EFFECT OF LYOPHILIZED TESTES FROM BULL (*Bos indicus*), RAM (*Ovis aries*), AND BUCK (*Cabra hireus*) ON OCCURRENCE OF PHENOTYPIC MALE IN NILE TILAPIA (*Oreochromis niloticus*) FRY

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ABSTRACT
The effect of lyophilized testes from a bull, ram, and buck on masculinization, growth, and survival of Oreochromis niloticus was investigated. A commercial diet was supplemented with the lyophilized bull, ram, and buck testes at 60, 80 and100 mg/kg diets, Control (CT1) was not supplemented while Control (CT2) was supplemented with 40mg/kg Methyl-testosterone. Diets were fed to satiation daily for 28 days to triplicate groups of randomly distributed O. niloticus fry. The feeding trial was continued with commercial diets for 12 weeks. In the end of which fifty fish were randomly selected from each aquarium for phenotypic male and female determination. Mean final weights and survival rates were recorded. Percentage phenotypic male was significantly highest (P<0.05) in fish fed bull testes treated diets (60 mg/kg=82.46%; 8 0mg/kg=82.35% and100 mg/kg=82.93%) and least in the buck groups (60 mg/kg=36.98%; 80mg/kg=36.97% and 100 mg/kg=36.69%). Intersex was highest in the buck groups (60 mg/kg=45.78%; 80 mg/kg=46.13% and 100 mg/kg=46.38%), while the bull and CT1 groups recorded none. MWG and SGR were significantly high (P<0.05) in CT2 than in other groups. The result of this study revealed that lyophilized testes of bull successfully masculinized O. niloticus

Keywords: Sex-reversal, intersex, tilapia, feed, hormone.

INTRODUCTION

Global production of tilapia has grown to over 5 million tonnes in the mid-2010s with Africa only accounting for 20% of this (FAO, 2017). Nile tilapia production in sub-Saharan Africa accounts for less than 4% of global production. However, the species is essentially an important one in aquaculture. According to FAO (2018), Nile tilapia occupied the 4th position contributing 8% of total aquaculture production coming next to the carps. This position is attributed to its tolerance to adverse environmental conditions; survival in low dissolved oxygen, fluctuating salinity, relatively fast growth, and efficient food conversion (Penna-Mendoza et al, 2005). Despite these attributes, one major impediment in tilapia production at a commercial scale is its precocious reproduction and utilization of most of its energy for gonadal developments instead of somatic growth. This problem is however addressed using a mono-sex production system.

According to Angienda *et al* (2010), male mono-sex cultures are preferred to female ones because of the differential growth in favour of males as it channels metabolic energy towards growth. The ability of male tilapia to also benefit from anabolism enhancing androgens, as reported by Fontainhas-Fernandes *et al*. (1994) also makes it preferred. Stocking of all-male will result in a faster-growing population because the production of offspring is eliminated, and so, problems associated with competition for nutrients, space, and light due to overpopulation arising from prolific breeding are overcome.

To achieve an all-male population, Anderson *et al* (2003) reported that increasing the sex hormone level at an early stage affects the final sex of a fish population independent of the genetic sex. This is because the early period (fry stage) is characterized by a sexually underdeveloped fry. Therefore, monosex production is achievable through the supplementation of diets with reproductive hormones to achieve sex-reversal. Other techniques for mono-sex production include manual sexing, hybridization, and genetic manipulation (Gupta and Acosta, 2004).

At present, various natural and synthetic hormones have been used in the production of masculinized tilapia, in which oral administration of synthetic hormones is most common (El-Sayed, 2006). However, the consumption of steroid-treated tilapia has raised some concerns in the wake of this cultural practice. The use of synthetic hormones has been under increasing public criticism due to their possible health and environmental impacts (Phelps and Carpenter, 2002). Therefore there is the need to search for non-synthetic (natural) hormonal sources for sustained production of properly-sized tilapia for human consumption.

Natural sources of testosterone that are locally available, as feed supplements may provide alternatives to synthetic androgens, which also is an anabolic steroid for tilapia sex reversal. Varying levels of success (ranging from 65-86%) have been reported on the use of the testes of some animals (Bull, hog, carabao, and boar) as a feed supplement for tilapia sex-reversal (Meyer *et al*., 2008; Odin and Boliver, 2011; Orose *et al*., 2018). There is little information on the use of testes from ram and buck as a feed supplement for sex reversal. This study is therefore aimed at evaluating lyophilized testes from

a bull, ram, and buck in the masculinization of *Oreochromis niloticus* and the effect on the growth and survival of this species.

MATERIALS AND METHODS

Breeding families (2 males with mean weight 200±5.13 g and 4 females with mean weight 70±2.52 g) were stocked into 1x1x1 m Hapas with 1.6 mm mesh size situated in concrete tanks at the Wet Laboratory of the Department of Fisheries and Aquaculture Management, University of Ibadan. The temperature was maintained at 28-30^{o}C while breeding activity was monitored daily. After 2 months of feeding and monitoring, fertilized eggs were observed from the mouth of female broodstocks. Eggs were collected into a plastic aquarium (25 L capacity) and hatched using a high-pressure aerator (to oscillate the eggs till hatching). Hatchlings break and feed on their egg yolks for 6 days, during which no exogenous food was introduced. During this period, larvae swam to the surface and descended to the bottom by gravity. This changed to horizontal swimming when the yolk was absorbed. On the 6th day, one hundred fry were randomly stocked into plastic troughs each (25 L).

Feed preparation

Testes of sexually matured bull, ram, and buck (ages 3 years, 2 years, and 2 years respectively) were collected from a municipal abattoir. The testes were skinned and freed from the epididymis, weighed, sliced, and completely homogenized without dilution using an electric blender. The homogenized testes were then freeze-dried within 72 hours at -40^{o}C. The various lyophilized testes andthe synthetic hormone were dissolved in 5 ml 95% ethanol and sprayed on a commercial feed (45% crude protein <0.2 g mash Aqualis$^{@}$). The control (CT1) was also sprayed 5 ml 95% ethanol. These were air-dried and packed in labeled polyethylene bags. Fry were fed diets BL1 (Feed containing 60 mg bull testes/kg feed), BL2 (80 mg bull testes/kg feed), BL3 (100 mg bull testes/kg feed), RM1 (60 mg ram testes/kg feed), RM2 (80 mg ram testes/kg feed), RM3 (100 mg ram testes/kg feed), BK1 (60 mg buck testes/kg feed), BK2 (80 mg buck testes/kg feed), BK3 (100 mg buck testes/kg feed), CT1 (no supplement) and CT2 (40 mg/kg 17 α-methyltestosterone supplemented diet).

Diets were fed 5 times daily (satiation) to triplicate groups of fry for 28 days in a completely randomized design. After 28 days, fish were further fed commercial diets (45% crude protein <0.1 g mash) to satiation for 12 weeks. Fish per aquarium were bulk weighed biweekly and the feed size adjusted accordingly and survival was also monitored.

Sex determination

At the end of a 112-day culture period, sex was determined through a histological examination using the gonadal squash method (Guerrero and Shelton, 1974). Thirty-five percent of the fish population per treatment was sacrificed and the gonad was excised. Each gonad was stained using Harris' hematoxylin with a 10X dilution and then crushed by placing an additional slide on top and pressing the two together. These were examined under stereo-microscope at 100X magnification along the length of each gonad and recorded as male, female, or intersex.

Statistical analysis

The sex ratio was analyzed using frequency distribution while growth and survival rates were subjected to analysis of variance (ANOVA) using SPSS 22 software at a significance level of 95%. The variance of significance was verified using Duncan's test.

RESULTS

Testosterone concentration in the serum of animals used is presented in table 1. The bull serum had a concentration of 0.94 ng/ml, followed by buck serum with 0.76 ng/ml and the least concentration of 0.48 ng/ml recorded in ram serum.

Table 2 presents the percentages of sexes recorded at varying levels of testes supplementation compared with the control diets. The percentage of males was high in the group fed diets supplemented with bull testes as earlier mentioned. However, increased inclusion levels of either bull, ram, or buck did not result in any significant variation within treatments. Fish-fed diets without hormone supplement CT1 had a significantly higher percentage of males (62.69%) than those fed ram and buck testes at all inclusion levels.

The CT1 group had the highest percentage population of females 37.31%, closely followed by RM1, RM2, and RM3 (36.09, 36.26, and 36.77% respectively). The female population was significantly lower ($P<0.05$) in CT2 (15.04%). Increased inclusion levels of any of the three testes did not result in variation in the percentage of females among treatments.

No intersex was recorded in BL1, BL2, BL3, and CT1 groups. Intersex was however significantly high in BK1, BK2, and BK3 groups (45.78%, 46.13%, and 46.38% respectively), followed by RM2, RM1, and RM3 groups (16.63%, 16.56%, and 15.52% respectively).

After 28 days of feeding, final weight and mean weight gain varied significantly among treatments as presented in table 4. Final weight and mean weight gain (1.22 g and 1.21 g respectively) were significantly higher in CT2, while the least values were observed in the BL2 group (0.78 g and 0.77 g respectively). The specific growth rate ranged

between 16.86% in BL2 through 16.91% in BK1 to the highest value of 18.44% in The CT2 group. Percentage survival was significantly lowest (P<0.05) in CT1 (78.11%), with the highest values recorded in BL3 and BK3 (94.81% and 97.26% respectively).

DISCUSSION

The result from this present study shows the skewness of the general population, including fish, fed diets without supplement, towards more of the male except in the group fed buck testes that have more of intersex. Overall the mean percentage of males was highest in fish-fed bull testes. Despite the testosterone concentration been higher in buck serum compared to ram, it has a lower percentage of the male population. However, the intersex population in fish-fed buck testes was significantly higher. This is contrary to the findings of Costa and Paula (2006) where a positive and significant correlation between serum total testosterone and the volume of Leydig cells in the testes were reported. The values of serum total testosterone signify the capacity of the Leydig cells to secrete testosterone hormones in the animal testes.

Fish-fed bull testes had no intersex population, similar to the group without testes supplement. Lyophilized bull testes in this present study gave higher male populations in tilapia than values of 61.33, 57.0, and 53.0% from dehydrated testes of hog, carabao, and cattle respectively reported by Odin *et al* (2009). The use of heat in dehydrating testes diminishes testosterone level (Lue et al., 2000) and therefore may be responsible for the variation in the two studies, as testosterone in testes was preserved under very low temperature in this present study. Also, the percentages of phenotypic males from fish-fed diets supplemented with lyophilized bull testes are relatively higher than 65% males obtained from the same lyophilized testes fed ad-libitum to tilapia for 28 days reported in Phleps *et al* (1996). However, the percentage of males from the sex-reversed population obtained from buck and ram in this present study is lower than the 85% reported by Haylor and Pascual (1991) feeding Tilapia fry with fresh ram testes for 80 days, 95% reported by White (2008) feeding frozen bull testes ad-libitum for 30 days and 87% and 83% reported by Meyer et al (2008) using fresh bull and fresh hog testes respectively.

The result from lyophilized testes of buck, ram, and 17 Alpha MT showed a significant percentage of incomplete sex differentiation (Intersex). Intersex fish are described as having ovarium and testicle in one gonad, which could be as a result of androgen concentration in diet ordosage of testes as attested to by the work of Yustiati *et al* (2018) and Mateen and Ahmed (2007) where intersex was recorded in Nile tilapia fed graded level of bull testes.

Results from the hormonal assay would suggest an expected better performance from buck (with 0.76 ng/ml) in masculinizing *O. niloticus* than ram (with 0.48 ng/ml). However, the RM group had a higher male population than the BK. However, the latter had a higher mean intersex population, considering that the intersex population is assumed to be sterile.

Results of the SGR and MWG showed that mean weight gain and SGR were significantly higher (P<0.05) in the CT2 group, with the least values recorded in BL2. Despite the testes of animals' protein content which is said to contribute to fish growth in the works of Odin and Bolivar (2009), this cannot be validated from this present study as testes supplement did not positively affect growth, even though inclusion levels werestaggered. However, groups treated with lyophilizedtestes gave a better survival rate than the control groups. This signifies that the natural sources of thehormone have no negative effect on the fry compared to the synthesized hormone. The highsurvival rate in the present study agrees with the findings of White (2008) where fry was fed with animal testes in outdoor tanks for 30 days. Water quality parameters of all the rearing troughs were maintained at pH (7.2-7.8), dissolved oxygen (4.5-6.5 mg/l), and temperature (25-28^{0}C). These fall within the recommended range suggested by El-Sayed (2006) for tilapia culture. Similarly, the recommended optimum temperature of 26-28^{o}C (Phelps and Popma, 2000) for tilapia sex reversal is adhered to in this study.

Conflict of Interest

The authors declare that they have no conflict of interest in this work.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors.

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Table 1: Total testosterone in the serum of bull, ram, and buck (ng/mL ± SEM)

Treatment	Testosterone ng/ml
Bull	0.94±0.02
Ram	0.48±0.00
Buck	0.76±0.00

Table 2. Percentage sex of fish at varying inclusion levels of bull, ram, and buck testes.

Treatment	Male (%)	Female (%)	Intersex (%)
BL1	82.46±0.29^{e}	17.51±0.26^{bc}	0.00±0.00^{a}
BL2	82.35±0.37^{e}	17.62±0.32^{c}	0.00±0.00^{a}
BL3	82.93±0.08^{e}	17.10±0.06^{bc}	0.00±0.00^{a}
RM1	47.33±0.27^{b}	36.09±0.06^{d}	16.56±0.22^{cd}
RM2	47.11±0.57^{b}	36.26±0.44^{d}	16.63±0.95^{d}
RM3	47.70±0.39^{b}	36.77±0.38^{de}	15.52±0.42^{c}
BK1	36.98±0.05^{a}	17.22±0.49^{bc}	45.78±0.16^{e}
BK2	36.97±0.02^{a}	16.89±0.32^{bc}	46.13±0.34^{e}
BK3	36.69±0.30^{a}	16.67±0.19^{b}	46.38±0.27^{e}
CT1	62.69±0.30^{c}	37.31±0.29^{e}	0.00±0.00^{a}
CT2	72.76±0.40^{d}	15.04±0.15^{a}	12.22±0.27^{b}

Mean values with the same superscript along column are not significantly different (p>0.05)

Table 3. Some growth parameters of *Oreochromis niloticus* fed diets for 28 days

Treatment	Initial Wgt (g)	Final Wgt (g)	MWG (g)	SGR (%)	Survival (%)
BL1	0.006	0.821±0.00^{g}	0.814±0.00^{g}	17.54±0.02^{b}	88.92±0.03^{de}
BL2	0.007	0.782±0.00^{h}	0.773±0.00^{h}	16.82±0.01^{e}	92.18±1.14^{bcd}
BL3	0.008	1.014±0.01^{c}	1.006±0.01^{c}	17.15±0.11^{cd}	94.81±0.74^{ab}
RM1	0.007	1.021±0.01^{c}	1.013±0.01^{c}	17.62±0.25^{b}	90.37±1.48^{cde}
RM2	0.007	0.843±0.00^{f}	0.835±0.00^{f}	17.10±0.00^{cde}	87.74±1.07^{ef}
RM3	0.008	0.991±0.00^{d}	0.984±0.00^{d}	17.21±0.00^{c}	91.41±0.30^{bcd}
BK1	0.008	1.045±0.00^{b}	1.036±0.00^{b}	16.91±0.06^{de}	93.95±0.62^{b}
BK2	0.007	1.005±0.00^{cd}	0.998±0.00^{cd}	17.78±0.10^{b}	93.33±0.00^{bc}
BK3	0.006	0.827±0.00^{g}	0.820±0.00^{fg}	17.55±0.03^{b}	97.26±0.26^{a}
CT1	0.007	0.936±0.00^{e}	0.929±0.00^{e}	17.50±0.02^{b}	78.11±2.55^{g}
CT2	0.007	1.225±0.00^{a}	1.218±0.00^{a}	18.44±0.00^{a}	84.87±0.43^{f}

Mean values with the same superscript along column are not significantly different (p>0.05)

Highlights

Bull testes supplement resulted in 83% male population in fish fed diets. However, buck testes had a more intersex population showing it could not complete the masculinization of *O. niloticus.*