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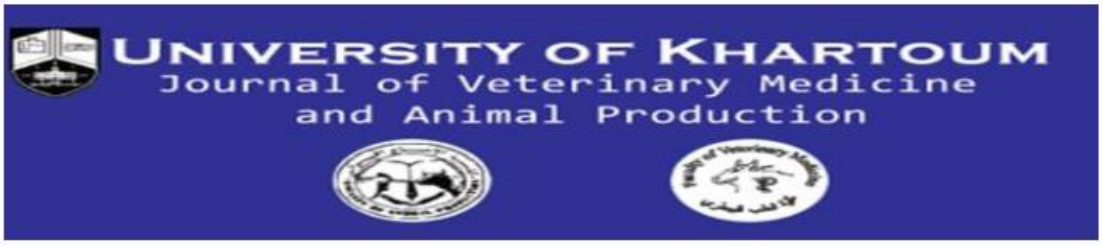

Evaluation of the potential of freeze dried ram testes on masculinization and growth performance of Nirwana fish (*Oreochromis niloticus*)

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Abstract

This study aimed to evaluate the potentiality of freeze–dried ram testes (FDRT) on growth performance and masculinization of Nile Tilapia (*Oreochromis niloticus*). 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 were 20.08 MJ/Kg and 45%, respectively. The experimental treatments were freeze-dried ram testes (FDRT) 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 parameters (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 ram testes 90ng/ml obtained the highest growth rate with mean 0.022 followed by fry fed with 60, 30 ng/ml and control group, with means 0. 020, 0, 019 and 0.016, respectively. There was no significant difference ($p > 0.05$) among the different treatments. Results revealed that Nile tilapia fry fed with 90ng / ml FDRT gave the highest percentage of a male population with mean 75.55 ± 2,22%, followed by fry fed 60, 30ng/ml FDRT and control group; with means 71.11 ± 5.87, 60.00 ± 3.85and 55±5.87%, respectively.

Key words: Nile Tilapia, Nirwana, masculinization, growth performance, ram testes.

المستخلص

اجريت هذه الدراسة لتقييم اثر خصي الضان علي اداء نمو وانقلاب الجنس في اسماك البلطى النيلي جنس نيروانا . عدد 1296 من صغار اسماك البلطي عمر يوم ، وزعت عشوائيا علي عدد 27 حوض زجاجي . حيث كانت طاقه الهضم 20.08ميقاجول/كيلوجرام ونسبة البروتين 45% . غذيت الاسماك على نسب مختلفة من الخصي المجفدة بمعدلات 30، 60 ، 90 نانوجرام /مل. تمت تغذية الاسماك ثلاث وجبات في اليوم بمعدل 20% من وزن الجسم . تم قياس جودة المياه اسبوعيا حيث شملت درجه الحرارة ، الاس الهيدروجيني، الاكسجين الذائب في الماء و الامونيا . كانت نتائج خواص الماء في الحدود المثلي الموصي بها لتربية الاسماك . تم قياس الوزن اسبوعيا . الاسماك التى غذيت على معدل 90نانوجرام / مل من مسحوق خصي الضان المجفدة حققت اعلى معدل نمو مقارنة بالمعاملات الاخرى بمتوسط 0.022 تلتها المجموعه

التى غذيت علي معدل 60 نانوجرام / مل، 30 نانوجرام / مل و مجموعه الكنترول بمتوسطات 0.020، 0.019 و 0.016 . حيث لم تكن هناك فروق معنويه بين المعاملات . اظهرت النتائج ان الاسماك التى غذيت على معدل 90 نانوجرام / مل حققت اعلى معدل للذكور بمتوسط 75.55±2.22 % ، تليها المجموعه 60، 30 نانوجرام / مل و مجموعه الكنترول ($p>0.05$). بمتوسطات 71.11±5.87، 60.00±3.85 و 55±5.87% على التوالى.

الكلمات المفتاحية: اسماك البلطى النيلي جنس نيروانا، وانقلاب الجنس، اداء النمو، خصي الضان

Introduction

The Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) is the main genus *Oreochromis* with potential foraquaculture due to its rusticity and rapid growth, reaching commercial weight in small interval of time; great capacity for adaptation to confinement and the various systems of farming, high capacity for hybridization : which allows the characters units desired; tolerance to wide variations in salinity, temperature and concentrations of dissolvedoxygen, high resistance to diseases, high quality meat with its clear color and high acceptance by the consumer (Boscolo *et al*., 2001; Drummond *et al*., 2008). The androgen 17 α-Methyltestosterone (MT)- an anabolic steroid- is being widely used in the production of all male population in aquaculture especially the *Oreochromis* spp ; due to their precocious sexual maturity and a high reproductive efficacy, resulting in overpopulation in ponds (Desprez *et al.,* 2003: Kefi *et al*., 2013)**.** The use of natural hormone may provide means of sex alternation in tilapia, particullary where synthetic steroidsare less available and highly expensive. Naturally occuring 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* ., 2017). The consumption of fish flesh containing synthetic hormone residues is potentially hazardous to consumers health. Bull and ram testes are by-products of the sloughterhouse and could be a potential source of testosterone for tilapai sex reversal. The successful masculinization 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 synthetic hormone has been under increasing public criticism due to their possible health and environmental impact. As a result the use of methyletestosterone for masculinization of Nile tilapia is either limited by the U. S. Food and Drug Administration or prohibited in Eurorpe (Mc Andrew, 2000).

The objective of this study was to determinethe efficiency of freeze-dried testes from ram in production of phenotypic male of *O. niloticus*(strain Nirwana) and their influence on growth performance, survival rate.

Materials and methods

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 were treated with freeze-dried testes for 30 daysin aquaria.

Experimental design of aquaria

The aquaria were made from fiber in rectangular shape. The aquarium dimension was 30 cm width 50 cm length, and 60 cm height. Each aquarium was equipped with an appropriated airator. Twenty seven aquariawere set in the laboratory following the compeleterandomized design (CRD) for three levels according to concentration of hormone (30, 60 and 90ng/l) each one contains three replications. During this study water parameters : pH , dissolved oxygen, ammonia and temprerature were observed weekly .

Preparation of the bull testes and experimental diet

Mature ram testes were selected and brought at Ciroyom- Bandung. Testes were skinned and cut 1x1cm, then inserted

into the tube freeze in freezer for 24 hr at temperature -75c and pressure -0.1 Mpa. After that testes were blended then sieved fine (0.42 mm). A testicle sample was taken to determine the proximate analysis and concentration of testosterone per gram by using ELISA. 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 30 days. The lyophilized testes diets were sealed in polyethylene packets and stored at refrigation .

Culture Technique

Fry was stocked at a rate of 50 fries per aquarium.The aquaria were cleaned every day. A siphon house was used to remove the accumulated feaces and food and then aquaria were immediately refilled with fresh tap water. The average weight of fry was 0.006 g /fry. All fry were obtained from one parent. The aquaria were empted every day. A siphon house was used to remove the accumlated feces and feed the aqurium were immediatly refilled with fresh tap water. Fish in all treatments were fed at 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.

Method of 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).

Statistical analysis

Data were analysed using One-way ANOVA of SPSS 21.0 for Windows. Means were separated by Duncan's test at a 5% significance level.

Results

Sex determination

The fry of Nile tilapia (Nirwana) fed with FDRT 90ng/ml obtained the highest percent male with a mean 75.55± 2.22%, this result followed by fry fed with 60, 30 ng/ml FDRT and control with means 71.11± 5.87, 60.00± 3.85, and 55.55± 5.87 %, respectively. The treatments fed with a high level of freeze-dried ram testes were of significant difference ($p>0.05$) compared with control group. The finding of this study (Table1) revealed that the freeze dried ram testes have a significant effect on the masculinization of Nirwana fry.

Table 1: The percentage of male female and intersex of Nirwana fry by different treatments at end of experimental period.

Treatment	Male %	Female %	Intersex%	Survival rate
FDRT30 ng/ml	60. 00 ± 3.85^{a}	40.0± 5.87^{bc}	2.22 ±2.22^{a}	95.13±0.69^{bc}
FDRT60 ng/ml	71.11 ± 5. 87^{ab}	24.44± 4.44^{abc}	4.44 ± 2.22^{a}	90.97±1.38^{abc}
FDRT90 ng/ml	75.55 ± 2.22^{b}	13.33 ±2.22^{a}	11.11± 2.22^{a}	85.41±3.18^{a}
Control	55.55± 5.87^{a}	44.44±5.87^{c}	0.00 ± 0.00^{a}	97.91±0.00^{c}

^{a,b} means with different superscript in the same column are significantly different $p<0.05$

FDRT= freeze dried ram testes

Performance of Nile tilapiaduring the experimental period

The growth rate of Nile tilapia (strain Nirwana) in this study increased significantly with an increase of the level of the concentration of hormone in the diet. The analysis of variance shows a significant difference ($p< 0.05$) among the treatment at 5% probability level of DMRT. After the 30-day treatment period

result revealed that Nile tilapia fry fed with freeze-dried ram testes 90ng/ml obtained the highest growth rate among other treatments with mean 0.026g. There was no significant difference among the fry fed on30, 60 ng/ml and the untreated group (Control) with a mean of 0.02g, 0.016 and 0.016, respectivly. Feed conversion ratio ranged from 0.083, which obtained in control group to 0.056, which obtained in 90 ng/ml of FDRT;it was significantly different ($p < 0.05$) compared with other groups.(Table 2).

Water quality

Water quality parameters: such as pH temperature (T), dissolved oxygen (DO) and ammonia; were all found to be within the desirable optimum range. Statistical analysis revealed that there were no significant differences ($p > 0.05$) found between treatments during experiment period (Table 3).

Table 2: Post hatching performance of *Nile tilapia* fed diets with different levels of ram testes.

Parameters	FDRT30ng/dl	FDRT60ng/dl	FDRT90ng/dl	CONTROL	Level of significance
Initial weight (g)	0.006	0.006	0.006	0,006	N.S
Final weight (g)	0.57^{a}	0.62^{ab}	0.68^{b}	0.50^{a}	*
Growth rate (g)	0.019^{a}	0.020^{ab}	0.022^{b}	0.016^{a}	*
Feed conversion ratio	0.076^{bc}	0.070^{abc}	0.066^{ab}	0.083^{c}	*
Feeding period (days)	30	30	30	30	-

^{a,b} means with different superscript in the same row are significantly different $p<0.05$
FDRT= freeze dried ram testes . N.S= not significant; *= $p<0.05$

Table 3: Water quality during the growing period of fry of *Nile tilapia* (Nirwana)

Treatment	DO	pH	Ammonia	TºC
DRT30	7.5±0.23	8.1±0.15	0.01±0.02	25-27
FDRT60	7.8±0.47	8.1±0.02	0.01±0.001	25-27
FDRT90	7.8±0.23	8.1±0.013	0.01±0.002	25-27
Control	7.8±0.34	8.3±0.06	0.01±0.001	25-27
Standared	>5 mg/L	6.5-8.5 mg/L	0.2 mg/L	25-30

FDRT= freeze dried ram testes . DO= dissolved oxygen.
T = temperature

Table (4) :The percentage of sex reversal of Niletilapia after 30 days treatment period.

Treatment	Male %	Survival rate
FDRT 30ng/dl	60.00 ± 3.85^{a}	95.13 ± 0.69^{bc}
FDRT 60ng/dl	71.11 ± 5.87^{ab}	90.97 ± 1.38^{abc}
FDRT 90ng/dl	75.55 ± 2.22^{b}	85.41 ± 3.18^{a}
Control	55.55 ± 5.87^{a}	97.91 ± 0.00^{c}

FDRT= freeze dried ram testes

Discussion

Performance of Nile tilapia during the experimental period

The growth rate of Nile tilapia in this study increased significantly with increase of the level of testicular tissue inclusion level. The analysis of variance shows a significant difference ($p< 0.05$) among the treatment at 5% probability level of DMRT. After the 30-day treatment period, result revealed that Nile tilapia fry fed with freeze dried ram testes 90 ng/dl obtained the highest growth rate among other treatments with mean 0.022. The untreated group (Control) obtained the lower growth rate with a mean of 0.016 which significantly differed from the group treated with high level of freeze dried ram testes and there is no difference with groups fed with 30 and 60 ng/dl of FDRT. The result refer that the animal meal contains high level of protein which turn to apparent high growth rate of Nile tilapia fry fed with freeze dried testes diet from ram. However, this could possibly be due to increased testosterone effect which is a known anabolic agent for improvement in the quality of dietary protein. Several studies were in agreement with this result that testosterone produce muscle hypertophy by increasing muscle protein synthesis (Bhasin *et al* 2001). Also Mishirgi and Yousif (1988) who found feeding lamb testicular meal to tilapia resulted in increasing growth rate. Fasina *et al* (2008) found that fish fed with goat testes meal grew faster than control group fed with commercial diet. Saber *et al* (2004) who found feeding ram testes meal to fish it supported fish growth in diets rich in ram testes. Feed conversion ratio (FCR) improved with by the increase of dietary FDRTand FDBTwhich could be due to the improvement in the rate of body gain in tilapia fish. Feed conversion ratio ranged from 0.083 obtained in control group to 0.056 obtained in FDBT 90 ng/dl was significantly different ($p< 0.05$) compared with other groups.This finding was ingreement with Ramjie *et al* (2013) who obtained high growth rate with fry fed with bull carabao and boar testes compared with control group. The result was agreed with Seazar *et al* (2013) who found that feeding freeze-dreid bull testes resulted in high gowth rate compared with untreated fry. The high growth rate confirmed by the finding of earlier studies regarding the effect of 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.

The reading of the water quality during experimental period was found within tolerable limits of tilapia aquaculture (Bardach *et al.,* 1972). Pheleps 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 is 6.0- 9.0. Phelps and Popma (2000) stated that obtimum temperature for sex reversal of Nile tilpia falls in between 26-28^{0}C.

Survival rate

The data on the survival rate of Nile tilapia during experimental period (30 days) is shown in Table (4).The analysis of variance of survival rate shows that there was a significant difference among treatments ($p>0.05$) after expermintal period. The highest survival rate obtained in control group with mean 97. 91% this was followed by those fry fed with FDRT30, FDRT60 and FDRT90 ng/dl with means 95.13±0.69, 90.97±1.38 and 85.41±3.1%, respectively. The fry fed with FDRT90 ng/dl obtained lowest survival rate due to high level of hormone in the feeding which lead to decrease of the water quality in the aquarium during night. The high survival rate of fry of Nile tilapia obtained in this study agreed with Odien *et al*., (2009) who obtained high survival rate (92.27±0.02 - 86.93±0.08%) with fry of Nile tilapai fed with different lyophilized testes from animal. White (2008) obtained high survival rate (88-

95%) of fry fed with different animal testes. The survival of fry during sex reversal treatment is dependent on factors such as feeding, temperature, stocking density and other environmental conditions (Bocek *et al* ., 1992).

Percentage of sex reversal

The fry of Nile tilapia (sp. Nirwana) fed with FDRT 90ng/dl obtained the highest percent male with a mean 75.55± 2.22% followed by fry fed with FDRT60, FDRT30 ng/dl and control with means 71.11±5.87, 60± 3.85, and 55.55±5.87%, respectively. The treatments fed with high level of freeze dried ram testes were significantly different ($p<0.05$) compared with control group. Following the chi-square test ($\alpha \leq 0.05$) it was found that the fresh dried ram has a significant effect on the masculinization of Nile tilapia fry. These results are in agreement with Phelps *et al* (1996) who reported that the percentage of sex reversal produced from lyophilized testes from animal is 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) where 61.33, 57 and 53% males were obtained from 23-day treatment period of dehyrated hog, carabao and cattle testes, respectively. This study agreed with Saezar *et al.,* (2011) who reported that fed fry of Nile tilapia with dried bull testes at different percentage mixed 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 population is lower compared with that obtained by Ramjie *et al.* (2013) 80.67; 79.33; 72.67 % sex reversal produced in out door tanks from lyophilized testes from bull, boar and caraboa, respectively. However, this result is lower compared to reported 85% male population, of sex reversal of Nile tilapia fry fed with fresh ram testes for 80 days (Haylor and Pascual, 1991). Also compared to 93% phenotypic males produced from *ad libtum* feeding of tilapia fry fed with frozen bull testes for 30 days treatment period (White, 2008) and to the reported 87, and 83% male population of Nile tilapiafry fed with *ad libtum* fresh bull testes and fresh hog testes, respectively. According to Phelps and Popma (2000) the age and size of fry and the environmental factors such as temperature can effect growth of gonadal differentioation and in turn the treatment duration needed. These findings were in agreement with Mishrigi and Yousif (1988) who fed lamb testicles meal to tilapia fish and found that the male population in fish fed 50% lamb testicles (gave 72%); fish fed 25% lamb testicles (gave58%) and the control group gave 35%. Also this result was in agreement with 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 study showed that the high percentage of male popuation is related to the high level of concentration of hormone in the diet. The significant difference of phynotypic male and intersex in treatment at level of 90ng/dl freeze dried ram testes were effective in sex reversal of Nile tilapia fry.

Conclusions

Freeze dried ram testes resulted in masculinization of *Nile tilapia* fry after 30-day treatment period but the precent of male population was not high as synthetic hormone. The sex reversal rates of tilapia fry using freeze dreid ram testes were found to be higher than other treated fry. Freeze dried ram testes gave higher growth rate and feed conversion ratio. Therefore, develop a procedure for use of freeze dried testes from animal to reduce the risk caused by using synthetic hormone is considered for future investigation.

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