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Effect of keeping 17α-methyl testosterone hormonal feeds at different ambient temperatures on mono-sex male tilapia fingerlings production

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Abstract
Early expansion of tilapia culture was limited as tilapia sexually matures at an early age, which results in overcrowding and in stunted growth in culture systems such as ponds and tanks. Methods for culturing exclusively mono-sex male tilapia have been developed to address these problems and until now the commonest of these is by treating hatchling with a male hormone, such as methyl testosterone (MT) or ethyl testosterone (ET) before the primal gonadal cells of females are differentiated into ovarian tissue. The effect of keeping 17α-MT feeds at different ambient temperatures (i.e. refrigerator, room and sun; designated FHF, RHF and SHF respectively) on mono-sex male tilapia fingerling production was investigated. Swim-up Nile tilapia fry at initial mean weight of 0.03 ± 0.00 g were stocked at a density of 1 500 per m^3 in 12 hapas, each of dimensions 1.0 x 1.0 x 1.0 m. The hapas were randomly put into four groups, with each consisting of three hapas. Each group of fry was separately fed with each feed type whilst the last group was fed with a non-hormonal feed (NHF) at a two-hour time interval for 28 successive days. The fry were then transferred to 12 larger hapas, each of dimensions 5 x 2 x 1.2 m and they were all fed with a non-hormonal feed at *ad libitum* until they attained sizes ≥ 20 g for sexing. Percentages of males recorded in FHF, RHF and SHF were similar (90.24 ± 2.01%, 91.34 ± 0.95% and 92.49 ± 0.42% respectively) and they were significantly higher (Tukey's HSDT, $P <$ .05) than that (64.90 ± 3.32%) of NHF. Hence, keeping 17α-MT feeds at different ambient temperatures did not have effect on mono-sex male tilapia fingerling production.

Keywords: testosterone hormonal, mono-sex, fingerlings

1. Introduction
Formerly, production of relatively large-sized tilapia in culture systems such as ponds was highly limited as tilapia sexually matures at an early age, resulting in overcrowding and stunted growth.
This problem has been addressed due to different methods such as manual sexing, hormonal administration and hybridization that have been developed for the production and culturing of exclusively mono-sex male tilapia. Of these methods, hormonal administration has been found to be easy and most effective [1, 2]. Of the hormones, the synthetic 17α-methyl testosterone (MT) has been widely used [1, 3]. The hormone administration involves treating the fry with a male hormone, such as methyl testosterone (MT) or ethyl testosterone (ET) before the primal gonadal cells of females are differentiated into ovarian tissue. Production of mono-sex males is preferred to that of the females as the males grow and reach a larger ultimate size faster than the females. Culturing of mono-sex fish species has been recognized as the most effective way of avoiding early sexual maturation and uncontrolled reproduction in culture systems [4, 5, 6].
Currently, the most widely cultured fish species in the country is the Nile tilapia, *Oreochromis niloticus*. However, the main problem with the culturing of this fish, just like that with other tilapia species, is its early sexual maturation and prolific breeding ability. These characteristics of the fish bring about overpopulation and crowding of stocked mixed-sex fish ponds resulting in stunted fish [4, 5, 6]. Another problem associated with the culturing of the mixed-sex stock is a wide variation in sizes of the males and the females at harvest. Hence, for the production of relatively uniform and large-sized fish within a reasonable time period, stocking of all male tilapia fingerlings in culture systems, particularly ponds is recommended.
The standard hormone treated procedure for tilapias involves adding MT to powdered fry/starter tilapia feed, which is then administered to batches of fry of similar age during the short period of their early development when they are most susceptible to the masculinisation effect of this hormone [7]. Treatment with MT begins from the second or third day after the fry are released from maternal care. The first-feeding (and still sexually undifferentiated) tilapia fry are fed with the powdered feed containing 30-60 mg MT/kg of the feed. The MT hormone-incorporated feed is fed to the fish between 1 to 2 hours time interval during the day for 28-31 days. A hormonal feed is usually prepared by dissolving the measured hormone in an appropriate quantity of ethanol (95-99%). Then the hormone solution is mixed thoroughly with the feed until all the feed is moist. The moist feed is air-dried as the hormone is known to break down when it is exposed to direct sunlight or high temperatures. Although most hatchery operators adhered to this protocol in the hormonal feed preparation, yet after air-drying of the feed, the dried feed is mostly kept in the hot sun in the field close to the facilities in which the fry are fed. This practice may contribute to a significant number of female fish observe in harvested fish in grow out facilities, especially in cages. For ponds, the consequence is obvious by the excessive breeding of stocked purported all-male fingerlings. Hence, the present study was designed to investigate the percentage of male tilapia fingerlings that were produced from a batch of hatchery hatched tilapia fry fed with MT hormonal feeds kept at three different temperature conditions, namely in a refrigerator, in a room and in the sun/field during the feeding period. The

overall objective of the study was to assess the efficacy of 17α-methyl testosterone hormonal feeds in mono-sex male tilapia fingerling production when the feeds were kept under different temperature conditions.

2. Materials and Methods

2.1 Study area

The evaluation of the commercial tilapia feeds with varying crude protein levels was conducted at the Aquaculture Research and Development Centre (ARDEC) of Water Research Institute (WRI) of the Council for Scientific and Industrial Research (CSIR), Ghana. The area lies between latitude 6° 13′ North and the longitude 0° 4′ East at Akosombo in the Eastern Region of Ghana.

2.2 Hormonal feed preparation

Exactly 60 mg of 17α-methyl testosterone was dissolved in 100 ml ethanol (95%) and the resulted solution was mixed thoroughly with a pre-milled and sieved 40% crude protein commercial tilapia feed (*Raanan*) at a rate of 60 mg hormone per kilogramme of the feed. The moist feed was spread thinly on a clean and dry surface for air-drying. In all, 6 kg of the hormonal feed was prepared. The hormone-mixed dry feed was divided into three equal portions (2 kg each) into separate plastic containers. The containers were labelled accordingly and they were kept separately in a refrigerator (FHF) at a constant temperature of 4 °C, in a room (RHF) and in the sun (SHF) throughout the feeding (treatment) period.

2.3 Pond preparation and hapa installation

An earthen pond of 0.2 ha in size at the Aquaculture Research and Development Centre (ARDEC), Akosombo was used for the study. It was treated by liming and then filled with water from the Volta Lake in about a week's time. This was followed by installation of twelve (12) hapas each of dimensions 1.0 x 1.0 x 1.0 m (1.0 m^3). Each hapa was separated from others at a distance of about 2.0 m to avoid easy drifting of contents of one system into another and to enhance water exchange [8]. About four-fifths (0.8 m) of the hapa heights were constantly submerged in the pond water by ensuring periodic topping up of the water when the level fell due mainly to evaporation and seepage.

2.4 Collection, incubation of eggs and stocking of fry

Fertilized eggs were collected from the mouth of female Nile tilapia breeders and the eggs were subsequently incubated in the hatchery until they were hatched and their yolk sacs were completely absorbed. The mean weight of the swim-up fry was determined by counting and putting 100 individuals in a pre-weighed container containing water using a digital electronic balance. The mean weight was calculated as follows:

$$\text{Mean weight of fry (g)} = \frac{(W_{cw} + W_f) - W_{cw}}{100}$$

Where:
W_{cw} = weight of container and water in grams
W_f = weight of fry in grams

The procedure was repeated five times, and in all cases, the mean weight was found to be the same (i.e. 0.03 g). A weight of exactly 45.0 g (i.e. 1 500 fry) was measured and stocked in each of the 12 hapas.

2.5 Hormonal treatment and sampling of fry

Feeding of the stocked fry commenced on the day following stocking with the four feed types namely, a hormonal feed kept in a refrigerator (FHF), a hormonal feed kept in a room (RHF), a hormonal feed kept in the sun (SHF) and a non-hormonal feed (NHF), the control. Each feed type was randomly assigned to three hapas. The fry in each of the treatments were fed at 20% of their body weight five times daily at two hours interval commencing at 0800 GMT for 28 successive days. Weight gain by the fry was determined weekly by bulk weighing of the survived fish in each hapa of each treatment. On the day following the 28th day of feeding, all the fish in each hapa of each treatment were removed from the various hapas and the weights and numbers were determined.

2.6 Post hormonal treatment nursing of fry

The post-treated fry were transferred to 12 larger (5.0 x 2.0 x 1.2 m) hapas which were labelled accordingly. The fry were fed with a non-hormonal 40% crude protein feed at *ad libitum* until they attained sizes ≥ 20.0 g for manual sexing so as to determine the proportions of males and females (sex ratio) in each treatment. The fish from each treatment were sexed by checking the genital papillae with the help of a magnifying glass. Five per cent (5%) of each sex group from all treatments was randomly sampled and sacrificed using a lethal dose of anesthetic (Benzocaine, Sigma). After which the sex of each group of each treatment was confirmed by microscopic analysis of gonad squash [9].

2.7 Measurement of temperature and water quality parameters

The ambient temperatures of the room and the field/sun where the feeds were kept were measured three times daily (0800, 1200 and 1600 hours) during the study period. On each sampling day, temperature, dissolved oxygen (DO), pH, nitrite, total ammonia and total alkalinity of the water within and outside the experimental hapas were measured. Temperature was measured with a thermometer (TESTO 110). DO was measured with oxygen meter (YSI Environmental model no: DO 200) and pH was measured with a pH meter (Hanna model no: HI 98128). Nitrite and total ammonia were measured using a spectrophotometer (UV mini-1240). Total alkalinity was measured using a digital titrator (HACH).

2.8 Growth performance of Nile tilapia Fry

The growth performance of the tilapia fry under each treatment was determined using the following formulae:
Mean weight gain (g) = final mean weight - initial mean weight

$$\text{Daily mean weight gain (g day}^{-1}) = \frac{\text{final mean weight - initial mean weight}}{28 \text{ days}}$$

$$\text{Mean specific growth rate (\% day}^{-1}) = 100 \times \frac{[\ln(\text{final mean body weight}) - \ln(\text{initial mean body weight})]}{28 \text{ days}}$$

$$\text{Mean feed conversion ratio} = \frac{\text{quantity of feed fed (g)}}{\text{live weight gain (g)}}$$

$$\text{Mean survival rate (\%)} = \frac{\text{mean number of fry harvested}}{\text{mean number of fry stocked}} \times 100$$

2.9 Data analyses

The data on fish growth performance, ambient temperature of hormonal feeds and water quality parameters were tested for normality using the Kolmogorov-Smirnov test and homogeneity using the Levene's test so as to establish normality and homogeneity of the data. All percentages and ratios were arcsine transformed to normalize the data before analyses. Statistical analyses were carried out using one-way analyses of variance (ANOVA) to test differences among the various parameters measured. Tukey's honest significant difference test (THSDT) was used to identify specific differences between pairs of treatments. Differences were considered significant when $P \leq .05$.

3. Results and Discussions

3.1 Storage of hormonal feeds

The temperature of the refrigerator was maintained at 4 °C throughout the feed storage period during the study. However, recorded temperatures in the room and in the sun (field) varied during the period. The minimum figures recorded were 25.5 and 23.7 °C for room and field respectively whilst the maximum were 36.1 and 40.7 °C respectively (Table 1).

Table 1: Minimum, maximum and mean figures (± Standard Deviation, SD) of the ambient temperatures (°C) recorded in the room and in the field/sun during the 28 days of hormonal feeding to Nile tilapia fry

Location	Min. Temp. (°C)	Max. Temp. (°C)	Mean Temp. (°C)
Room (RHF)	25.5	36.1	30.4 ± 2.4
Field/Sun (SHF)	23.7	40.7	29.2 ± 1.9

During the study the lowest (4 °C) temperature was recorded in the refrigerator whilst the highest (40.7 °C) was recorded in the sun (field). This suggest that the feed (SHF) kept in the sun had the greatest exposure to ambient temperature than the others (FHF and RHF).

3.2 Water quality parameters

The minimum, maximum and mean figures (± Standard Deviation) of water quality parameters recorded during the feeding of the mixed-sex *O. niloticus* fry with the hormonal and the non-hormonal feeds are shown in Table 2.

Table 2: Minimum, maximum and mean figures (± SD) of the water quality parameters recorded in the various hormonal and non-hormonal feed treatments during the 28 days of feeding to Nile tilapia fry

Parameter	Minimum	Maximum	Mean (± SD)
Temperature, °C	27.9	33.8	31.2 ± 0.3
pH	5.73	7.00	6.41 ± 0.33
DO, mg L^{-1}	3.5	8.5	6.3 ± 1.6
Nitrite, mg L^{-1}	0.012	0.018	0.010 ± 0.002
Total ammonia, mg L^{-1}	0.44	0.63	0.50 ± 0.04
Total alkalinity, mg L^{-1}	73.0	87.0	81.0 ± 0.4

Temperature ranged from 27.9 to 33.8 °C, pH ranged from 5.73 to 7.00, DO ranged from 3.5 to 8.5 mg L^{-1}, Nitrite ranged from 0.012 to 0.018 mg L^{-1}, Total Ammonia ranged from 0.44 to 0.63 mg L^{-1} and Alkalinity from 73.0 to 87.0. The mean figures were 31.2 ± 0.03 °C, 6.41 ± 0.33, 6.3 ± 1.6 mg L^{-1}, 0.010 ± 0.002 mg L^{-1}, 0.50 ± 0.04 mg L^{-1} and 81.0 ± 0.4 mg L^{-1} for Temperature, pH, DO, Nitrite, Total Ammonia and Alkalinity respectively. There were no significant differences (ANOVA, $P > 0.05$) among recorded water quality parameters within and among the various treatments. The water quality parameters recorded in all the treatments during this study were consistent with the standard range of ideal water for tilapia rearing [10]. Hence, the water temperature and the chemical quality prevailed in the hapas during the study did not have any adverse effect on the survival, growth performance and well being of the experimental fish.

3.3 Growth performance and feed utilizations of Nile tilapia fry

The growth performance of mixed-sex Nile tilapia fry fed with both the hormonal and the non-hormonal feeds in terms of final mean weights, mean weight gains, specific growth rates, feed intake, feed conversion ratios, feed efficiency and survival rates are shown in Table 3.

Table 3: Growth performance and feed utilization of mixed sex Nile tilapia fry fed with hormonal feeds kept separately in a Refrigerator (FHF), in a Room (RHF), in the Sun (SHF) and a non-hormonal one (NHF) for 28 days

Parameter	Feeds			
	FHF	RHF	SHF	NHF
Initial Mean Weight (g)	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0	0.03 ± 0.0
Final Mean Weight (g)	0.29 ± 0.0	0.28 ± 0.0	0.30 ± 0.0	0.29 ± 0.0
Mean Weight Gain (g)	0.26 ± 0.1	0.25 ± 0.1	0.27 ± 0.3	0.26 ± 0.2
Mean Specific Growth Rate (% day^{-1})	0.08 ± 0.0	0.08 ± 0.0	0.08 ± 0.0	0.08 ± 0.0
Mean Feed Intake (g $fish^{-1}$)	0.31 ± 0.1	0.28 ± 0.2	0.30 ± 0.1	0.29 ± 0.1
Mean Feed Conversion Ratio	1.2 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.1
Mean Feed Efficiency (%)	83.3 ± 0.1	90.9 ± 0.1	90.9 ± 0.1	90.9 ± 0.1
Mean Survival Rate (%)	72.8 ± 13.9	70.6 ± 10.4	73.2 ± 7.2	71.4 ± 11.2

The recorded figures for each parameter among the treatments were similar and there were no significant differences (ANOVA, $P > 0.05$) among them. Final mean weights ranged from 0.28 to 0.30 g, mean weight gain ranged from 0.25 to 0.27 g, specific growth rate was the same (0.08 % day^{-1}) in all treatments, feed intake ranged from 0.28 to 0.31 g $fish^{-1}$ and survival ranged from 70.6 to 73.2%. The similarity in growth performance observed between both hormone and non-hormone treated *O. niloticus* fry at the end of the 28 day period in this study suggests that the hormone did not have any effect on growth and survival of the fry. This observation agreed with the findings of Pechsiri and Yakupitiyage [11] who found that there were no significant differences ($P > 0.05$) in final mean weight (g), specific growth rate (%day^{-1}), feed conversion ratio and survival rate of mono-sex diploid and triploid *O. niloticus.* Similar results

were recorded on Muskellunge, *Esox masquinongy* [12].
The weekly growth pattern of the treated and the untreated *O. niloticus* fry is demonstrated in Figure 1. At the commencement of feeding, the mean weights of the fry in all the treatments (SHF, RHF, FHF and NHF) were the same (0.03 g).

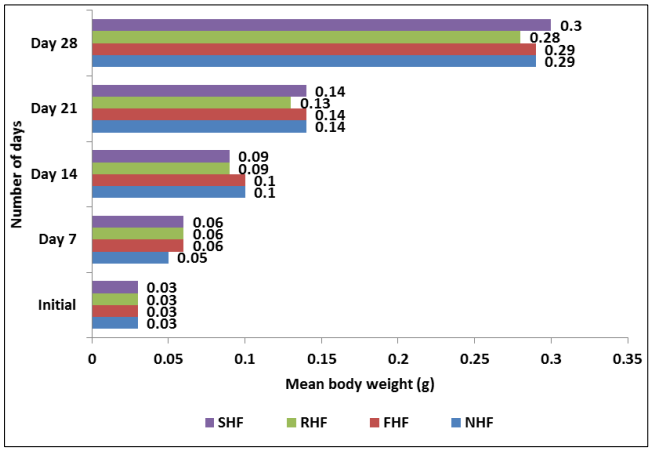

Fig 1: Growth pattern of mixed-sex Nile tilapia fry fed with 17α-methyl testosterone hormonal feeds kept separately in a Refrigerator (FHF), in a Room (RHF) and in the Sun (SHF) and that of non-hormonal (NHF) for 28 days

On the seventh day, all the mean weights were 0.06 g each, except that of NHF which was 0.05 g. On the fourteenth day, the mean weights of RHF and SHF were 0.09 each whilst those of FHF and NHF were 0.10 g each. On the twenty-first day, all the treatments were 0.14 g each except RHF which was 0.13 g. On the twenty-eighth day, RHF was 0.28 g, SHF was 0.30 g whilst both FHF and NHF were 0.29 each. Results of this study disagreed with those of others. Even though some researchers agreed that growth of treated fry of *O. niloticus* with 17α-methyl testosterone is superior to the untreated one [13, 14, 15, 16], this was not observed in the present study as there was no significant differences in growth parameters in both the non-hormonal and the hormonal treatments. The results of this study agreed with that of [17], who did not observe any significant differences ($P < 0.05$) among growth parameters between control and treated tilapia fry. Similar findings were reported by Phelps *et al.* [18], Smith and Phelps [19], Celik *et al.* [20], Junior *et al.* [21] and Kefi *et al.* [22]. In addition, the experiments conducted by Soto [23] on *O. niloticus* and Hossain *et al.* [24] on *Clarias gariepinus* showed no evidence of enhanced growth when androgens were administered. The results of this study also disagree with the findings of Robles Basto *et al.* [25], who stated that the use of androgenic hormone in mono-sex fish production has anabolic effect that enhances growth and protein synthesis, resulting into greater muscle mass gains. The similarity in the final mean weights observed in all the treatments in the current study, could be due to the short culture period (28 days), which agreed with findings of Anani and Agbo [26].

3.4 Sex ratio of Nile tilapia

The results of the study indicated that the percentage (90.24 to 92.49%) of males in the hormonal treated groups deviated significantly (Tukey's HSDT, $P <. 05$) from that (64.90%) of the non-hormonal treated (NHF) group. However, there was no significant differences (ANOVA, $P > 0.05$) among percentage of males obtained from the hormonal treatments in which the feeds were kept at different ambient temperatures, namely in a refrigerator (FHF), in a room (RHF) and in the sun, SHF (Table 4). The percentage of males and females is graphically shown in Figure 2.

Table 4: Percentage (± SD) of males observed in each treatment

Treatment	Number of Fish Examined*	Percentage Males ± SD
Refrigerator, FHF	858	92.49 ± 0.42[a]
Room, RHF	1 640	90.24 ± 2.01[a]
Field/Sun, SHF	1 149	91.34 ± 0.95[a]
Non-Hormonal, NHF	1 266	64.90 ± 3.32[b]

*Represents the total number of fish which survived in each treatment during the post hormonal treatmentnursing of fry in hapas

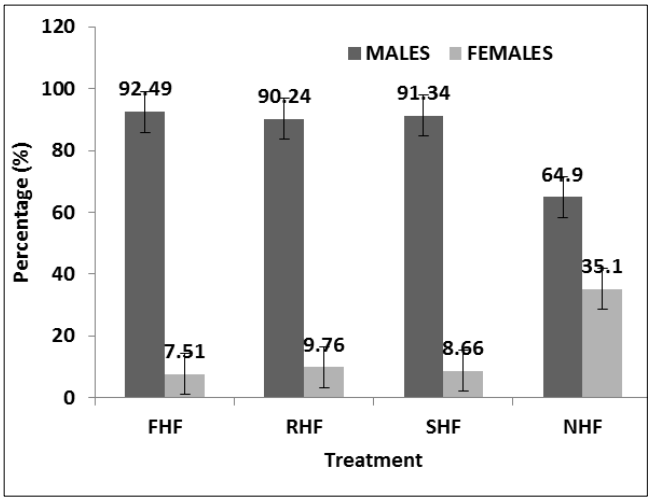

Fig 2: Percentage of males and females Nile tilapia obtained following feeding the fry with non-hormonal feed (NHF) and hormonal feeds kept separately in a Refrigerator (FHF), in a Room (RHF) and in the Field/Sun (SHF) for 28 days

The percentage (90.2 to 92.5%) of males recorded in the various hormonal treatments in this study was close to the 94.4% observed by Kefi *et al.* [27] in *O. andersonii*. However, the results were lower than the 97.8% males reported by Phelps *et al.* [18] and the 96.8% males observed by Green and Teichert-Coddington [1], who conducted their study in hapas for 28 days at 60 mg MT/kg feed for *O. niloticus* which was similar to the protocol of the current study.

4. Conclusion

The hormonal tilapia feed kept in the refrigerator, (FHF) had the highest (92.49 ± 0.42%) percentage of males, whilst that of the room (RHF) had the least (90.24 ± 2.01%). However, no significant differences (ANOVA, $P > 0.05$) were found among treatments (FHF, RHF and SHF). The percentages of males recorded in the hormonal treated feeds were significantly higher (Tukey's HSDT, $P < 0.05$) than that (64.90 ± 3.32%) of the non-hormonal (NHF). Hence, keeping 17α-methyl testosterone hormonal feeds at different ambient temperatures did not have effect on mono-sex male tilapia fingerlings production.

5. Acknowledgement

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