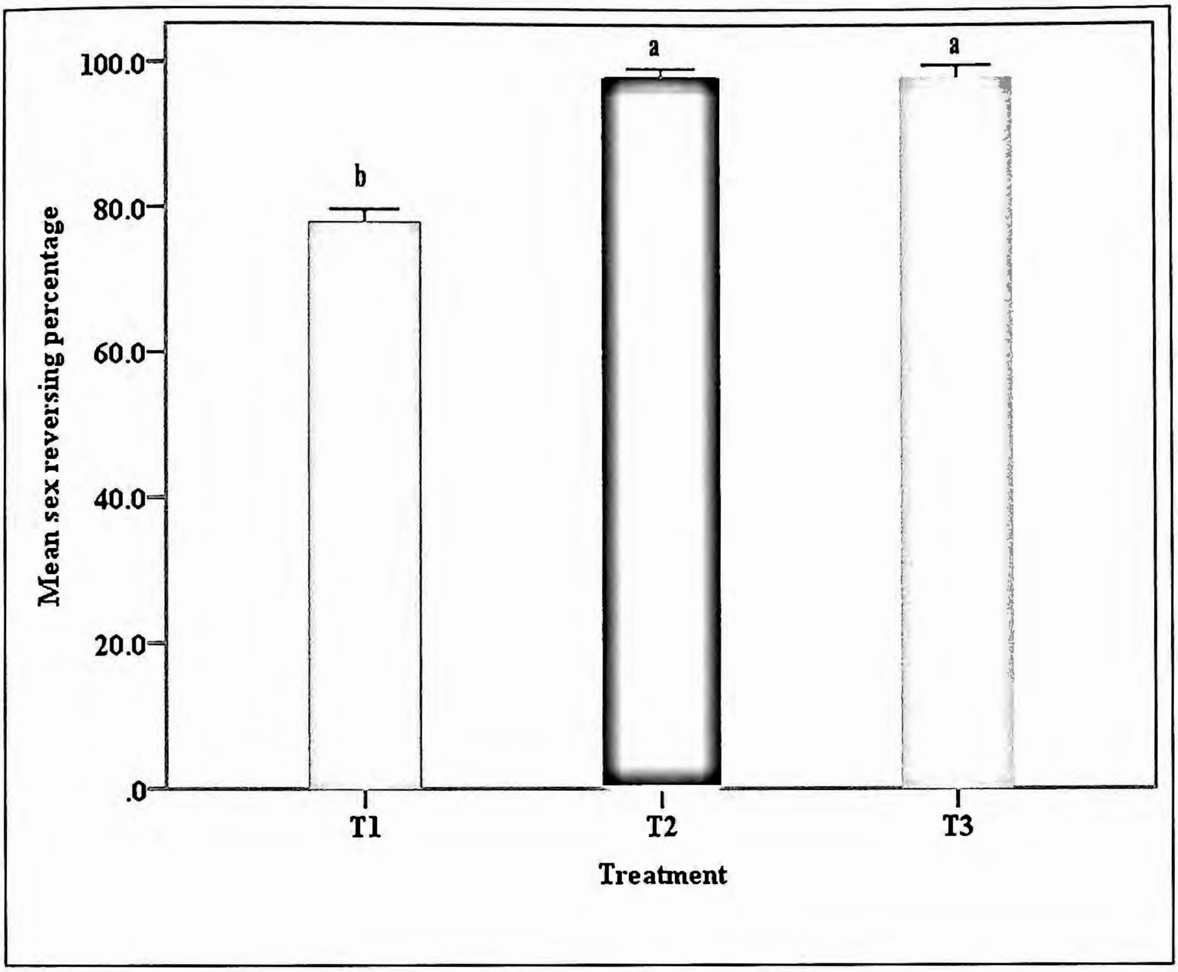

Figure 9: Effects of hormonal masculinization on fish mean sex reversing percentage (Mean ± SD) were shown after 6 months.

The mean sex reversing percentage of fish of T_1, T_2 and T_3 fish were compared. Values accompanied by different letters are statistically significantly different ($p < 0.05$, $n = 4$) which showed that T_1 were statistically significantly different from other treatments and there was no difference between T_2 and T_3.

4.5 Effects of 17-alpha methyltestosterone hormone on FCR

Experimental fish showed best FCR (1.2) in T_2 and T_3 hormonal treated feed. All hormonal treated fish showed better FCR performance than control fish (1.7) (Figure 10).

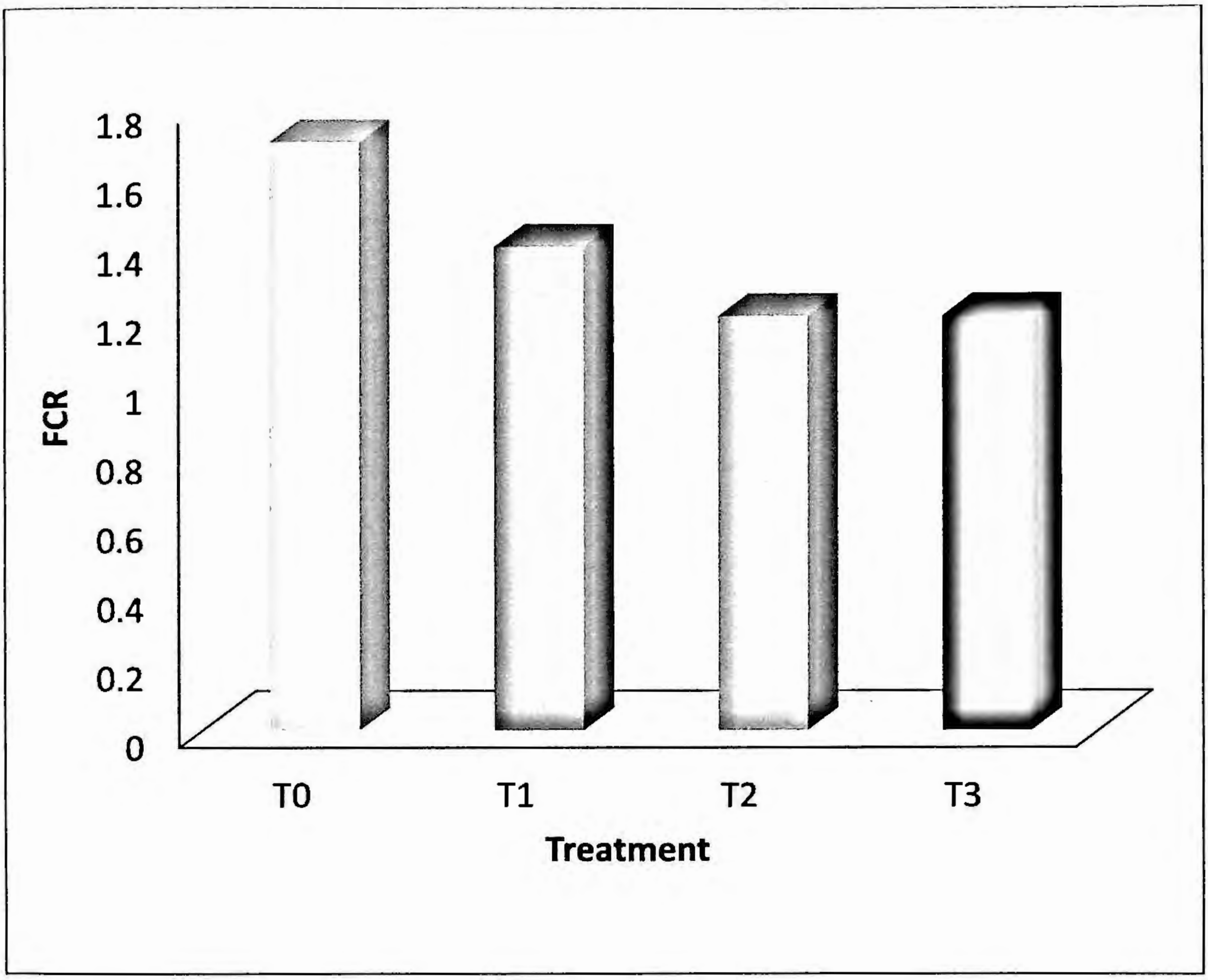

Figure 10: FCR value.

4.6 Effects of 17-alpha methyltestosterone hormone on survival rate

Effects of hormonal masculinization on survival rate of tilapia are recorded in Table 5. The highest survival rate was 87.6% in T_2 and lowest was 65.7% in T_0 in the experiment.

Table 5: Effects of hormonal masculinization on survival rate.

Treatment	Survival Rate (%)
T_0	65.7
T_1	78.1
T_2	87.6
T_3	84.9

4.7 Water quality parameter

Water quality parameter was determined fortnightly basis using analysis kit. Table 6 presented the range of water quality parameter during overall experiment.

Table 6: Water quality parameters

Water quality parameter	Air temperature (°C)	Water temperature (°C)	pH	DO (mg/L)	Ammonia (mg/L)	Salinity (ppt)
Value	19.9-31	26.2-34.6	7.8-8.9	5-8	0-0.6	0.4-1

CHAPTER -V
DISCUSSION

DISCUSSION

5.1 Effects of 17-alpha methyltestosterone in the growth performance of the Nile tilapia

Growth parameters were observed significantly higher in Treatment-2 in the present study. T_2 (60 mg MT hormone) have the highest growth in terms of weight when comparing with other treatments. In final sampling, it showed that the average weight of each treatment such as T_0, T_1, T_2 and T_3 were 481.567, 505, 533.5, 530.367 g, respectively. T_2 showed higher growth in terms of length when comparing with other treatments. The average length of each treatment such as T_0, T_1, T_2 and T_3 were 29.03, 29.3, 29.967, 29.67 cm, respectively at the end of the experiment. Present study found the highest length gain in T_2R_3 (29.03 cm) treatment and lowest was T_0 R_3 (27.93 cm) treatment. Length gain was calculated in the every sampling to visualize actual growth trends among the treatments.

Ferdous *et al.,* (2011) found significant influences of MT hormone on the growth performance of Nile Tilapia (*Oreochromis niloticus*). All the MT treatments showed more average body weight and weight gain than the control treatment, which make evident the findings of present study. In his experiment, T_5 (70 mg MT /kg) showed 0.27 g gain in weight, T_4 (60 mg MT/kg) 0.16 g, T_3 (50 mg MT/kg) 0.14 g, T_2 (40 mg MT/kg) 0.12 g and T_1 (control) 0.098 g after the 28 days hormone treatments. The specific growth rate found higher in MT treatments groups than the control treatment which also agree with the present study findings.

Varadaraj *et al.,* (1994) observed higher growth when fry fed 17-alpha methyltestosterone hormone. Hanson *et al.,* (1983) found use of 60 ppm MT-treatment provides higher growth than control treatments.

Present study findings also in line with Dan and Little (2000), who compared growth performance of different strains of Nile Tilapia and observed MT treatment showed larger size than mixed sex fish in a final length of the farmed fish.

Tayamen and Shelton (1978) and Jensi *et al.,* (2016) found faster growth of hormone treated *O. niloticus* in their experiment which also very similar to the findings of the present study.

Sarbajna *et al.,* (2010) stated that the hormone treated groups gain better weight (0.76±0.016g) compared to the control groups (0.38±0.028g).

Hormone treated monosex tilapia showed a significant higher mean body weight than the control mixed-sex population at the end of the study. In addition, hormone treated fish also showed higher weight gains during the culture period which is agree with present study findings.

5.2 Effects of 17-alpha methyltestosterone on fish mean sex reversing

Ferdous *et al.,* (2011) showed that hormone treated group gave a significantly higher male than the control group. Maximum male population (94.28 %) was obtained at the dose of 60 mg MT/kg while the minimum male proportion (88.57%) was found for the dose of 40 mg MT/kg at the end of the 28 days hormonal treatment. Fifty (50) and 70 mg MT/kg showed 91.43% males at the end of the hormonal treatment. Results of this study have shown that the male population was slightly higher than that of 60 mg/kg of hormone.

17α-methyltestosterone hormone application was proven tools for the production of male tilapia. Greater than 90% male populations were obtained in the different study by using different dose of 17α-methyltestosterone. Romerio *et al.,* (2000) found 98% of male population by using a dose of 60 mg MT/kg of feed. Jae-Yoon *et al.*, (1988) acquired 97% of male population of *Oreochromis niloticus* at dose rate of 10 mg MT/ kg. Vera–Cruz and Mair (1994) found 95 to 98% male populations by using 40 mg MT/kg and 99% with 60 mg MT/kg at the end of 25 days hormone treatment by diet. Smith and Phelps (2001), found 99-100 % male Nile Tilapia when given MT at 60 mg/kg of feed. All the study agree with the present study that 17α-methyltestosterone hormone treatment (especially 60 mg MT/kg) has significant role for the production of male population of Nile Tilapia.

Present study agrees with the findings of Okoko (1996). He found 97 males at the dose rates of 60 mg/kg. He further reported that higher dose rates of MT/kg of feed resulted in no increase of male percentage. Jensi *et al.,* (2016) found slight lower percentages (93.3%) of male population in case of 60 mg kg-1 17 α-MT.

According to Muir and Little (1991), high percentages of male tilapia fry production might be influenced by several factors such as treatment duration, concentration of hormone, feeding frequency, size and age and stocking density.

However, hormone treatment could be considered for the masculinization of tilapia fry. Present study found T_2 (60 mg MT/kg) as effective dose for the masculinization of Nile Tilapia in the hatchery. T_3 (80 mg MT/kg) also found effective in case of sex-reversal of the fry but it might increase the cost of the hatchery operations.

5.3 Effects of 17-alpha methyltestosterone on FCR

Experimental fish showed best FCR (1.2) in T_2 and T_3 hormonal treated feed. All hormonal treated fish showed better FCR performance than control fish (1.7).

Beaven and Muposhi (2012) observed a value of food conversion ratio (FCR) 1.686 for MT hormone treated diet fish individuals and 1.98 for those individuals which diet not treated with MT hormone. That makes clear evidence of effects of the 17-alpha methyltestosterone on FCR in case Nile Tilapia which also make evident the findings of the present study.

Ahmed *et al.*, (2002) found better FCR value in case hormone treated fish at the end of the experiment which agree with the findings of the present study. He found a range of 1.54 to 1.88 FCR value in his experiment.

5.4 Effects of 17-alpha methyltestosterone on survival rate

The highest survival rate was 87.6% in T_2 and lowest was 65.7% in T_0 in this experiment. Highest mortality was observed in the SRT tank during the experimentation.

Jensi *et al.,* (2016) observed that following 21 days of treatment with SRT tanks containing 50 mg/kg and 60 mg/kg 17-methyltestosterone hormone, the mean survival rate was 87.33% and 90.83%, respectively. With doses of 50 mg/kg and 60 mg/kg 17-methyltestosterone hormone, survival rates of 78.5% and 80.06% were found after 90 days of pond rearing.

Beaven and Muposhi (2012) found a significant high survival rate (77%) in the individuals whose was treated with MT hormone diet compared to the non-MT hormone treated diet (89.08%) over the three months study period.

5.5 Water quality parameters

Maintaining appropriate water quality is critical for culture organisms' survival and optimal growth. Tilapia growth, survival, and feed consumption are all influenced by environmental conditions (Fry, 1971 and Brett, 1979) and aquaculture almost depends on the water quality. It is often assumed that a proper range of water quality parameters ensures better aquatic organism and aquatic environment management. The temperature of the water is one of the most critical water quality parameters that affect aquatic organisms' development, food intake, reproduction, and other biological activities. In general, every 10°C rise in temperature within the range of temperature that the animal can tolerate doubles or triples the metabolic demand for oxygen in aquatic species.

Water quality is considered the foremost factor for the controlling the health and disease in case of both fish hatchery operations and culture. In this study periodic sampling for the water quality parameters has done according to the treatment pond. In the study period, range of surface temperature (°C), water temperature (°C), pH, ammonia (mg/L), dissolved oxygen ((mg/L) and salinity (ppt) was observed 19-31°C, 26.2-34.6°C, 5-8, 0-0.6 mg/L, 7.8- 8.9 mg/L and 0.4-1 ppt, respectively. All the parameter values were in the suitable ranges for the growth of the experiential fish.

Dewan *et al.,* (1991) recorded temperature range of 25.9 to 34.5°C in a fish polyculture pond which agree with the present study findings. Akter *et al.,* (2015) reported a temperature range from 25 to 30°C, pH 6.90 to 8.90, dissolved oxygen 3.70 to 5.00 mg/L in the studied ponds, and these findings are almost agree with the present study.

Aminul (1996) stated that the water temperature ranged from 25-35°C is suitable for the fish culture. Tilapia were categorized as thermophilic fish and so fishes was not badly affected during the experimentation. During the study, the water temperature in experimental ponds fluctuated from 26.2 to 34.6 degrees Celsius, depending on the treatment which was more or less similar to Asaduzzaman *et al.,* (2005), Kunda *et al.,* (2008), Rahman (2005), FAO (1988) and DoF (2009).

The pH of a water body is used as a productivity index and is an important aspect in fish rearing. Fishes' growth rate, physiological activities and metabolic rate are all affected by an acidic pH (Swingle, 1967). In this study, pH ranged from 5-8 during the experimentation period. The ideal value of pH was observed 7-9 (Boyd, 1998). The pH value found from this study agreed with the findings of Ahmed (2004), Ali *et al.,* (2004), Fatema (2004), Asaduzzaman (2005) and Asaduzzaman *et al.,* (2006). A value of pH 7.0 to 9.0 and 8.0 to 9.5 were found by Kunda *et al.,* (2008) and Alam *et al.,* (1997), respectively, which differed somewhat from the findings of the current investigation.

The most significant and vital of the gases found in dissolved state in natural waters is oxygen. Because oxygen (O_2) has a direct influence on feed intake, disease resistance, and metabolism, maintaining optimum DO levels in water is critical for effective production. For fish, a sub-optimal level is extremely stressful. Except for anaerobic bacteria, all aquatic species require a constant supply of dissolved oxygen. As a result, it's essential to keep dissolved oxygen levels at 3.5 ppm all of the time.

In this study, dissolved oxygen ranges from 7.8- 8.9 mg/L in the four treatments during culture period. The range of dissolved oxygen acceptable for fish culture, according to DoF (1996), is 5.0 to 8.0 mg/L. The value of dissolved oxygen in this study agree with the findings of Alam *et al.,* (1997), Ali *et al.,* (2004) and Asaduzzaman (2005) who found that dissolved oxygen levels ranged from 4.0 to 7.0, 4.3 to 6.9, and 1.2 to 7.2 mg/L, which was similar to the current study. Boyd (1998) found the ideal concentration of Dissolved oxygen 5-15 mg/L which make the present study findings more fruitful.

Ammonia is a critical characteristic for successful fish production. Ammonia in water can be found in two forms: non-toxic ammonium ions ($NH4^{+}$) and harmful un-ionized ammonia (NH_3). The ideal ammonia range for fish farming was 0.1 mg/L (Boyd, 1998). Because of natural fish metabolism and microbiological breakdown of organic waste, ammonia is present in fish pond water. Many types of fish and shrimp can be poisoned by as little as 0.6 mg/L free ammonia (NH3), producing gill irritation and respiratory difficulties. The toxic ammonia form is more concentrated in water with a higher temperature and pH. A range of the value of the ammonia 0-0.6 mg/L was records in this study. The current study's conclusions were similar to those of Boyd (1998), DoF (2009).

CHAPTER-VI
CONCLUSION

CONCLUSION

The study conclude that 17 α-methyltestosterone had a positive and significant impacts on the growth performances, survival rate and Food Coversion Ratio (FCR) of Nile tilapia (*Oreochromis niloticus*). The study showed that 60 mg/kg 17 α-methyltestosterone hormone dose by diet might be useful to production of monosex populations of Nile tilapia (*Oreochromis niloticus*). It also showed that higher dose than the 60 mg/kg moreless similar effects on the growth performance and sex ratio but it might increase the cost. The findings of this study will help to increase Nile tilapia production from the culture pond and will minimize the cost for the production of the monosex populations in hatchery.

CHAPTER-VII

RECOMMENDATIONS AND FUTURE PERSPECTIVES

RECOMMENDATIONS AND FUTURE PERSPECTIVES

The study recommended the following points to raise the production of Nile Tilapia in Bangladesh.

- A dose of 60 mg/Kg 17 α methyltestosterone hormone could be used to produce monosex populations in the hatchery.
- Doses higher than 60 mg had produced similar sex ration so it could be avoid to decrease the cost of the operations in hatchery.
- Mixed population should avoid in case tilapia culture in fish pond.
- Water quality should be in optimum conditions like the present study, to produce better production of both fry in hatchery and fish in the pond in case of Nile Tilapia.
- Monosex Nile Tilapia population could rapidly be cultured to raise the fish production in Bangladesh.

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APPENDICES

APPENDICES

Appendix-1: Average weight data of the fishes at different sampling (g)

Treatment	1st Sampling	2nd Sampling	3rd Sampling	4th Sampling	5th Sampling	6th Sampling	7th Sampling	8th Sampling	9th Sampling	10th sampling	11th sampling	12th sampling
	Weight data of the fishes, g											
	15	30	45	60	75	90	105	120	135	150	165	180
$T_0 R_1$	1.5	6.8	16.2	38.4	63.2	102.4	128.1	195.2	219.5	297.6	388.9	482.9
$T_0 R_2$	1.4	6	16.5	38.1	62.8	101.9	127.5	194.8	218.1	298	387.3	481.2
$T_0 R_3$	1.7	6.5	15.9	`37.6	61.6	102	126.5	194.2	219.9	296.1	388.1	480.6
$T_1 R_1$	1.8	7.7	18.6	43.2	69.8	116.6	148.2	216.6	258.2	332.6	429.5	506.2
$T_1 R_2$	1.6	7.5	18	42.6	70.5	117	147.1	217.2	259	331.1	429.6	503.2
$T_1 R_3$	1.9	7.9	19.2	43.5	68.6	116.5	149.6	216.7	257.6	332.8	430.1	505.6
$T_2 R_1$	2	10.8	23.8	48.2	78.5	128.9	161.3	225.9	273.1	351.1	456.7	532.5
$T_2 R_2$	2.3	11	24.6	49.9	79	129.6	162.6	224.6	272.6	352.1	457	534.9
$T_2 R_3$	2	9.8	25.4	48.1	80.6	127.6	160.6	226.5	274	356	456.2	533.1
$T_3 R_1$	2.4	9.9	23	48.9	78.1	128.3	166	225.5	272.3	350	455.2	530
$T_3 R_2$	2	10.2	22.9	48.6	77	127.5	165.2	224	274.6	350.2	456	531.6
$T_3 R_3$	2.5	9.7	23.4	47.8	78	127	167.5	226	270.3	350.6	454	529.5

Appendix-2: Average length data of the fishes at different sampling of platy fish (cm)

Treatment	1st Sampling	2nd Sampling	3rd Sampling	4th Sampling	5th Sampling	6th Sampling	7th Sampling	8th Sampling	9th Sampling	10th sampling	11th sampling	12th sampling
	Length data of the fishes, cm											
$T_0 R_1$	4.5	5.9	9.2	13.1	16.2	17.8	18	21.8	23	26	27.1	29
$T_0 R_2$	4.4	5.7	9.2	13	16.1	17.7	18.1	21.5	23	26.2	27	29.1
$T_0 R_3$	4.5	5.6	9.1	13	16	17.8	17.8	21.4	23.1	25.7	27.2	29
$T_1 R_1$	4.7	6.1	10	14	16.9	18.1	19.1	22.8	24.4	26.3	27.9	29.4
$T_1 R_2$	4.6	6.1	10.1	13.8	17	18.2	19	22.8	24.5	26	27.9	29.2
$T_1 R_3$	4.8	6.2	10.5	14.3	16.5	18	19.3	22.7	24.3	25.9	28	29.3
$T_2 R_1$	5.1	8.9	11.1	14.6	17.5	18.4	20	23.1	25.2	26.8	28.2	29.8
$T_2 R_2$	5.3	8.9	11.2	15	17.5	18.5	20.2	23.1	25	26.8	28.1	30
$T_2 R_3$	5	8.7	11	14.8	17.8	18.2	20.1	23.2	25.1	27	28.3	30.1
$T_3 R_1$	5.1	8.6	11	14.3	17.4	18..4	20.2	23	25.2	26.8	28.1	29.7
$T_3 R_2$	5	9	10.8	14.4	17.3	18.2	20	23.1	25.3	26.8	28.2	29.7
$T_3 R_3$	5.4	8.8	11	14.1	17.2	18	20.1	23.1	25	26.5	28	29.6

Appendix-3: Average weight of fish (g)

Treatment	1st Sampling	2nd Sampling	3rd Sampling	4th Sampling	5th Sampling	6th Sampling	7th Sampling	8th Sampling	9th Sampling	10th sampling	11th sampling	12th sampling
	Weight data of the fishes, g											
T_0	1.53333	6.43333	16.2	38.25	72.5154	102.1	127.367	194.733	219.167	297.233	388.1	481.567
T_1	1.76667	7.7	18.6	43.1	69.6333	116.7	148.3	216.833	258.267	332.167	429.7333	505
T_2	2.1	10.5333	24.6	48.7333	79.3667	128.7	161.5	225.667	273.233	353.067	456.6333	533.5
T_3	2.3	9.93333	23.1	48.4333	77.7	127.6	166.233	225.167	272.4	350.267	455.0667	530.3667

Appendix-4: Average length of fish (cm)

Treatment	1st Sampling	2nd Sampling	3rd Sampling	4th Sampling	5th Sampling	6th Sampling	7th Sampling	8th Sampling	9th Sampling	10th sampling	11th sampling	12th sampling
	Length data of the fishes, cm											
T_0	4.46667	5.73333	9.16667	13.0333	16.1	17.7667	17.9667	21.5667	23.0333	25.9667	27.1	29.03333333
T_1	4.7	6.13333	10.2	14.0333	16.8	18.1	19.1333	22.7667	24.4	26.0667	27.93333333	29.3
T_2	5.13333	8.83333	11.1	14.8	17.6	18.3667	20.1	23.1333	25.1	26.8667	28.2	29.96666667
T_3	5.16667	8.8	10.9333	14.2667	17.3	18.1	20.1	23.0667	25.1667	26.7	28.1	29.66666667

Appendix-5: Weight (g) and length (cm) gain, SGR of fish

Treatment	Weight Gain, g	Length Gain, cm	SGR
T_0 R_1	482.65	27.93	4.203391
T_0 R_2	480.95	28.03	4.201432
T_0 R_3	480.35	27.93	4.200739
T_1 R_1	505.95	28.33	4.22957
T_1 R_2	502.95	28.13	4.226268
T_1 R_3	505.35	28.23	4.228911
T_2 R_1	532.25	28.73	4.25771
T_2 R_2	534.65	28.93	4.260208
T_2 R_3	532.85	29.03	4.258335
T_3 R_1	529.75	28.63	4.255095
T_3 R_2	531.35	28.63	4.25677
T_3 R_3	529.25	28.53	4.254571

Appendix-6: Value of water quality parameter

Water quality parameter	SRT-1 (april)	SRT-1 (april)	Nurseru-1(15 days)	nursery 2(30 days)	During 45 days	During 60 days	During 75 days	During 90 days	During 105 days	During 120 days	During 135 days	During 150 days	During 165 days	During 180 days
Temperture (air)	31	31.2	29.5	19.9	29	32.1	33.6	31.7	33.5	32.8	28.8	27.6	27.1	26.8
Temperture (water)	31.6	32.1	30.5	31.1	31.2	33.2	34.6	32.2	34.2	33.1	29.2	27.8	27.9	26.2
pH	8.7	8.6	8.4	8.7	8.8	8.5	7.8	8.9	7.9	8	8.3	7.8	8.8	8.5
DO	6.5	6.8	6	5.8	5.5	8	6.5	6.8	6.3	5	8	7.5	6.5	6.4
Ammonia	0	0	0.1	0.3	0.5	0.1	0.4	0.2	0.4	1	0.6	0.1	0.2	0.5
Salinity	0.5	0.8	1	0.5	0.9	0.8	1.5	1	0.8	0.4	0.5	0.9	0.5	0.7

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Research article

Effect of hormonal masculinization on growth performance of tilapia (*Oreochromis niloticus*)

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A B S T R A C T

The present study was conducted in the Meridian hatchery, Sonagazi, Feni, to evaluate the effects of 17α-methyltestosterone hormone on the growth performance of Nile Tilapia *Oreochromis niloticus*). Brood fishes were collected from NAMSAI farm, Thailand and BFRI, Bangladesh and then stocked in the breeding hapa. A total of 1600 brood fishes were used in 10 hapas where stocking density was maintained at 5 fishes/ sq. m and sex ratio was maintained at 3:1. Eggs were collected from the fish mouth, disinfected and finally placed on the hatching trays and jars. The hatched larvae were placed in the 12 SRT tank and different doses of 17α-methyltestosterone hormone applied. The experimental treatments were T_0 (Control, without MT), T_1 (50 mg MT/kg), T_2 (60 mg MT/kg), T_3 (80 mg MT/Kg) and each treatment had three replications. The study showed significantly higher growth performance in the T_2 treatment. The results showed that the mean weight of fish was significantly higher under T_2 (533.5±1.249 g), mean length was under T_2 (29.97±0.153 cm) and SGR was under T_2 (4.259±0.0013) in comparison with the other three treatments. The highest survival rate 87.6% was obtained in T_2. The highest value of FCR (1.8) was observed in T_1 and the lowest (1.2) was in both T_2, T_3, respectively. The highest sex reverse percentage was found in T_2 (98%) followed by T_3 (97.883%) and T_1 (77.833%). There was a strong interaction between 17α-methyltestosterone hormone and growth performance and sex reversal of Nile Tilapia ($p < 0.05$, n=4). This study suggests that the use of 17α-methyltestosterone hormone can improve the growth performance of Nile Tilapia.

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1. INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is a native fish species of Egypt that has become popular worldwide mainly as a valuable fish, easy to breed, and grows in various aquaculture systems (El-Sayed, 2006). In Bangladesh, UNICEF introduced Nile Tilapia (*Oreochromis niloticus*) in 1974, but this was not flourishing due to a lack of fruitful research. Then it was further introduced in Bangladesh at 1987 by BFRI from Thailand (Gupta et al., 1992). Since then, development agencies and scientists have developed technologies for better cultural techniques at the farm level. Tilapia is likely to be the most important of all aquaculture fish in the 21st century.

Tilapia has specific favorable characteristics, like tolerance to adverse environmental conditions, can survive at low dissolved O_2, euryhaline, relatively fast growth, and efficient food conversion. These characteristics make tilapia one of the best choices for farmers (Yi et al., 1996; Penna-Mendoza et al., 2005). Despite having many good characteristics, one of the main impediments in tilapia production at a commercial scale is its precocious reproduction. It attains sexual maturity early and reproduces after every 4-6 weeks in the pond. The mono-sex culture technique can be used to control this unwanted reproduction of tilapia by culturing all-male tilapia in the pond. Tilapia has sexual growth dimorphism in which males grow faster and have more standard size than females (Muir and Little, 1991).

There are four strategies for mono-sex male culture i.e., the manual process by visual examination; hybridization; gene manipulation; and masculinization via steroid hormone. At the time of hatching, tilapia fry is sexually undeveloped. Hence, during the early period of gonad differentiation, changes in sex hormone level can affect the final sex independently of the genetic sex (Andersen et al., 2003). One of the most common techniques for producing mono-sex populations is steroid-induced sex inversion. This involves the administration of synthetic androgens or estrogens for differentiating the fry. The production of mono-sex tilapia with 17α-methyltestosterone (MT) is well established and could be incorporated in starter feed with different doses of MT hormone (Popma and Green, 1990). The present study is aimed to evaluate the effects of different doses of 17α-methyltestosterone hormone in masculinization and growth performance of Nile Tilapia.

2. MATERIALS AND METHODS

Study area

The experiment was conducted on Nile Tilapia (*Oreochromis niloticus*) in the Meridian hatchery which was located at Sonagazi upazila in Feni district for 12 months. The study area's coordinates were obtained using 'Google Maps' software, and a map was generated by 'Arc-GIS' software (Figure 1).

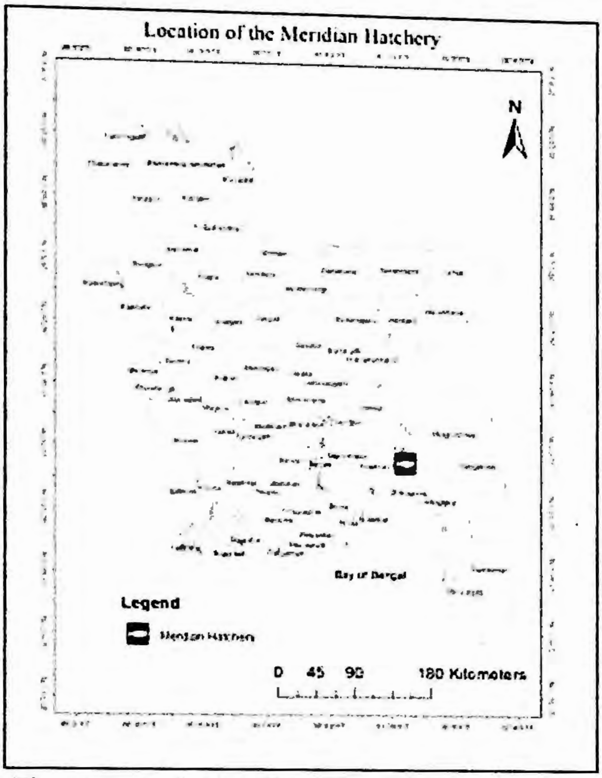

Figure 1. Study area (Meridian Hatchery)

Preparation of the brood hapa and collection, stocking, and feeding of the brood fishes

Brood fishes were collected from Bangladesh Fisheries Research Institute (BFRI), Mymensigh and NAMSAI farm, Thailand. A total of 10 hapas of 32 sq. m. (8 m x 4 m) was used for the breeding purpose and water height was maintained 1 meter for each hapa. A total of 1600 brood fishes were used in the 10 hapas and stocking density was maintained at 5 fishes/ sq. m and sex ratio was maintained 3:1 (120 females:40 male). Feeding was done in relation to the 1% per day of the bodyweight of the brood fishes and feeding frequency was twice/day. The protein content of the feed was maintained at 30% in the brood fish feed.

Egg collection, disinfection and incubation

After 3 weeks of stocking, eggs were collected from the mouths of the brood fishes and classified into various categories. The eggs were collected and washed in clean water to remove any foreign materials. The eggs were formalin-fixed (2 ml formalin/liter solution) for 2-3 minutes before being washed with clean water. Finally, the eggs were disinfected by keeping them in a 0.9% NaCl solution. The eggs were incubated using both tray and jar methods. After the disinfection, eggs were moved to the tray and jar, and sufficient water flow was maintained for hatching. During the incubation period (5-7 days) continuous aeration was ensured for proper hatching.

Transfer of swim up fry to SRT (Sex Reversed Tilapia) hapa

Swim-up fry were moved to SRT hapa for MT feed treatment after hatching. A total of 12 hapas were used, each with an area of 18 sq. m. (6 m x 3 m). Nine of the 12 hapas were used for hormonal treatment, while three were used to apply a control treatment (normal feeding). All of the hapas were placed in the 40 decimal ponds. Swim-up fry stocking density was 3 thousand per hapa. The average weight of the swim-up fry was 0.0117 g.

Preparation of MT hormone treated feed

A stock solution was prepared by using 5 gm 17α-methyltestosterone with 10 litre 95% ethyl alcohol. The solution was preserved at 4°C temperature in the refrigerator. Then, to prepared MT mixed feed for experimentation, 50 mg, 60 mg and 80 mg of MT and 120 ml solution and 120 ml additional alcohol (for rinsing and better distribution) were mixed with 1 kg feed respectively. The mixture of the feed had been completely dried at room temperature or sunlight of the early morning and then sealed in air-tight black container and stored in refrigerators until use to retard bacterial or fungal contamination.

Sex-reversed protocol and treatments of the experiment

To measure sex reversal percentages and the impact of MT on growth efficiency, three separate doses of MT hormone were used as treatments (T_1 = 50 mg MT/kg hormone, T_2 = 60 mg MT/kg hormone, T_3 = 80 mg MT/kg hormone). T_0 was the control treatment (without MT hormone). Nine (9) of the 12 SRT hapas were used for hormone treatment (Three for each treatment). Twenty-eight (28) days of hormonal treatment (MT feed feeding protocol) was maintained for the sex reversal in the sex reversal tank.

Fry grading and transfer to nursery and grow out pond

The fry were graded using a plastic net (mesh size: 4 mm). The average weight of the fry was 0.25 g/fry. The graded 2800 fry were then moved to each nursery hapa. Each hapa was 40.5 sq. m (9 m x 4.5 m) and water height was maintained at 1.5-2 m for each hapa. A total 12 hapas were used for the experiment; 9 for hormone-treated fry and 3 for control feed treated fry. Feed was given according to the 20% body wt. and 32% protein ratio was maintained during feed formulation. After a month of rearing, the fry were moved to the grow-out pond. Water quality was periodically monitored during the whole experimentation period.

Sampling and evaluation of the growth performance and survival rate

Sampling was done every 15 days interval in the nursery and grow-out pond. Weight and length were measured and data were recorded. To evaluate the growth performance of tilapia under different hormone treatments following growth parameters were calculated based on the collected data.

Mean weight (g)

$$= \frac{\text{Sum of final weight value of each treatment}}{\text{No of replications of each treatment}}$$

Mean length (cm)

$$= \frac{\text{Sum of final length value of each treatment}}{\text{No of replications of each treatment}}$$

Weight gain (g) = Final body weight – Initial body weight

Length gain (cm) = Final length of the fish body – Initial length of the fish body

$$\text{Survival rate (\%)} = \frac{\text{Final no. of live fishes}}{\text{Initial no. of fishes}} \times 100$$

Specific Growth Rate (SGR)

$$= \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{duration in days}} * 100$$

$$\text{Condition Factor (CF)} = \frac{\text{Weight of fish}}{(\text{Length of fish})^3} * 100$$

Specifying of sex rates

To specify sex rates, aceto-cramine solution was used. 1 gm carmine powder was mixed with 100 ml glacial acetic acid (45%) to prepare 1% aceto-cramine solution. After dissection of the fish, gonads were taken carefully in the glass slides and 1 drop of aceto-cramine solution was added. Then the slide was placed under the microscope and gonad was observed. Web-like structured gonad was identified as female fish, and finely grainy gonad as male fish. Determination of the sex was carried out in each sampling.

Statistical Analysis

The one-way analysis of variance (One way ANOVA) was performed using SPSS (Statistic Package for social science) version IBM SPSS Statistics 23 software to determine the significant differences among means. For all tests, a criterion of $P<0.05$ was used to determine statistical significance.

3. RESULTS

Growth performance and sex reversal of Nile Tilapia

Growth parameters were significantly higher in the T_2 treatment that was shown in Table 1. Among hormonal masculinization treatments, significant differences ($p<0.05$) were observed for final weight, weight gain, final length, SGR, and CF. The mean weight of each treatment such as T_0, T_1, T_2 and T_3 were 481.57±1.19, 505.0±1.58, 533.5±1.24 and 530.36±1.09 g (Figure 2) and mean weight gain were 481.31±1.19, 504.75±1.58, 533.25±1.24 and 530.11±1.09 g (Figure 3), respectively. The mean length of each treatment such as T_0, T_1, T_2 and T_3 were 29.03±0.05, 29.3±0.15, 29.97±0.15 and 29.67±0.05 cm (Figure 4) respectively. The study found the highest mean SGR (4.25±0.00) and CF (2.03±0.00) in the T_2 treatment (Figure 5 and 6).

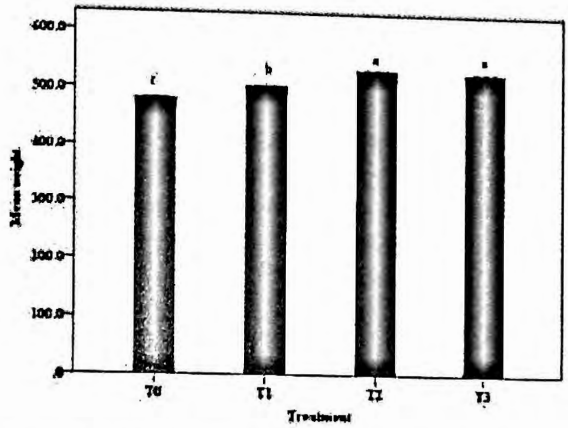

Figure 2. Effects of hormonal masculinization on fish weight

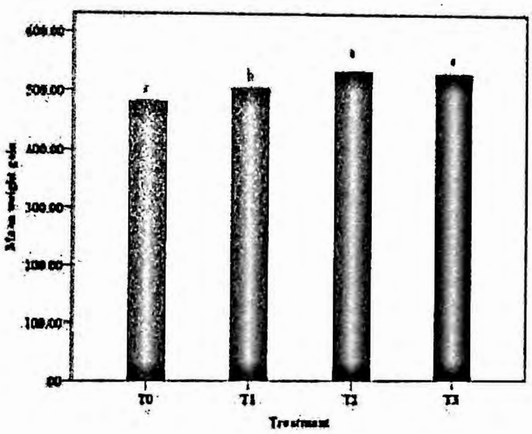

Figure 3. Effects of hormonal masculinization on fish weight gain

Table 1. Growth performance of tilapia

	T_0	T_1	T_2	T_3	Level of Significance
Mean weight (g)	481.57±1.19^c (478.61-484.53)	505.0±1.58^b (501.06-508.94)	533.5±1.24^a (530.39-536.61)	530.37±1.09^a (527.64-533.09)	0.000
Mean weight gain (g)	481.31±1.19^c (478.35-484.28)	504.75±1.58^b (500.81-508.69)	533.25±1.24^a (530.15-536.35)	530.11±1.09^a (527.39-532.84)	0.000
Mean Length (cm)	29.03±0.05^d (28.89-29.18)	29.3±0.15^c (29.05-29.55)	29.97±0.15^a (29.59-30.346)	29.67±0.05^b (29.52-29.81)	0.000
SGR	4.20±0.00^c (4.19 -4.20)	4.22±0.00^b (4.22-4.23)	4.25±0.00^a (4.26-4.26)	4.25±0.00^a (4.25-4.25)	0.000
CF	1.96±0.13^b (1.93-2.00)	2.00±0.01^{ab} (1.97-2.04)	1.98±0.027^b (1.92-2.05)	2.03±0.00^a (2.00-2.05)	0.012

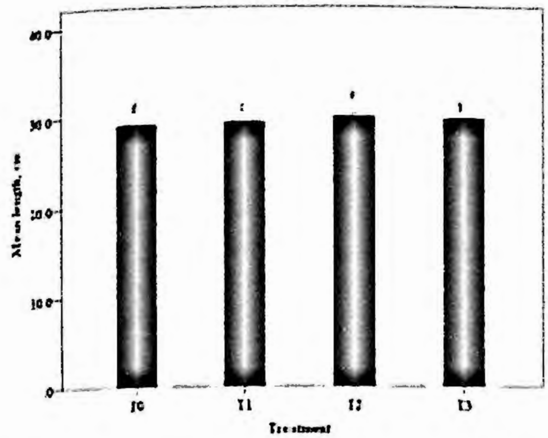

Figure 4. Effects of hormonal masculinization on fish length

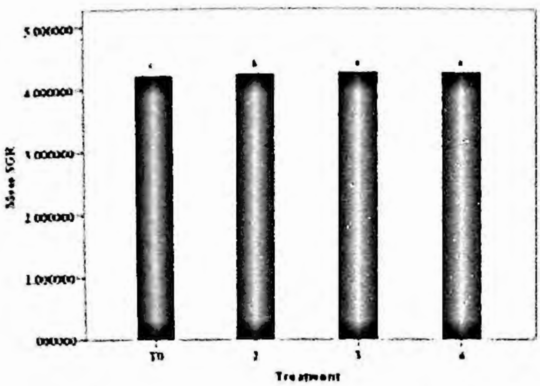

Figure 5. Specific Growth rate of fish at different treatment

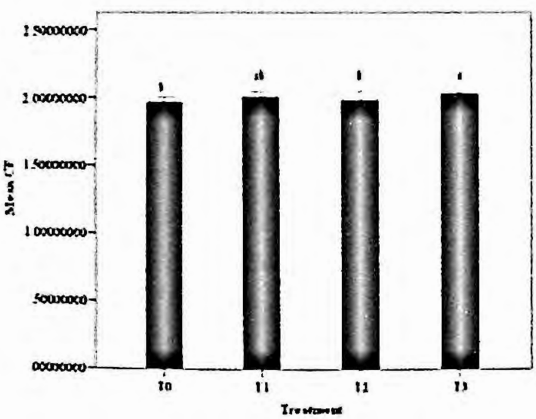

Figure 6. Condition factor of fish at different treatment

Effects of hormonal masculinization on sex reversal

The sex reversing percentages of T_1, T_2 and T_3 were 77.83±0.76, 98.0±0.5 and 97.83±0.76, respectively (Figure 7). T_2 showed the highest sex reverse percentage among all the treatments.

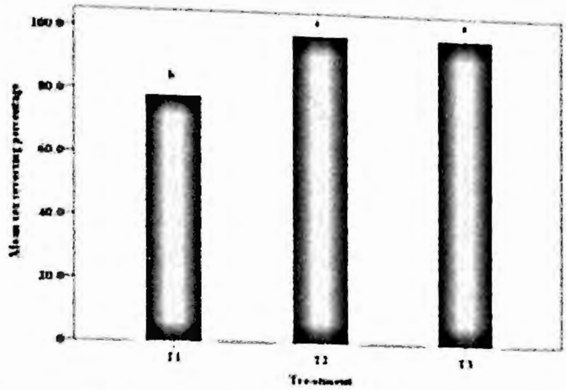

Figure 7. Percentage of fish sex reversing at different treatment

Effects of hormonal masculinization on FCR

Experimental fish showed the best FCR (1.2) in the T_2 and T_3 hormone-treated feed. All hormonally treated fish showed better FCR performance than control fish (1.7) (Figure 8).

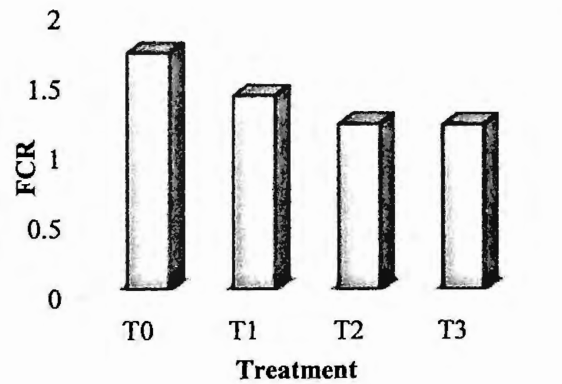

Figure 8. FCR value

Effects of hormonal masculinization on survival rate

Effects of hormonal masculinization on the survival rate of tilapia are recorded in Table 2. The highest survival rate was 87.6% in T_2 and the lowest was 65.7% in T_0 in the experiment.

Table 2. Effects of hormonal masculinization on survival rate

Treatment	Survival Rate (%)
T_0	65.7
T_1	78.1
T_2	87.6
T_3	84.9

4. DISCUSSION

Effects of hormonal masculinization in the growth performance

Growth parameters were observed significantly higher in the Treatment-2 (60 mg MT hormone/kg) of the present study (Table 01). Hanson et al. (1983) found that the use of 60 ppm MT-treatment provides higher growth of Nile Tilapia than control treatments which agreed with the present study. Hormone-treated group showed higher growth than the control group which agreed with Varadaraj et al. (1994), Tayamen and Shelton (1978), Jensi et al. (2016) and Sarbajna et al. (2010); who observed higher growth when fry fed 17-alphamethyl testosterone hormone.

Effects of hormonal masculinization on sex reversal

The highest mean percentage of sex reversal was observed in the T_2 (60 mg MT hormone/kg) in the present study (Figure 7). Ferdous et al. (2011) found that hormone-treated group gave a significantly higher male population of Nile Tilapia than the control group. Vera–Cruz and Mair (1994), Smith and Phelps (2001) and Okoko (1996) also found higher sex reversal percentage using 60 mg MT/kg dose of hormone. Hence, hormone treatment could be considered for the masculinization of Tilapia fry. The present study found T_2 (60 mg MT/kg) as an effective dose for the masculinization of Nile Tilapia in the hatchery. T_3 (80 mg MT/kg) was also found effective in case of sex-reversal of the fry but it might increase the cost of the hatchery operations.

Effects of hormonal masculinization on FCR

Experimental fish showed the best FCR (1.2) in T_2 and T_3 hormonal treated feed (Figure 8). All hormone-treated fish showed better FCR performance than control fish (1.7). Beaven and Muposhi (2012) observed a value of food conversion ratio (FCR) of 1.6 for MT hormone-treated diet fish individuals and 1.98 for those individuals not treated with MT hormone. That provides clear evidence of the effects of 17-alpha methyltestosterone on FCR in the case of Nile Tilapia which also makes the findings of the present study evident. Ahmad et al. (2002) found a better FCR value in the case of hormone-treated fish at the end of the experiment which too agrees with the findings of the present study.

Effects of hormonal masculinization on survival rate

The highest survival rate was 87.6% in T_2 and the lowest was 65.7% in T_2 (Table 2). The highest mortality was observed in the SRT tank during the experimentation. Beaven and Muposhi (2012) found a significantly high survival rate (89.08%) in the individuals that were treated with MT hormone diet compared to the non-MT hormone-treated diet (77%) over a three-month study period. Jensi et al. (2016) found the highest survival rate (80.06%) in the 60 mg/kg hormone-treated fish tank which is substantiated by the present study.

5. CONCLUSION

The study concludes that 17αmethyltestosterone has a positive and significant impact on the growth performances, survival rate, sex reversal, and Food Conversion Ratio (FCR) of Nile tilapia (*Oreochromis niloticus*). The study has evidenced that 60 mg MT/kg 17α-methyl testosterone hormone dose through diet might be useful in the production of mono-sex populations of Nile tilapia (*Oreochromis niloticus*). It has also been observed that a higher dose than 60 mg/kg has more or less similar effects on the growth performance and sex ratio of Nile tilapia but that might increase the production cost. The findings of this study will help to increase Nile tilapia production from the culture pond and will minimize the cost for the production of mono-sex Nile tilapia populations in the hatchery.

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