**MASCULINIZATION OF NILE TILAPIA (*Oreochromis niloticus* L*.*) USING LYOPHILIZED TESTES FROM CARABAO (*Bubalus bubalis carabanesis* L*.*), BULL (*Bos indicus* L.) AND**

**BOAR (*Sus domesticus* L.)**

**Ramjie Y. Odin1 and Remedios B. Bolivar2**

*1College of Fisheries, Mindanao State University – Maguindanao, Datu Odin Sinsuat, Maguindanao, Philippines*

*2Freshwater Aquaculture Center, College of Fisheries, Central Luzon State University, Muñoz, Nueva Ecija, Philippines*

***Abstract***

The study was conducted to evaluate the use of lyophilized testes from carabao (*B. b. carabanesis*), bull (*B. indicus*) and boar (*S. domesticus*) in the masculinization of Nile tilapia (*O. niloticus*) fry, specifically, their efficacy in producing phenotypic males and their influence on the growth and survival rate of Nile tilapia fry on a 28-day treatment period in outdoor tanks.

The experimental treatments evaluated were: Treatment I- lyophilized testes from carabao, Treatment II- lyophilized testes from bull, Treatment III- lyophilized testes from boar, Control I- methyltestosterone (MT)- treated diet and Control II- untreated diet. Percent phenotypic males, specific growth rate and survival rate were determined after 28 days of treatment in outdoor tanks.

Results revealed that Nile tilapia fry fed with MT-treated diet gave the highest percent phenotypic males with a mean of 96.67%. Those fry fed with lyophilized testes from bull, boar and carabao gave means 80.67, 79.33 and 72.67%, respectively. There was a significant difference (P<0.05) among the treatments. Based on the Chi-square test (α ≤ 0.05), the higher percentages of males produced from androgen-treated fry which are significantly different from that of untreated fry showed that lyophilized testes diets and MT-treated diet were effective in masculinizing Nile tilapia fry.

Lyophilized testes from bull, carabao and boar gave higher specific growth rate of tilapia fry with means 15.85, 15.29 and 14.82%, respectively. Tilapia fry fed with lyophilized testes from carabao and boar did not differ significantly (P>0.05) from MT-treated fry but differed significantly (P<0.05) from those untreated fry. Those fry fed with lyophilized testes from bull were found to be significantly different (P<0.05) from the two controls. All the experimental treatments gave relatively high survival rate of the tilapia fry with no significant differences (P>0.05).

**INTRODUCTION**

Tilapia (*Oreochromis niloticus* L*.*) has been regarded as one of the major produced and consumed aquaculture commodities in Asia. The tilapia world production has grown rapidly at 2,515,908 metric tons in 2007 (Fitzsimmons, 2008). One of the developed management aspects considered to contribute to this growth is the production technology of monosex fingerlings through sex reversal. The production of all-male tilapia through hormone manipulation became a common methodology in the aquaculture of tilapia. Male tilapia is preferred over the female one because of its fast growth. Oral administration of sex hormones is employed to control the sexual development of this species and produce monosex fish. Various natural and synthetic hormones have been used to sex-reverse tilapia fry. At present, successful production of masculinized tilapia is done through oral administration of synthetic androgen hormone-treated feed at 30-60mg/kg of diet for about three to four-week period (Shelton et al., 1978; Guerrero and Guerrero, 1988; Jo et al., 1988; Vera Cruz and Mair, 1994). The dosage of hormones incorporated in diets for sex reversal of tilapia varies widely from 10-70 mg of hormone/kg of diet (Abucay and Mair, 1997; Mateen and Ahmed, 2007). The use of 17α-methyltestosterone is by far the most common practice for many aquaculturists since it has been proven both effective and relatively inexpensive means of masculinizing fry of at least 95% for various tilapia species (reviewed by Macintosh and Little, 1995; Green et al., 1997; Phelps and Popma, 2000; and El-Sayed, 2006). However, some concerns have been raised on the consumption of steroid-treated tilapia in the advent of this culture practice. The use of synthetic hormones has been under increasing public criticism due to their possible health and environmental impacts. As a result, the use of methyltestosterone for sex reversal of food fish is either licensed by the U.S. Food and Drug Administration or prohibited in Europe (Penman and McAndrew, 2000). Potential disadvantage of synthetic hormone treatment is the increased risks of long term exposure of workers handling MT during food preparation and feeding which may cause adverse effects on their health (Green et al., 1997). There have been reports that hormones in the form of either active metabolites excreted by the treated fish or leachates from uneaten food can build up in a closed water system (Abucay and Mair, 1997). Hence, the waste water from the culture system with MT treatment for sex reversal can have unknown effects on the untargeted elements of the pond ecosystem.

The rapidly increasing demand for organic food in the world market has become a consideration in the aquaculture of tilapia. The demand for organic fish is rapidly increasing, while the supply is very inadequate (Aquaculture Production Technology Ltd., 2006). Most consumers want tilapia to be organically produced and with reduced or eliminated use of synthetic hormones. The idea is no antibiotics and chemicals, reduced environmental repercussions and recycled water and waste products. In Israel, organic aquaculture started at kibbutz Geva fish farm in 2000 with blue tilapia (*O. aureus*) as main species of the polyculture (Milstein and Lev, 2004). Similarly, Premier Organic Farms Group, Inc. in the US is now able to produce a superior farm-raised organic tilapia to supply the ever expanding organic market.

Among the alternatives which can be considered to mitigate the problem on using synthetic steroid for sex reversal of tilapia is the use of testes from animals which can be a potential substitute to synthetic MT. The testes from farmed animals like carabao, bull and boar which are readily available from any local market and abattoir in the country can be a good source of natural testosterone. Haylor and Pascual (1991) reported successful tilapia sex reversal using ram’s testes. Phelps et al. (1996) also obtained a 65% male population using bull testes. Meyer et al. (2008) reported successful use of bull and hog testes in sex reversal of Nile tilapia fry. White (2008) also obtained high percent male of tilapia fry after sex reversal treatment using frozen bull testes. The animal testes coming from carabao, bull and boar can be potential sources of dietary testosterone. There are only few studies conducted evaluating the use of animal testes in masculinizing tilapia fry. Hence, these natural sources of testosterone can therefore be investigated for sex reversal of Nile tilapia fry.

This study was conducted at the Freshwater Aquaculture Center, Central Luzon State University, Science City Muñoz, Nueva Ecija, Philippines. The Nile tilapia fry in this study were treated with lyophilized testes for 28 days in outdoor tanks.

The general objective of this study was to evaluate the use of lyophilized testes from carabao (*B. b. carabanesis*), bull (*B. indicus*) and boar (*S. domesticus*) in the masculinization of Nile tilapia (*O. niloticus*) fry. Specifically, the study determined the efficacy of lyophilized testes from carabao, bull and boar in producing phenotypic males of *O. niloticus* fry and their influence on the growth and survival rate of *O. niloticus* fry. A simple cost and return analysis was also considered in this study.

**MATERIALS AND METHODS**

Fifteen net enclosures (1 x 1 x 1 m) with 1.6 mm mesh size were set in 15 outdoor tanks (3 m3) following the Complete Randomized Design (CRD) for three treatments and two controls. The experimental treatments evaluated were: Treatment I- lyophilized testes from carabao, Treatment II- lyophilized testes from bull, Treatment III- lyophilized testes from boar, Control I- methyltestosterone (MT)- treated diet and Control II- untreated diet. These were replicated three times.

Each net enclosure was stocked with 500 fry. The net enclosures were extended at least 20 cm above the water surface to prevent the fry from escaping and were moored into the tank’s bottom. The experimental units were provided with continuous flow of water, regular cleaning and water exchange. The net enclosures were washed and cleaned once a week during sampling.

A total of 7,500 tilapia fry (0.008 to 0.009 g) from the artificial incubation units of the GIFT Foundation were used in this study. These fry were of the same cohort and were taken from Generation 11 of the selected GIFT strain.

The testes from carabao (*B. b. carabanesis*), bull (*B. indicus*) and boar (*S. domesticus*) were collected at Hiyas Agro-Commodity Center in Guiguinto, Bulacan and at the Balagtas Municipal Abattoir in Balagtas, Bulacan. The age, carcass body weight and size of the testes from each animal were recorded.

The fresh testes were skinned and freed from epididymides, weighed, sliced and completely homogenized without dilution using a countertop blender. The homogenized testes were then lyophilized at the Chemistry Laboratory of De La Salle University, Philippines after freezing for a minimum of 24 hours. The testes were completely lyophilized within 72 hours using a cascade-type freeze dryer equipment. The freeze dryer can accommodate up to 6 kg of raw testes per run. Lyophilization of frozen and homogenized testes was done by placing them in a vacuum with -40oC temperature to remove moisture from below zero frozen state before returning it to ambient room temperature of approximately 20oC. The low processing temperature and absence of liquid water help to maintain the color, flavor and texture of the testes samples. After lyophilization, 20-25% of the weight of the raw animal testes was recovered. The resultant crumbs were pulverized and sieved before feeding to the tilapia fry for 28 days. The lyophilized testes diets were sealed in polyethylene packets and stored at room temperature.

The sex reversal of Nile tilapia fry was done through oral administration of the experimental diets for 28 days. The lyophilized testes diets and the controls were given at a rate of 20% of the fish body weight per day during the first week with gradual reduction down to 10% of fish weight until the end of treatment. The feeding frequency was five times daily during daylight, 7 days per week. Growth and survival rate were recorded every week. After the 28-day treatment period, the fish were further reared and were fed with fry mash until they reach the age of 2-month old.

Sex determination through histological examination was done following the gonadal squash method of Guerrero and Shelton (1974). After the fish reaches age of 2-month old, 50 fish which is 10% of fish population from each net enclosure were sacrificed and gonad was excised. In determining the phenotypic sex through the squash method, some criteria were used to identify male and female gonadal tissue: presence of cyst-like structures containing spermatogonia and spermatocytes and appearance of oocytes at different stages of development (Figures 1a and b).

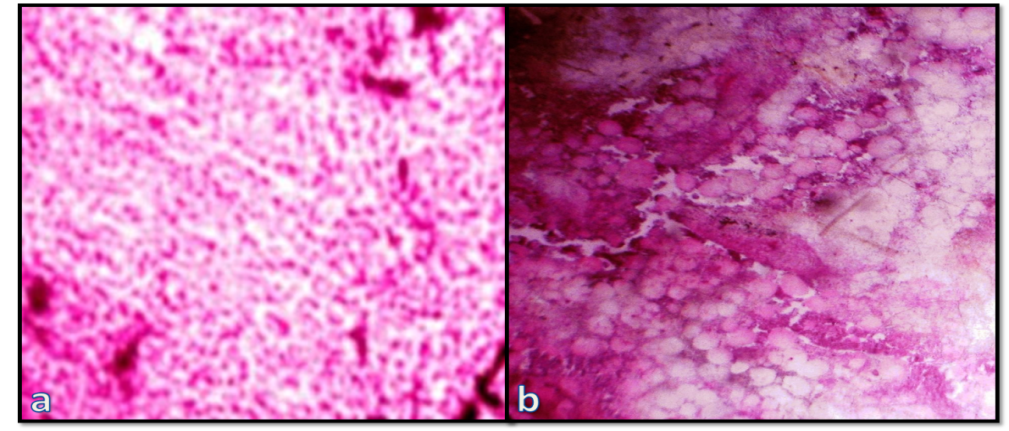


Figure 1. Tilapia gonad: (a) male; (b) female (Odin, 2009)

Water quality parameters such as temperature, dissolved oxygen and pH were monitored daily. Temperature and dissolved oxygen were measured using a YSI Model 55 DO meter while pH was measured using Fisher Model AB-15. Continuous water flow was provided to maintain desirable range of water quality parameters. Fifty percent of the total water volume of the tank was changed every other day to ensure optimum water exchange and good water quality throughout the treatment period.

The testosterone was analyzed using the Immulite 2000 analyser by a solid-phase, chemilumiscent enzyme immunometric assay. The serum collection was done after blood samples were collected from carabao, bull and boar and centrifuged at 5000 rpm for three minutes at 4oC. Serum total testosterone was analyzed since it was observed to have a positive and significant correlation with the volume of Leydig cells in the testes (Costa and Paula, 2006). This means that the value of serum total testosterone is related to the capacity of the Leydig cells to secrete testosterone in the animal testes (Ewing et al., 1979).

The proximate composition of every lyophilized testes diets were also chemically analyzed to determine the crude protein, lipid, ash, fiber and moisture content of the testes diets following the standard methods of AOAC (1980). The proximate analyses of the experimental diets were done at the Nutrition Laboratory of the Philippine Carabao Center, Science City of Muñoz, Nueva Ecija.

The analyses of data were done with the statistical package of Sirichai Statistics Version 6.00. Data gathered were subjected to Analysis of Variance (ANOVA) to determine significant differences among treatments. Comparison of means was done at 5% level by Duncan’s Multiple Range Test (DMRT). Sex ratio data were analyzed using the Chi-square test (α ≤ 0.05) to determine the efficacy of the treatments. Sample distributions violating assumptions were log-transformed before analysis. The data, expressed as percentages, were arc sine-transformed before analysis. Differences were regarded as significant at P < 0.05.

**RESULTS AND DISCUSSION**

**Phenotypic Males**

The data on the percent phenotypic males of Nile tilapia fry after the 28-day treatment period are shown in Table 1. The results show that there was a significant difference (P<0.05) among the treatments at 5% probability level of DMRT.

Table 1. Summary of the results from the 28-day sex reversal treatment of Nile tilapia (*Oreochromis niloticus*) fry using lyophilized testes diets and controls

|  |  |  |  |
| --- | --- | --- | --- |
| Treatments | Phenotypic males (%) | Specific growth rate (%) | Survival rate  (%) |
| Treatment I | 72.67 ± 3.91b | 15.59 ± 1.26ab | 92.27 ± 0.02 |
| Treatment II | 80.67 ± 2.24b | 15.85 ± 1.24a | 89.67 ± 0.00 |
| Treatment III | 79.33 ± 1.66b | 14.82 ± 0.22ab | 88.07 ± 0.05 |
| Control I | 96.67 ± 1.97a | 14.12 ± 0.31bc | 92.13 ± 0.07 |
| Control II | 46.00 ± 4.17c | 13.20 ± 0.40c | 86.93 ± 0.08 |

\*In a column, means followed by a common letter are not significantly different at 5% level by DMRT

Tilapia fry fed with MT-treated diet (Control I) obtained the highest percent male with a mean of 96.67 ± 1.97%. Those fry fed with lyophilized carabao testes (Treatment I), lyophilized bull testes (Treatment II) and lyophilized boar testes (Treatment III) attained means 72.67 ± 3.91, 80.67 ± 2.24, and 79.33 ± 1.66% males, respectively. The treatments were not significantly different (P>0.05) but were significantly lower than MT-treated group and significantly higher than untreated group (P<0.05). Following the Chi-square test (α ≤ 0.05), it was found out that the lyophilized testes diets and the MT-treated diet have a significant effect on the masculinization of Nile tilapia fry (Figure 2). The treatment groups and the MT-treated group were significantly skewed towards males and deviated from the theoretical 50:50 sex ratio.

Figure 2. Percentage of Nile tilapia (*O. niloticus*) fry classified as male and female under the lyophilized testes treatments and the controls after the 28-day treatment period. Note: Asterisks indicate significant differences in proportion of males from the untreated control (from Chi-square test; α ≤ 0.05)

One of the factors that may be considered to contribute to the percent males produced from the 28-day sex reversal treatment using lyophilized testes is the presence of testosterone in the animal testes. The total testosterone from serum of each animal was analyzed using chemilumiscent enzyme immunometric assay to determine the levels of testosterone (Table 2).

Table 2. Total testosterone from serum of carabao, bull and boar

|  |  |  |
| --- | --- | --- |
| Treatments | No. of Samples | Concentration (ppb) |
| Treatment I (carabao) | 2 | 2.57 |
| Treatment II (bull) | 3 | 9.83 |
| Treatment III (boar) | 3 | 13.61 |

As reported by Costa and Paula (2006), there is a positive and significant correlation between the serum total testosterone and the volume of Leydig cells in the testes. The values of serum total testosterone signify the capacity of the Leydig cells to secrete testosterone hormones in the animal testes (Ewing et al., 1979). Hence, the animal testes might contain concentrations of testosterone. The levels of total testosterone observed on the serum attested the presence of the androgen hormone in the testes of the animals. The testosterone in the testes is assumed to be preserved using lyophilization process which in turn promote sex reversal of tilapia fry after the 28-day lyophilized testes treatment. Furthermore, the animals from which the testes were collected were all characterized as sexually matured (Roth and Myers, 2004; Dewey and Ng, 2001; The University of Tennessee Health Science Center, 2009). Researchers have reported that mature animals have increased levels of testosterone (Becker and Snipes, 1968; Costa and Paula, 2006; Lindner, 1959; Lindner and Mann, 1960). During the age of sexual maturity of animals, testosterone level and potency is assumed to increase significantly. This idea explains the possible sex reversal of tilapia fry when treated with lyophilized testes from animals which contained concentration of potent testosterone.

Figure 3. Total testosterone and percent males produced from 28-day sex reversal treatment using lyophilized testes

The total testosterone levels from serum of each animal and the percent males produced from lyophilized testes diets are shown in Figure 3. Treatment I with 2.57 ppb of total testosterone resulted to 72.67% males. Treatment II with 9.83 ppb of total testosterone had 80.67% males. Treatment III with 13.61 ppb of total testosterone gave 79.33% males. The highest percent males were obtained from Treatment II while the lowest percent males were found in Treatment I. Treatment III with the highest total testosterone did not obtain the highest percent male. The reason for this may be accounted to the indigestible parts of the boar testes which might have affected the digestibility of the lyophilized boar testes diet. A tough, white fibrous connective tissue capsule, the tunica albuginea, surrounds each testis and extends inward to form septa that partition the organ into lobules (Darling, 2009). It was observed that the boar testes contained the thickest and toughest tunica albuginea among the testes from other animals. During the preparation of lyophilized testes diet, the tough and fibrous septa inside the testes were not removed. These probable indigestible parts of the testes remained in the diet. In this study, it is assumed that the fry treated with lyophilized boar testes diet assimilated less amount of testosterone since some parts of the diet were indigestible in the fish body. This may explain the reason why the Treatment III with the highest total testosterone level did not obtain the highest percent males of tilapia fry after the 28-day sex reversal treatment with lyophilized testes diet from boar.

The percentages of phenotypic males produced out of the lyophilized testes from animals are relatively higher than 65% males obtained from the 28-day treatment period of lyophilized bull testes fed *ad libitum* to tilapia fry, as reported by Phelps et al. (1996). Likewise, the results are also higher than the reports of Odin et al. (2009) where 61.33, 57 and 53% males were obtained from 23-day treatment period of dehydrated hog, carabao, and cattle testes, respectively. The relatively low percent males obtained from dehydrated animal testes treatment might be affected by the diminished and suppressed testosterone level of the testes due to heat exposure (Lue et al., 2000). In this study, the high percent males obtained is assumed to be favored by the high concentration of testosterone in the testes diets which was preserved under very low temperature processing during the lyophilization process.

However, these results are lower compared to the reported 85% male population of sex-reversed tilapia fry fed with fresh ram testes for 80 days (Haylor and Pascual, 1991), to the reported 93% phenotypic males produced from *ad libitum* feeding of tilapia fry with frozen bull testes after 30-day treatment period (White, 2008) and to the reports of Meyer et al. (2008) where percent males obtained from tilapia fry fed *ad libitum* with fresh bull testes and fresh hog testes were 87 and 83%, respectively. The lower percentages of males obtained from tilapia fry fed with lyophilized testes from carabao, bull and boar might be attributed to the restricted feeding of tilapia fry with testes diets at 20% feeding rate in this study. Hence, it is assumed that fry which were not masculinized consumed fewer hormones than the required minimum amount for sex reversal during the gonadal differentiation period.

According to Phelps and Popma (2000), the age and size of the fry and the environmental factors such as temperature can impact growth and affect gonadal differentiation and in turn the treatment duration needed. In this study, the first feeding fry with less than 9 mm initial length were used. Apparently, the ample amount of high protein from lyophilized testes diets fed to the fry and the high temperature during the treatment period might have contributed to the fast growth of the tilapia fry, reaching a length greater than 18 mm on the 14th day of the treatment period. This length is greater than the minimum harvestable size recommended for effective sex reversal of tilapia (Phelps and Popma, 2000; and Phelps, 2006). Hence, since growth was too fast, it may be necessary to reduce the quantity or quality of diet to reduce the growth rate and obtain effective sex reversal.

Another factor which may be considered to affect the percentages of males produced from the treatments is the flow-through system maintained throughout the experiment period. It is well established that hormones administered for sex reversal are metabolized and eliminated from the body of fish (Lone and Matty, 1981; Gomelsky et al., 1994). Abucay and Mair (1997) observed sex reversal of untreated fish reared within a system previously used with hormone treatment. He also mentioned that sex reversal treatments are more successful in closed water systems where metabolites and leachates can build up. In this study, the active metabolites of the testosterone excreted by treated fry during sex reversal and the hormones which leak from the uneaten food might have been prevented to build-up, diminished and lost from the system since continuous flow of water and regular water exchange were maintained during the treatment period. The shortened exposure of the treated fry to active metabolites of testosterone due to its loss in turn, may have affected the percentages of males produced in this study.

The percent males obtained from MT-treated group were found to be highest among the groups. This result conformed to the reports of Shelton et al. (1978), Guerrero and Guerrero (1988), Jo et al. (1988), Vera Cruz and Mair (1994) that oral administration of testosterone-treated feed (30-60mg/kg feed) to tilapia fry during a three to four-week period yields populations composed of ≥ 95% males.

The significant difference of phenotypic males in Treatments I, II, and III from that of the untreated group showed that lyophilized testes diets from bull, boar and carabao and the MT-treated diet were effective in masculinizing Nile tilapia fry. However, the higher percentage of males obtained in MT-treated group compared to those treated groups with lyophilized testes showed the greater potency of the synthetic androgen under the condition of this study. This might be due to the fact that 17α- methyltestosterone contained concentrated form of synthetic androgen. Synthetic androgens are generally more potent than natural androgens for masculinizing fish (Yamamoto, 1969).

**Growth Rate**

Data on the specific growth rate of Nile tilapia fry after the 28-day treatment period are shown in Table 1. The analysis of variance shows a significant difference (P<0.05) among the treatments at 5% probability level of DMRT (Figure 4).

Figure 4. Specific growth rate of Nile tilapia (*Oreochromis niloticus*) fry after the 28-day treatment period. Note: Treatments with different letter superscripts indicate mean values that are significantly different at 5% level by DMRT

After the 28-day treatment period, results revealed that Nile tilapia fry fed with lyophilized carabao testes (Treatment I), lyophilized bull testes (Treatment II) and lyophilized boar testes (Treatment III) obtained the highest specific growth rate among other treatments with means 15.59 ± 1.26, 15.85 ± 1.24 and 14.82 ± 0.22%, respectively. There was no significant difference (P>0.05) found among the treatments using lyophilized testes. Tilapia fry fed with MT-treated diet (Control I) obtained a mean of 14.12 ± 0.31% with no significant difference from Treatments I, III and untreated fry. The untreated group (Control II) with a mean of 13.20 ± 0.40% significantly differed from the groups treated with lyophilized testes diets.

These results may be attributed to the fact that animal meal contains higher protein content which in turn results to apparent high specific growth rate of Nile tilapia fry fed with lyophilized testes diets from carabao, bull and boar. The proximate analysis evaluated on the lyophilized testes from such animals revealed their high crude protein (CP) content (Table 3). Consequently, those tilapia fry fed with lyophilized testes had the highest specific growth rate since these diets contain the high crude protein content of about 64.85 to 71.69%.

Table 3. Chemical composition of the experimental diets

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Treatments | Crude Protein (%) | Moisture Content (%) | Crude Fat (%) | Crude Fiber (%) | Ash (%) |
| Treatment I\* | 64.85 | 2.89 | 13.59 | 0.43 | 10.24 |
| Treatment II\* | 71.69 | 1.97 | 13.16 | 0.25 | 10.17 |
| Treatment III\* | 70.20 | 2.98 | 12.33 | 0.27 | 9.84 |
| Control I\*\* | 33.00 | 12.00 | 6.00 | 5.00 | 12.00 |
| Control II\*\* | 33.00 | 12.00 | 6.00 | 5.00 | 12.00 |

\*Analyzed using AOAC (1980) method

\*\*Guaranteed proximate analysis of TATEH Aquafeeds, SANTEH, Feeds Corp

The results on the specific growth rate of the tilapia fry fed with lyophilized testes diets conformed to the results of the study conducted by Phelps et al. (1996) who reported that growth increase of tilapia fed *ad libitum* with trout chow feed containing lyophilized bull testes may range from 0.7 to 2.0 g after 28-day treatment period in outdoor tanks. Likewise, White (2008), reported that tilapia fry fed with frozen bull testes and oven-dried bull testes for 30-day treatment period gained a mean weight of 0.79 and 0.94 g, respectively. Odin et al. (2009) also reported significant high growth rate of Nile tilapia fry fed with dehydrated testes from carabao, cattle and hog at 20% feeding rate after a 23-day treatment period in hapas in earthen ponds. Fashina-Bombata and Somotun (2008) obtained an average length of 2.9 cm for fry of ‘Wesafu’, a sub-group of cichlid, after 25-day feeding trial of goat testes meal with 47.33% crude protein.

In this study, the specific growth rates found among those fry treated with lyophilized testes came out to be as high as the results of similar studies. This may be due to the similar culture conditions provided during the treatment period such as the minimal stocking density of 500/unit, continuous flow of water and good water quality of outdoor tanks which ensure optimum conditions for the growth of the tilapia fry.

The trend of the growth of Nile tilapia fry during the 28-day treatment period revealed that tilapia fed with lyophilized testes consistently increases rapidly in terms of body weight (g) followed by those treated with 17α-MT diets and the control with the lowest growth increase (Figure 5). This may be due to higher protein content of the testes diets fed compared to those control treatments.

Figure 5. Growth of the Nile tilapia (*Oreochromis niloticus*) fry during the sex reversal treatment

Several dietary protein requirements of several tilapia species have been estimated to range between 20-56% (El-Sayed and Teshima, 1991). De Silva and Perera (1985) reported that the optimum dietary protein level for optimum growth of Nile tilapia fry was 30% crude protein. As reported by Ahmad et al. (2004), the growth performance of Nile tilapia fry was highest at 45% protein diets. Al-Hafedh (1999) found out that better growth of this species was obtained at high dietary protein levels (40-46%) rather than 25-35%. In this study, the lyophilized testes diets contain the highest protein level compared to the control diets which contain only about 33% crude protein. The lyophilized testes diets are also assumed to contain androgenic hormones which are beneficial for fish growth. The growth increase may be attributed to the androgenic steroids which promote release of growth hormone from pituitary somatotrops fish (Higgs et al., 1976). Hence, the presence of testosterone in the testes promotes anabolic effects which in turn lead to increased growth rate of tilapia fry after a 28-day sex reversal treatment period with lyophilized testes diets.

Tilapia fry fed with MT-treated diet did not differ significantly from those fed with lyophilized testes of carabao and boar. The MT-treated diet in this study contained a dose of 50mg/kg which is high enough to promote growth aside from sex-reversing the fry. As reported by Ahmad et al. (2004), the optimum effective dose of 17α-MT treated diet in promoting significant final weight, weight gain, and specific growth rate of Nile tilapia is 5 mg/kg. Similarly, Jo et al. (1995) found *O. niloticus* fry treated with MT at 5-25 mg/kg diet to be heavier than the control after the sex reversal period. Mateen and Ahmed (2007) also reported that different dose rates of MT significantly increased the growth of Nile tilapia fry than the control. However, in this study, it was found out that fry fed with MT-treated diet had no significant difference from untreated fry. Vera Cruz and Mair (1994) did not find significant effect of MT on the growth and survival of Nile tilapia fry during the treatment period with MT at 40 mg/kg diet.

The high growth of tilapia confirmed the findings of earlier studies regarding animal protein meal. El-Sayed (2006) stated that terrestrial animal by-products have been widely and successfully used as protein sources for tilapia due to their high protein content and essential amino acids.

**Survival Rate**

The data on the survival rate of Nile tilapia fry after the 28-day treatment period are shown in Table 1. Analysis of variance shows no significant difference (P>0.05) among treatments (Figure 6).

Figure 6. Survival rate of Nile tilapia (*Oreochromis niloticus*) fry after the 28-day treatment period

Results show that the survival rate among treatments did not differ significantly (P>0.05) after the 28-day treatment period. Tilapia fry fed with lyophilized testes from carabao (Treatment I) obtained the highest survival rate with a mean of 92.27 ± 0.02%. This was followed by those fry fed with MT-treated diet (Control I), lyophilized testes from bull (Treatment II), lyophilized testes from boar (Treatment III) and untreated diet (Control II) with means 92.13 ± 0.07, 89.67 ± 0.00, 88.07 ± 0.05 and 86.93 ± 0.08%, respectively.

The high survival rate of Nile tilapia fry obtained in this study confirmed the findings of White (2008) who obtained high survival rates (88-95%) of fry fed with animal testes and stocked in outdoor tanks with green water during a 30-day treatment period. The survival of fry during the sex reversal treatment are dependent on factors such as stocking density, feeding, temperature and other environmental conditions (Bocek et al., 1992). Vera Cruz (1991) stated that the sex reversal treatment unit influences the quality of fingerlings produced. In this study, the experimental units were set with continuous flow of water to ensure optimum water exchange and good water quality throughout the treatment period. Vera Cruz and Mair (1994) obtained >70% survival rate of tilapia fry utilizing outdoor tanks having at least once/day water exchange rate during the hormone treatment period. Vera Cruz and Mair (1994) also reported insignificant effect on the survival of *O. niloticus* fry during the treatment period of 40 mg/kg diet. The minimal stocking density of 500 fish per unit in outdoor tanks in this study might have favored the high number of fish surviving throughout the treatment period (Figure 7).

Figure 7. Number of fry surviving based on the original 500 fish per experimental unit during the 28-day treatment period

Apparently, the survival rates obtained in this experiment were higher compared to the results of earlier studies conducted on the use of animal testes in masculinizing Nile tilapia fry (Haylor and Pascual, 1991; Phelps, 1996; Meyer et al., 2008. In this study, the high survival rate of fry is assumed to be attributed to the continuous water flow and regular cleaning of the tanks and enclosure nets which in turn promoted optimum culture and environment conditions for the experimental fish during the treatment period.

**Simple Cost and Return Analysis**

The simple cost and return analysis of Nile tilapia fry treated with lyophilized testes on a 28-day treatment period is shown in Table 4. Treatments III and II with no significant difference (P>0.05) gave the highest gross income of PhP 306.58 and PhP 294.45, respectively. MT-treated diet (Control I) and Treatment I followed with a gross income of PhP 271.15 and PhP 263.17, respectively. The MT-treated group differed significantly (P<0.05) from Treatment III but did not differ significantly (P>0.05) from Treatments I and II in terms of gross income. The untreated fry (Control II) with the lowest gross income of PhP 167.42 differed significantly (P<0.05) from other groups.

In terms of cost, Treatment I, II and III gave the highest operating cost of Php 238.97, PhP 260.83 and PhP 197.78, respectively. The control treatments, MT-treated diet and untreated diet, obtained costs of PhP 136.02 and PhP 131.98, respectively. The cost for lyophilized testes diets significantly differed (P<0.05) from the control groups.

Table 4. Simple cost and return analysis of Nile tilapia (*Oreochromis niloticus*) fry fed with lyophilized testes vs controls

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Items | Treatments | | | | |
| I | II | III | MT-treated | Untreated |
| Gross Income (PhP) | 263.17c | 294.45ab | 306.58a | 271.15bc | 167.42d |
| Operating Cost (PhP) |  |  |  |  |  |
| 1. Fry | 100 | 100 | 100 | 100 | 100 |
| 1. Lyophilized testes diet | 111.37 | 133.23 | 70.18 | 0 | 0 |
| 1. MT-treated diet | 0 | 0 | 0 | 8.42 | 0 |
| 1. Fry-mash | 0 | 0 | 0 | 0 | 4.38 |
| 1. Labor | 23 | 23 | 23 | 23 | 23 |
| 1. Electricity | 4.60 | 4.60 | 4.60 | 4.60 | 4.60 |
| Total Cost | 238.97b | 260.83a | 197.78c | 136.02d | 131.98d |
| Net Returns (PhP) | 23.20b | 33.62b | 108.80a | 135.13a | 35.44b |

\*In a column, means followed by a common letter are not significantly different at 5% level by DMRT

In terms of net returns, those fry fed with MT-treated diet gave the highest significant net return of PhP 135.13 and this was followed by those in Treatment III with Php 108.80 net returns. There was no significant difference (P>0.05) found between the MT-treated group and Treatment III. Treatment I and II obtained a net return of PhP 23.20 and PhP 33.62, respectively. The untreated group with PhP 35.44 net return did not differ significantly (P>0.05) from those in Treatments II and I.

The low value of net returns in Treatments I and II was assumed to be greatly affected by the high cost incurred for the production and processing of lyophilized testes diets. Treatment III, on the other hand, obtained a high net return because of the low price of raw boar testes in the market. In order to determine the market price of the sex-reversed fingerlings produced from lyophilized testes, the cost of diet preparation of the lyophilized testes must be reduced.

The result of the simple cost and return analysis revealed that it is more economical to use synthetic MT-treated diet than lyophilized testes diet in masculinizing Nile tilapia fry. The MT hormone is synthetic product and more potent in masculinizing Nile tilapia fry. This product is also a concentrated form of androgen which can be easily stored and can be easily administered to the fish. On the other hand, the testes of carabao, bull and boar are readily available from any market and local abattoirs in the country. However, the preparation and processing of these animal testes into lyophilized form to ensure prolonged shelf-life and potency for sex reversal requires a complicated, sophisticated and extensive methodology. The use of lyophilized testes can be of great relevance to the production of organic tilapia.

**Water Quality**

Water quality parameters such as temperature, pH and dissolved oxygen were all found to be within the desirable optimum range. Statistical analysis revealed that there were no significant differences (P>0.05) found among the treatments in terms of the water quality parameters monitored during the 28-day treatment period (Table 5).

Table 5. Water quality parameters monitored during the 28-day treatment period

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Treatments | Temperature (oC) | | DO (mg/L) | | pH | |
|  | AM | PM | AM | PM | AM | PM |
| Treatment I | 27.95 | 29.46 | 5.3 | 8.0 | 8.2 | 8.3 |
| Treatment II | 27.97 | 29.38 | 5.4 | 7.7 | 8.1 | 8.3 |
| Treatment III | 27.94 | 29.40 | 5.3 | 8.2 | 8.2 | 8.3 |
| MT-treated | 27.96 | 29.39 | 5.3 | 7.8 | 8.1 | 8.3 |
| Untreated | 27.97 | 29.36 | 5.1 | 7.5 | 8.2 | 8.3 |

The readings on the water quality parameters during the experimental period demonstrated desirable levels suitable for sex reversal of Nile tilapia fry. The average temperature was recorded to be optimal at an average of 27oC in the morning. However, it was also found to be relatively high during afternoon with an average of 29oC. Phelps and Popma (2000) stated that optimum temperature suitable for sex reversal of tilapia fry falls between 26-28oC. In this study, high temperature readings which fell out of the maximum optimum range were recorded during the first to third week of the treatment period where the weather was sunny. The temperature readings started to drop to its optimum range on the last days of the treatment period where rainy weather was observed. The readings on dissolved oxygen (5.1 - 8.2 mg/l) and pH (8.1 - 8.3) recorded were all within the favorable conditions appropriate for sex reversal. Phelps and Popma (2000) suggested that dissolved oxygen concentrations should remain above 4 mg/l to ensure a strong feeding response. In terms of pH, it was mentioned that tilapia can best survive in pH of 6.0-9.0 (Popma and Masser, 1999).

**CONCLUSIONS**

Based on the results of this study, it can therefore be concluded that the objectives of this study were met. (1) Lyophilized testes from bull, boar and carabao were possible in masculinizing Nile tilapia fry after a 28-day treatment period in outdoor tanks but the percent phenotypic males produced is not as high as the synthetic MT. The sex reversal rates of tilapia fry using lyophilized testes were found to be significantly higher than the untreated fry. (2) Lyophilized testes from bull, carabao and boar gave higher specific growth rate of Nile tilapia fry after a 28-day treatment period in outdoor tanks. High survival rate of Nile tilapia fry fed with lyophilized testes from carabao, bull and boar were obtained after a 28-day treatment period in outdoor tanks. (3) The simple cost and return analysis revealed that it is more economical to use the synthetic MT-treated diet in masculinizing Nile tilapia fry rather than the lyophilized testes from carabao, bull and boar.

**RECOMMENDATIONS**

Based on the results of this study, the following recommendations are considered for future investigation: (1)Consider the influence of controlled and uncontrolled feeding in using lyophilized testes diets for sex reversal of Nile tilapia fry to come up with effective dose; (2) Consider the use of lyophilized testes diets for sex reversal of Nile tilapia fry in hapas in earthen pond; (3) Develop a procedure for the use of liquid nitrogen instead of lyophilization in the preparation method of freeze-drying the testes to reduce the cost of testes diets.

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