G.J.B.B., VOL.6 (1) 2017: 130-134 ISSN 2278 – 9103

GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

www.scienceandnature.org

IMPACT OF DIFFERENT LEVEL OF FREEZE-DRIED BULL TESTES ON GROWTH PERFORMANCE AND MASCULINIZATION OF Nile tilapia (*Oreochromis niloticus*) Nirwana

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ABSTRACT
This study aimed to evaluate the impact of freeze- dried bull testes (FDBT) on growth performance and masculinization of Nile Tilapia (*Oreochromis niloticus*) Nirwana. A total of 1296 fry 1 day old were randomly allocated to 27 experimental aquaria. The metabolizable energy concentration (ME) and crude protein percentage (CP) of the diet 20.08 MJ/Kg and 45% respectively. The experimental treatments evaluated were freeze-dried bull testes (FDBT) at different concentration levels of hormone 30, 60 and 90 ng/ml. Diets were randomly distributed among the experimental treatment aquaria using completely randomized design. The fry fed three times per day at a fixed rate of 20% body weight. Water parameter such as (temperature, pH, dissolved oxygen, and ammonia) were measured and recorded every week. The result of water parameters was within the recommended range for fish rearing. The total body weight was measured and recorded every week. The fry fed with freeze-dried bull testes 90ng/ml obtained the highest growth rate among other treatments with mean 0,026, followed by fry that fed with 60, 30 ng/ml and control group with means 0,021, 0,016 and 0,016There was no significant different among the other treatments. Results revealed that Nile tilapia fry fed with FDBT gave the highest percentage of a male population with mean 73,33%, followed by fry fed 60, 30ng/ml FDBT and control group with means 66,66 ±7,69 55, 57,77±8,01r and 55±5,87 %, respectively. The treatments fed with the high level of freeze-dried bull testes were a significant differences ($p>0.05$) compare with control group.

KEYWORDS: Testosterone, Nirwana, Growth rate, Bull testes.

INTRODUCTION

Nile Tilapia ranked second in the global world production and one of the major produced and consumed aquaculture commodities in the Asia. The tilapia world production has grown rapidly at 2,515,908 metric tons in 2007 (Fitzsimmons, 2008). *Nile Tilapia* reach sexual maturity early that resulted inbreeding in overstocked ponds, reduced production and farmed stocks of a generally low quality. Female reach maturity faster than male and used most of the energy for egg production, according to those males has better growth rate and feed conversion ratio than female and therefore reach marketable size sooner. Thus, all male population of tilapia is necessary for efficient production. To skew this problem there are several methods used to control sex ratio and increase the percentage of male population such as grading or sorting; hybridization; supermale and used of hormones. The production of mono sex male tilapia through androgen sex reversal is considered a reliable method for eliminating the problem of excessive reproduction and for increasing yield of harvestable size fish (Gerrero, 1982). The use of natural hormone may provide means of sex alternation in tilapia, particularly where the synthetic steroid is less available and high expensive.

Naturally occurring source of testosterone may be an alternative to using a synthetic androgen, which also is an anabolic steroid for tilapia sex reversal Seazar *et al.* (2013). Bull testes are a by product of the slaughterhouse and could be a potential source of testosterone for tilapia sex reversal. At the present successful masculinized of tilapia is done through oral administration of synthetic hormone in the diet feed at 30-60mg/kg for about 28 days (Selton *et al*., 1978; Guerrero and Mair, 1994). The effect of the synthetic hormone has been under increasing public criticism due to their possible health and environmental impact. As a result, the use of methyl testosterone for masculinization of Nile Tilapia is either limited by the U.S. Food and Drug Administration or prohibited in Europe (Mc Andrew, 2000). This study was conducted at the research pond, Faculty of Fisheries and Marine science, Universitas of Padjadjaran, Indonesia. The fry of Nile tilapia in this study was treated with freeze-dried bull testes for 30 days in the aquarium.

The general objective of this study is to evaluate the potentiality of freeze-dried bull testes in the masculinization

of Nile Tilapia, specifically, the study determined the efficiency of freeze-dried testes from bull in production phenotypic male of *O. niloticus* and their influence on growth performance, survival rate.

MATERIALS & METHODS

Twenty-seven aquaria (30x50x60) were set in the laboratory following the complete randomized design (CRD) for three treatment 30, 60 and 90ng/ml each one contained three replication; each aquarium was equipped with an appropriate aerator. During this study water parameters like temperature, pH, ammonia and dissolved oxygen were measured weekly. Bull testes were brought from the slaughterhouse. The testes were skinned and cut 1x1 cm sliced and completely homogenized without dilution using a countertop blender. The homogenized testes were freeze dried at the laboratory for minimum 24 hours. After 72 hours the testes were completely freeze- dried, using cascade-type freeze dryer equipment accommodates up to 6 kg fresh testes per run. Lyophilization of frozen and homogenized testes was done by placing them in a vacuum with -40ºC temperature to remove the moist from below zero frozen state before returning it to ambient room temperature of approximately 20 ºC, after freeze – dried 20-25% at the weight of fresh testes was recovered. The resultant crumbs were pulverized and sieved before feeding to the tilapia fry for 30 days. The freeze–dried testes diet was sealed in polyethylene packets and store at refrigerator.

Exeperimental trail

Nile tilapia strain Nirwana, were collected hatchery at Balai benih ikan ciparary- Bandung the average weight of fry ranged from 0.005 to 0.007g/fry all fry obtained from one parent. Fry was stocked at a rate of 50 per aquarium. The aquarium was emptied every day. A siphon house was used to remove the accumulated feces and feed the aquarium were immediately refilled with fresh tap water. Fish in all treatment were fed at the daily rate of 20% of body weight at three times intervals (8.00, 13.00 and 16pm time). The fish body weight was taken weekly using electronic balance.

Determination of sex

For all experimental groups, sex differentiation was studied using the histological technique while sex frequency and sex change were assessed using acetocarmine Squash method Guerrero and Shelton (1974).

RESULTS

Water quality

Water quality parameter such as pH, temperature, dissolved oxygen, and ammonia. Were all found to be within the desirable optimum range. Statistical analysis revealed that there were no significant differences (0.05) found between treatments during experiment period.

TABLE 1: Water quality during the growing period of fry of Nile Tilapia (Nirwana)

Treatment	DO	pH	Ammonia	T
FBBT30ng/ml	8.02±0.32	8.2±0.04	0.01±0.001	25-27
FDBT60ng/ml	7.7±0.42	8.2±0.01	0.01±0.001	25-27
FDBT90ng/ml	7.5±0.55	8.2±0.01	0.02±0.02	25-27
Control	7.8±0.34	8.3±0.06	0.01±0.001	2527
Standard	>5 mg/L	6.5-8.5 mg/L	0.2 mg/L	25-30 °C

DO= dissolved oxygen. T = temperature

Performance of Nile Tilapia (Nirwana) during the experimental period

The growth rate of *Nile tilapia* strain Nirwana in this study increased significantly with an increase of the level of the testicular tissue inclusion level. The analysis of variance shows a significant difference ($p< 0.05$) among the treatment at 5% a probability level of DMRT (Figure 2). After the 30-day treatment period, result revealed that Nile tilapia fry fed with freeze-dried bull testes 90ng/ml obtained the highest growth rate among other treatments with mean 0,026. There was no significant different among the fry fed on30 ng/ml and the untreated group (Control) with a mean of 0,016. Feed conversion ratio was significant differences (0.05) compare with fry fed on 30ng/ml and control group with mean from 0,083 which obtained in control group to 0,056 which obtained in FDBT90ng/ml it.

TABLE 2: Performance of Nile Tilapia (Nirwana) during the Experimental Period

Parameters	FDBT30ng/ml	FDBT60ng/ml	FDBT90ng/ml	CONTROL	L .S
Initial weight (g)	0,006	0,006	0,006	0,006	N.S
Final weight (g)	$0,49^{a}$	$0,63a^{b}$	$0,80^{b}$	$0,50^{a}$	*
Growth rate (g)	$0,016^{a}$	$0,021^{ab}$	$0,026^{b}$	$0,016^{a}$	*
Feed conversion ratio	$0,086^{c}$	$0,070^{abc}$	$0,056^{a}$	$0,083^{c}$	*
Feeding period(days)	30	30	30	30	-

FDBT= freeze dried bull testes.

L.S= Level of significant, N.S= not significant; *= $p<0.05$

Survival rate

The data on the survival rate of Nile tilapia during experimental period (30 days) are shown in the table (3). The analysis of variance of survival rate shows there was a significant deference among treatment ($p>0.05$) after an

experimental period. The highest survival rate obtained in control group and fry fed on 30 ng/dl with mean 97, 91 and 97, 22±1, 38%. The fry fed with FDBT90 ng/ml obtained lowest survival rate due to a high level of hormone in the feeding lead to decrease the water quality in the aquarium during night.

Sex determination

The fry of Nile tilapia (Nirwana) fed with FDBT 90ng/ml obtained the highest percent male with a mean 73,33%, this result followed by fry fed with 60, 30 ng/ml FDBT and control with means 66,66 ± 7,69, and 57,77 ±8,01, and 55,55 ±5,87 %, respectively. The treatments fed with a high level of freeze-dried bull testes were a significant difference ($p>0.05$) compare with control group. Following the chi-square test ($\alpha \leq 0.05$) it was found out that the fresh dried from bull have a significant effect on the masculinization of fry.

TABLE 3: The percentage of male, female and intersex of Nile tilapia, Nirwana after 30 days treatment period

Treatment	Male %	Female %	Intersex%	Survival rate
FDBT30 ng/ml	$57,77 \pm 8,01^{a}$	$37,78 \pm 8,89^{bc}$	$4,44 \pm 2,22^{a}$	$97,22 \pm 1,38^{c}$
FDBT60 ng/ml	$66,66 \pm 7,69^{ab}$	$26,66 \pm 10,18^{abc}$	$6,66 \pm 3,84^{a}$	$93,05 \pm 0,69^{abc}$
FDBT90 ng/ml	$73,33 \pm ,00^{ab}$	$20,00 \pm 3,85^{ab}$	$6,66 \pm 3,84^{a}$	$88,19 \pm 4,86^{ab}$
Control	$55,55 \pm 5,87^{a}$	$44,44 \pm 5,87^{c}$	$,00 \pm ,00^{a}$	$97,91 \pm 0,00^{c}$

DISCUSSION

The reading of the water quality during the experimental period within tolerable limits of tilapia aquaculture (Bardach *et al*., 1972). Phelps and popma (2000) suggested that dissolved oxygen had remained above 4 mg/ l. Popma and Maser, 1999) reported that the optimum range of pH that tilapia can survive in pH of 6.0-9.0. Phelps and Popma (2000) stated that optimum temperature for sex reversal of Nile tilapia falls in between 26-28c̊. The growth rate of Nile Tilapia (*O. niloticus*) Nirwana fed freeze-dried bull testes in this study increased significantly with increase the level of hormone in the diets. These result may be attributed to the fact that animal meal contains higher protein content which in turn to apparent high growth the rate of Nile tilapia fry fed with freeze- dried testes diet from bull, however, this could possibly be due to increased testosterone effect which is a know anabolic agent and to an improvement in the quality of dietary protein. Several studies were in agreement that testosterone produce muscle hypertrophy by increasing muscle protein synthesis Bhasin *et al.*(2001). The significant increase of the inclusion level of freeze- dried testes from bull might also explain the improvement in growth rate.

This was agreement with Fasina *et al.* (2008) who found that fish fed with goat testes meal grew faster than the control group fed a commercial diet. This finding was agreement with Ramjie *et al.* (2013) who obtained high growth rate with fry fed with the bull, carabao and boar testes compare with control group. The result agreed with Seazar *et al.* (2013) who fed dried bull testes and obtained high growth rate compare with untreated fry. The high growth rate confirmed the finding 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. Feed conversion ratio (FCR) improvement with the increase of dietary FDBT could be due to the improvement in the rate of body gain in tilapia fish. The high survival rate of fry of Nile Tilapia obtained in this study confirmed with Odien *et al* (2009) who obtained high survival rate (92.27 ±0.02- 86.93 ±0.08%) with fry of Nile tilapia fed with different lyophilized testes from the animal. White (2008) obtained high survival rate (88-95%) of fry fed with different animal testes. The survival of fry during sex reversal treatment are dependent on factors such as feeding, temperature, stocking density and other environmental condition Bocek *et al.* (1992). These results of male, female and intersex percentage are in agreement with Phelps *et al* (1996) who reported that the percentage of sex reversal produce out from lyophilized testes from the animal are relatively higher than 65% males obtained from the 28- day treatment period lyophilized bull testes fed ad libitum to tilapia fry. Likewise, the result is also higher than the report of Odien *et al.* (2009) were 61.33, 57 and 53% male were obtained from 23-day treatment period of the dehyrated hog, carabao and cattle testes, respectively. This study agree with Saezar *et al.* (2011) reported that the fed fry of Nile tilapia fed with dried bull testes at different percentage mix with feeding at a rate 25 and 50% dried bull testes , obtained 55 and 75% male respectively from 30-day treatment period . This percentage of male the population are lower with Ramjie *et al.* (2013) who obtained the percentage of sex reversal produce in outdoor tanks with means of 80.67, 79.33.72.67% from lyophilized testes from the bull, boar, and carbon respectively. However this the result are lower compared to the reported 85% male population of sex reversal of Nile tilapia fry fed with fresh ram testes for 80 days Haylor and Pascual (1991), to the reported 93% phenotypic males produced from *ad libtum* feeding of tilapia fry fed with frozen bull testes for 30 days treatment period White (2008). According to Phelps and Popma (2000) the age and size of fry and the environmental factors such as temperature can effect on the growth of gonadal differentiation and in turn the treatment duration needed. These findings were in agreement Ronald *et al* (1998) who found that freeze-dried bull testes resulted in 65% male population in fish fed 50% freeze-dried bull testes, 54% male population in fish fed 25% freeze-dried bull testes and 52% male population in the group under control. Finding in this the study which showed that the high

percentage of male population related with the high level of concentration of the hormone in the diet. The significant different of phenotypic male and intersex in treatment at the level of 90ng/ml freeze dried ram testes and 90ng/ml freeze-dried bull testes were effective in sex reversal of *Nile tilapia* fry. The result of gonad examination in the various treatments is presented in Table (3) and it showed that each of the treatment groups gave a high male: female: intersex ratio. A total of fifteen fish in each treatment were randomly collected in all the replicates and sex was confirmed by dissection through examination of their gonads (Fig 1, 2 and 3) as described by Mohamed (2015).

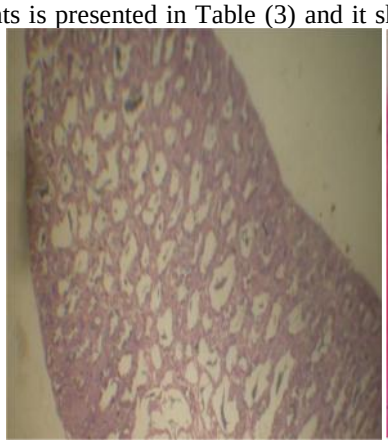

FIGURE1: Testicular tissue cells show seminiferous tubule.

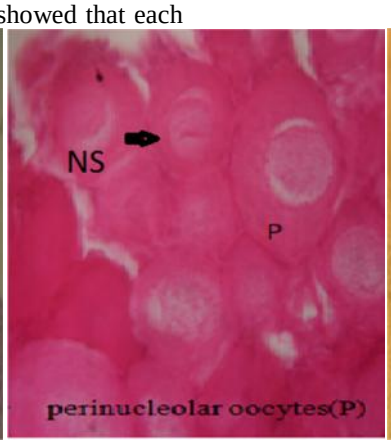

FIGURE2 : Ovarian tissue show various stages of oogcyte Nucleolus stages (NS) and Prei- nucleolus oocytes(P)

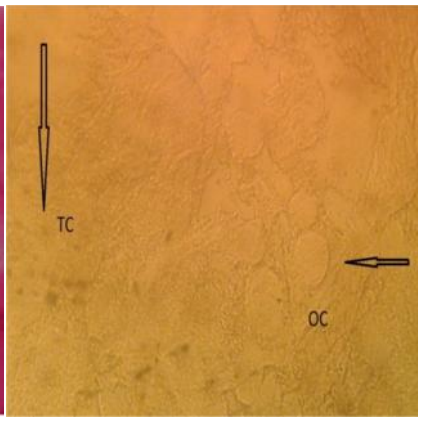

FIGURE3: Show Ovarian cell(CO) as well as Testicular cell (TC)

CONCLUSION

Based on the results of this study, it can conclude that the objectives of this study were met (1) freeze dried bull testes were possible in masculinization of Nile tilapia fry after 30-day treatment period but the percent of the male population is not high as the synthetic hormone. The sex reversal rates of tilapia fry using freeze dried 90 ng/ml were found to be higher than other treated fry. Freeze dried bull testes gave higher growth rate and feed conversion ratio.

RECOMMENDATION

Based on the result of this study, the following recommendation is considered for future investigation: develop a procedure for use a freeze dried testes from animal to reduce the risk that could possibly cause by using synthetic hormone. Freeze dried bull testes give the same percentage of male population. Freeze dried bull testes improve fry performance and fish flesh chemical composition and quality, and inclusion level of 60ng/ml ram and bull testes in Nile tilapia diet should not be exceeded 50-60 ng/ml to avoid increased rate of mortality.

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