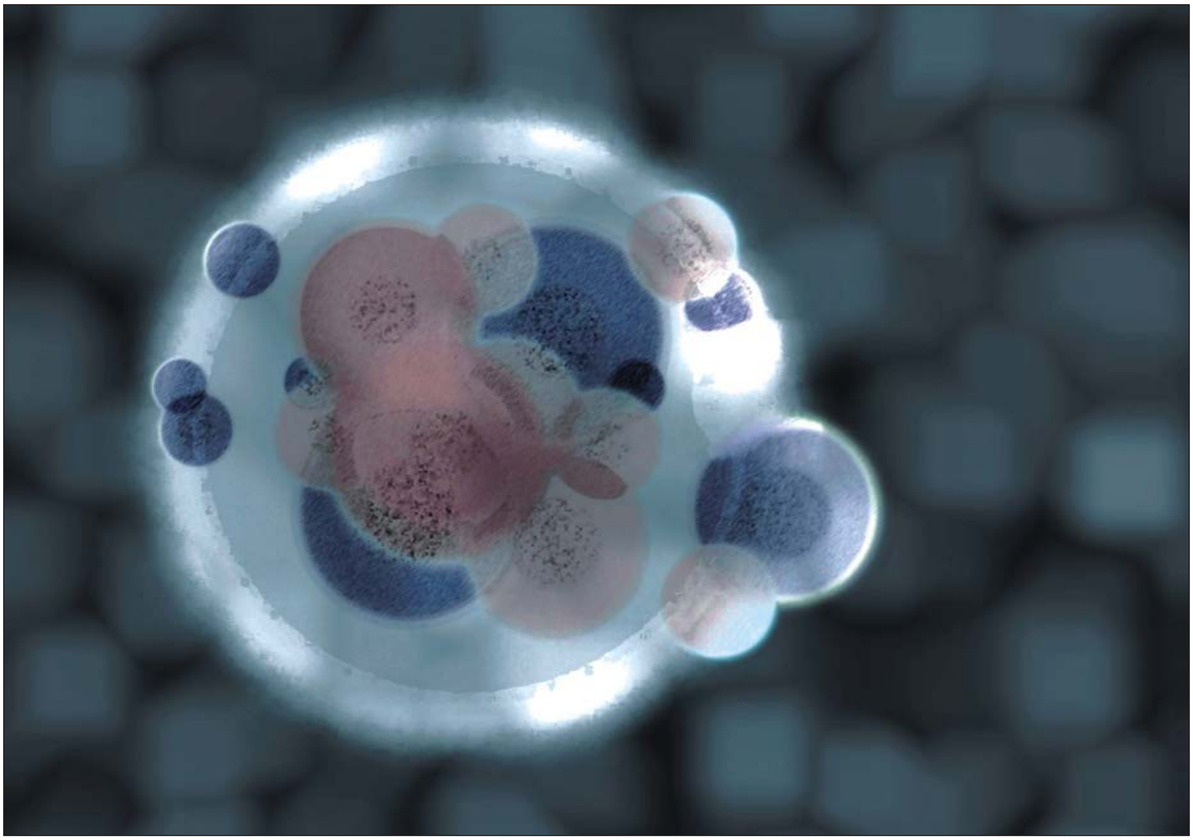

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Research Article
17α-methyltestosterone and Some Medicine Plants as Reproductive Controller Agents of *Oreochromis niloticus*

Ahmed I. Mehrim, Fathy F. Khalil, Fayek H. Farrag and Mohamed M. Refaey

Department of Animal Production, Faculty of Agriculture, Mansoura University, Al-Mansoura, Egypt

Abstract

Background and Objective: In recent years, many studies have tended to use herbal plants instead of drugs in fish farms, because they are safer and cheaper. This study aimed to compare the use of17α-methyltestosterone (17α-MT), pawpaw (*Carica papaya*) seeds powder (PSP) and neem (*Azadirachta indica*) leaves powder (NLP) as reproductive controller agents for *Oreochromis niloticus*. **Materials and Methods:** This experiment was divided into 2 consecutive periods, the 1st period was the treating period with dietary 17α-MT, PSP and NLP for 60 days. Then, the 2nd period was conducted for rearing the obtained fingerlings till the adult stage for 130 days. The treatments were (T_1) hormone untreated mixed-sex fry, (T_2) fry treated with 60 mg 17α-MT kg^{-1} diet for 28 days, (T_3) fry treated with 6 g PSP kg^{-1} diet for 45 days and (T_4) fry treated with 1 g NLP kg^{-1} diet for 60 days. **Results:** Fish treated with 17α-MT gave the highest significant values of growth performance, highest male percentage and economic efficiency followed by PSP, then NLP compared to the mixed-sex group. While, fish treated with PSP were the worst significantly effects on sperm quality parameters. Hence, it could be concluded that addition of PSP improved growth performance, food efficiency and economic efficiency of *O. niloticus* nearly the same results were obtained by using 17α-MT. **Conclusion:** This means it is recommended for using PSP as a natural reproductive controller agent for *O. niloticus*, besides its prospective economically and safety effects on fish environment, production and human health than using 17α-MT.

Key words: Nile tilapia, 17α-methyltestosterone, pawpaw, neem, sperm quality, economic efficiency

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Corresponding Author: Ahmed I. Mehrim, Department of Animal Production, Faculty of Agriculture, Mansoura University, Al-Mansoura, Egypt

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tilapia has become one of the most important and fastest-growing aquaculture species. According to FAO, tilapias are the second only to carps as the most widely farmed freshwater fish in the world[1]. In Egypt, tilapia production now takes the 2nd position after China in the world production and the 1st in Africa and Middle East. Locally, total fish production is more than 1 million t (1.82 million t), from which the fish culture is about 79.65% (1.45 million t) in year 2017. About 59.37% of the fish culture production is tilapia (967.30 thousand t)[2]. The success of tilapia production in Egypt stemmed from the drive to ensure monosex male production through sex reversal using 17α-methyltestosterone (17α-MT)[3]. Nile tilapia, *Oreochromis niloticus* is characterized many advantages such as short reproductive cycles, easy spawning, rapid growth, high feed conversion, high tolerance to environmental changes and feeding a wide range of natural food organisms or cheap artificial foods as well as firm flesh texture, neutral flavor and marketable request[4,5].

Despite all of aforementioned merits, tilapias have precocious maturity and uncontrolled reproduction, which often product in the overcrowded of production system with young fish where they sexually mature at about 20 g weight[6]. This uncontrolled reproduction of tilapia in culture system leads to small marketable-sized fish. Among the 4 major ways of producing all-male tilapia, i.e., manual/hand sorting, hybridization, genetic manipulation and hormonal sex reversal[6]. The technique of using androgenic hormones to sexually reverse to an all-male stock has become the most common practice in commercial culture[7]. The use of hormones for sex reversal is however, under increasing public scrutiny due to perceived potential health risks, environmental impacts and social constraints[5]. For instance marketing of treated fish is illegal in EU countries and India[8].

Recent years, several studies using medicinal plants as natural reproduction inhibitors is a new trend that may offer a solution for this problem. In addition, the medical plants are safer for human and environmental friendly, compared with artificial drugs[9]. Thus, some plants used for this purpose such as, pawpaw, *Carica papaya* seeds[7,10-14], neem, *Azadirachta indica* leaves[9,15-17], Moringa, *Moringa oleifera*[7], *Mangifera indica*[17]. In this respect, our previously results in some complementary studies to the present study concluded that addition of 6 g pawpaw (*C. papaya*) seeds powder (PSP) kg^{-1} diet for 45 days[18,19] and 1 g neem (*A. indica*) leaves powder (NLP) kg^{-1} diet for 60 days[20] of *O. niloticus* fry gave the best results of growth, survival, feed utilization and caused reduction of fertility parameters (e.g., gonadosomatic index, sex hormones and histological alterations in gonads). Additionally, in a recent study Ugonna *et al.*[21] demonstrated the possibility of using pawpaw (*C. papaya*) seed meal (PSM) as a naturally alternative agent of 17α-MT hormone to attain sex reversal of *O. niloticus*, which may be a cost-effective and ecofriendly alternative to synthetic hormones. Therefore, this study was conducted through 2 successive experimental periods to investigate the using dietary addition of 17α-MT, PSP and NLP as reproductive controller agents for *O. niloticus*. Also, to compare their effects on the mixed-sex *O. niloticus* (males and females) as a control group, regarding the growth performance, survival rate, feed utilization, whole fish body composition, testosterone hormone in blood and muscles, sperm quality parameters, ovarian measurements and the indicators of economic efficiency parameters.

MATERIALS AND METHODS

Study area: The current study was carried out at March-September, 2017 in Fish Research Laboratory, Animal Production Department, Faculty of Agriculture, Mansoura University, Dakahlia Governorate, Egypt. This study was divided into 2 consecutive experimental periods, the 1st period was the treating period with dietary 17α-MT, PSP and NLP, for 60 days. Then, the 2nd period (the rearing period) was conducted for rearing the obtained fingerlings till the adult stage for 130 days.

First period (the treating period)

Experimental design and conditions: A total of 1200 *O. niloticus* fry at one-day old and after absorbing the yolk sac with an average initial body weight 0.013 ± 0.002 g fry were randomly distributed to 4 treatments (three aquaria were refereed as a treatment). The treatments were (T_1) fry untreated hormone (mixed-sex fry, the control group), (T_2) fry treated with 60 mg 17α-MT kg^{-1} diet for 28 days according to Geer and Singh[22], (T_3) fry treated with 6 g PSP kg^{-1} diet for 45 days according to Farrag *et al.*[18] and Khalil *et al.*[19] and (T_4) 1 g NLP kg^{-1} diet for 60 days according to Refaey[20]. Fish were stocked at a rate of 100 fry/glass aquarium (90×40×50 cm). Each aquarium was filled with 108 L dechlorinated tap water and supplied with an air stone connected to electric compressor.

Experimental diet and feeding: Ripe fruits of pawpaw (*C. papaya*) were obtained from the local market to get the seeds, while neem (*A. indica*) leaves obtained from Faculty of Agriculture plantation, Mansoura University, Egypt. Then, the

Table 1: Ingredients and proximate chemical analysis (% on dry matter basis) of the basal diet in the treating period

Ingredients (g kg^{-1})	Percentage
Fish meal (60% CP)	540.00
Soybean meal (44% CP)	250.00
Corn gluten meal (60% CP)	150.00
Corn oil	50.00
Premix mixture[1]	10.00
Nutrients composition (%)	
Dry matter (DM)	91.88
Crude protein (CP)	45.22
Ether extract (EE)	9.00
Ash	7.82
Crude fiber	3.70
NFE[2]	34.26
Gross energy (MJ 100 g^{-1} DM)[3]	2.00

[1]Premix mixture containing per kilogram, vit. A (15 million I.U.), vit. E (15 mg), vit. B_1 (1.0 mg), vit. B_{12} (5.0 mg), vit. K_3 (2.5 mg), vit. B_6 (2.0 mg), pantothenic acid (10.0 mg), folic acid (1.2 mg), biotin (0.05 mg), vit. D_3 (3.0 million I.U.), copper (7.0 mg), manganese (100.0 mg), iodine (0.4 mg), Iron (40.0 mg), Zinc (50.0 mg), Selenium (0.15 mg) and anti-oxidant (125.0 mg), [2]NFE (Nitrogen free extract) = 100-[% CP+% EE+% Ash+% crude fiber], [3]Gross energy calculated based on 0.023, 0.039 and 0.016 MJ 100 g^{-1}DM for protein, lipid and NFE, respectively

seeds and leaves were cleaned and shade-dried in a drying oven at 50°C for 72 h. The dried seeds were milled into fine particle size (<250 µm) and kept in a dry, air-tight transparent plastic container. The basal diet formulation and its chemical analysis are shown in Table 1, containing 45.22% crude protein. The diet was prepared by milling and mixing the dry ingredients with oil, before starting the experiment. The control fish group fed the hormone free diet, while other fry treated with above the tested regent's. The PSP (T_3) and NLP (T_4) were added to the basal diet with level 6 and 1 g kg^{-1} diet, respectively. The diet was introduced manually 4 times daily at 9.00-11.00 am, 13.00 and 15.00 pm.

Fish fed the experimental diet at the rate of 30% of their live body weight in the 1st month. After 30 days of the experiment, it reduced gradually to 15% and then reduced to 10% of live body weight after 45 days until the end of the first period. Because the difficulty of weighing the fry in this age and to avoid the handling stress caused by the weighting, the amount of feed in the 1st week was doubled in the 2nd week, 3 fold in the 3rd week and 4 fold in the 4th week. At the end of the 4th week, random sample of fry per each aquarium was taken to adjust the feed intake. After that, fish were weighed biweekly to adjust the amount of food based on the actual body weight changes until the end of the treating period.

Accumulated wastes were removed from each aquarium 2 days a week by siphoning about 20% of the water volume/aquarium, then the equal volume of dechlorinated tap water was added. The water was aerated using air pump (BOYU, air-pump U9900, China) to permit suitable level of dissolved oxygen. The dissolved oxygen was 6-8 mg L^{-1}, water temperature was 25-27°C during the experimental period. Light period was providing a 12 h light: 12 h dark as a daily photoperiod.

Second period (the rearing period)

Experimental condition and feeding system: The rearing period was directly started after the end of the treating period. *O. niloticus* fingerlings were stocked at a rate of 50 fish/tank (3 tanks were refereed as a treatment). Each plastic tank (1 m^3) was supplied with an air stone connected with electric compressor. In this period, fish of all treatments (the same treatments in the treating period) fed the commercial diet only. The commercial diet used in the 2nd period contains 29.98% crude protein. It was purchased from Al-Badrashin manufacture for fish food, Giza Governorate, Egypt. The proximate chemical analysis of the commercial diet is 90.11% DM, 29.98% CP, 6.12% EE, 5.72% Ash, 58.18% total carbohydrate,1.950 MJ 100 g^{-1} DM gross energy (GE) and 15374.35 mg CP MJ^{-1} GE protein/energy ratio. Fish fed the experimental diets at a rate of 8, 6, 4 and 3% of their live body weight daily for 1st, 2nd, 3rd month and until the end of the experiment, respectively. Diets manually introduced to twice daily at 8 am and 14.00 pm. The amount of food was adjusted bi-weekly based on the actual fish body weight changes.

In each tank, accumulated wastes were removed by siphoning every 2 days a week. About 20% of the water volume/tank was changed during 2 months and then 40% of the water volume was changed until at the end of the rearing period, using equal volume from the fresh ground water. The water temperature ranged 24-25°C during the rearing period, through providing each aquarium by two thermo-heaters (Risheng aquarium glass heater, Model 2000, 100 W, China). The water was aerated using air pump (BOYU, air-pump U9900, China) to permit suitable level of dissolved oxygen, which within the accepted the range 5.2-6.6 mg L^{-1} for rearing *O. niloticus*. Light was providing a 12 h light: 12 h dark as a daily photoperiod by 3 electric lamps power 200 watt.

Sample collection and analytic methods: At the end of 1st and 2nd periods, fish in each treatment were weighed as a group to determine the final body weight. The growth performance and feed utilization parameters such as, final weight (FW, g), weight gain (WG, %), average daily gain (ADG, g/fish/day), specific growth rate (SGR, (%)/day), feed conversion ratio (FCR), protein efficiency ratio (PER) and survival rate (SR, %). These parameters were calculated/tank as following equations according to Halver and Hardy[23]:

$$WG = \frac{FW - IW}{IW} \times 100$$

$$ADG = \frac{FW - IW}{T}$$

$$SGR = \frac{FW/IW}{T} \times 100$$

where, IW and FW are initial and final weights (g), respectively and T is the time of the experiment (days):

$$FCR = \frac{\text{Dry feed intake (g)}}{\text{Fish live weight gain (g)}}$$

$$PER = \frac{\text{Body weight gain (g)}}{\text{Protein intake (g)}}$$

Only, at the end of the rearing period, adult *O. niloticus* males (n = 10 of each tank) and females (n = 10 of each tank, except T_2) the body weight (W) and the total length (TL) were measured individually, for calculating the condition factor[24]:

$$K = \frac{100 \times \text{Weight (g)}}{TL^3}$$

After that, fish abdominal cavity was opened to obtain the gonads and they were individually weighed too. Gonadosomatic index (GSI, %) was calculated as[25]:

$$GSI\ (\%) = \frac{\text{Gonads weight (g)}}{\text{Fish weight (g)}} \times 100$$

Also, at the beginning and the end of the 2nd period, fish samples (n = 3 of each tank) were collected and kept frozen (-20°C) till the chemical analysis of the whole fish body according to AOAC[26].

Testosterone analysis: Five males from each tank were randomly taken and anesthetized by pure clove oil (3 mL dissolved in 10 mL absolute ethanol and were added up to 10 L water) as a natural anesthetic material. Blood samples (5 mL) were collected from the fish by puncturing caudal venous with a syringe needle in dried plastic tubes and centrifuged for 20 min at 3500 rpm to obtain the blood serum. Serum samples were kept in deep freezer (-20°C) until the analysis was carried out. Samples of fish dorsal muscles (n = 6 of each treatment) were removed and crushed in ceramics brushed. About 2 g of muscle were homogenized with 10 mL distilled water. Then, it was filtered by Whatman™ quantitative filter paper to extract the hormone residues. The filtered liquid was used to determine the testosterone residues in the muscle samples. Testosterone was determined in both serum and the dorsal muscles extraction using commercial ELISA test kits catalog BC-1115 (BioCheck, Inc) according to Tietz[27].

Sperm quality parameters for males: Five adult males from each tank were netted and their abdomen was dried with a soft cloth to avoid contamination of sperm with water. Seminal fluid was collected into capillary tube by pressing the fish abdomen gently using the thumb and forefinger from the direction of the head to tail, then liquated to precooled clean 1.5 mL micro centrifuge tubes and immediately sperm quality parameters were analyzed. Sperm motility was assessed subjectively, using a microscope at×400 magnification, five (1-5) category classifications, corresponding to a motility of 0, 0-25, 25-50, 50-75% or <75%, were used according to Boussit[28]. While, sperm concentration was estimated microscopically using a Neubauer® counting chamber and sperm viability was measured by the eosin nigrosin staining method according to Parente *et al.*[29] on the final pooled semen samples. Whereas, motility duration was determined by the sperm activity as viewed under Olympus microscopic at×100 magnification to see when all the sperm got stopped[30].

Female reproductive parameters: Four adult females per tank were randomly chosen then individually weighed. Fish were sacrificed and the target organs (ovaries) were sampled. Eggs weight, number and diameter per each female were measured and counted. Eggs number was counted per gram and then related to ovary weight or body weight of fish. Then absolute fecundity (AF) and relative fecundity (RF) were calculated according to Bhujel[31] as:

$$AF = \frac{\text{Egg number in the ovaries}}{\text{Female}}$$

$$RF = \frac{\text{Absolute fecundity}}{\text{Female body weight (g)}}$$

Indicators of economic efficiency: The economic efficiency was calculated regarding to the total weight gain costs (as output) and food consumption costs (as input) regardless to any other costs during the second period only. The estimation was based on local retail sale market prices of all the dietary ingredients in Egypt at the time of the study.

Statistical analysis: All data were statistically analyzed using one-way analysis of variance (ANOVA) by used SAS[32] (version 9.2). All ratios and percentages were arcsine-transformed prior to statistical analysis. All mean were statistically compared for the significance (p<0.05) using Tukey's *post-hoc* test.

RESULTS

Growth performance and feed efficiency: There were no significant differences in FW, WG and FCR among treatments during the treating period ($p>0.05$, Fig. 1). While, fry in the control group gave the lowest SR compared to other treatments ($p<0.05$). No significant differences in SR among 17α-MT, PSP and NLP were observed ($p>0.05$).

For the rearing period, Fig. 2 showed that fish treated with 17α-MT gave the highest values of FW, WG, ADG and SGR, followed by PSP, then NLP compared to the control group ($p<0.05$). However, no significant differences in the SR among all treatments were found, which it ranged from 94-100%. Fish treated with 17α-MT, followed by PSP gave the best values of FCR and PER compared to the control group ($p<0.05$, Fig. 2).

Fish whole body composition: Significantly differences in the fish whole body composition traits among treatments were detected (Table 2). Fish treated with NLP recorded the lowest value in water and fat contents compared to other treatments ($p<0.05$). While, the control group, 17α-MT and PSP treatments gave the best values of CP content ($p<0.05$). Ash content was significantly increased in the control treatment among all treatments.

Fish sexual ratio: Figure 3 shows that fish treated with 17α-MT achieved the highest significant sexual ratio (97.22% males and 2.77% females), followed by PSP (68.00% males and 32.00% females) and the control (59.00% males and 41.00% females) than NLP (50.00% males and 50.00% females) ($p<0.05$).

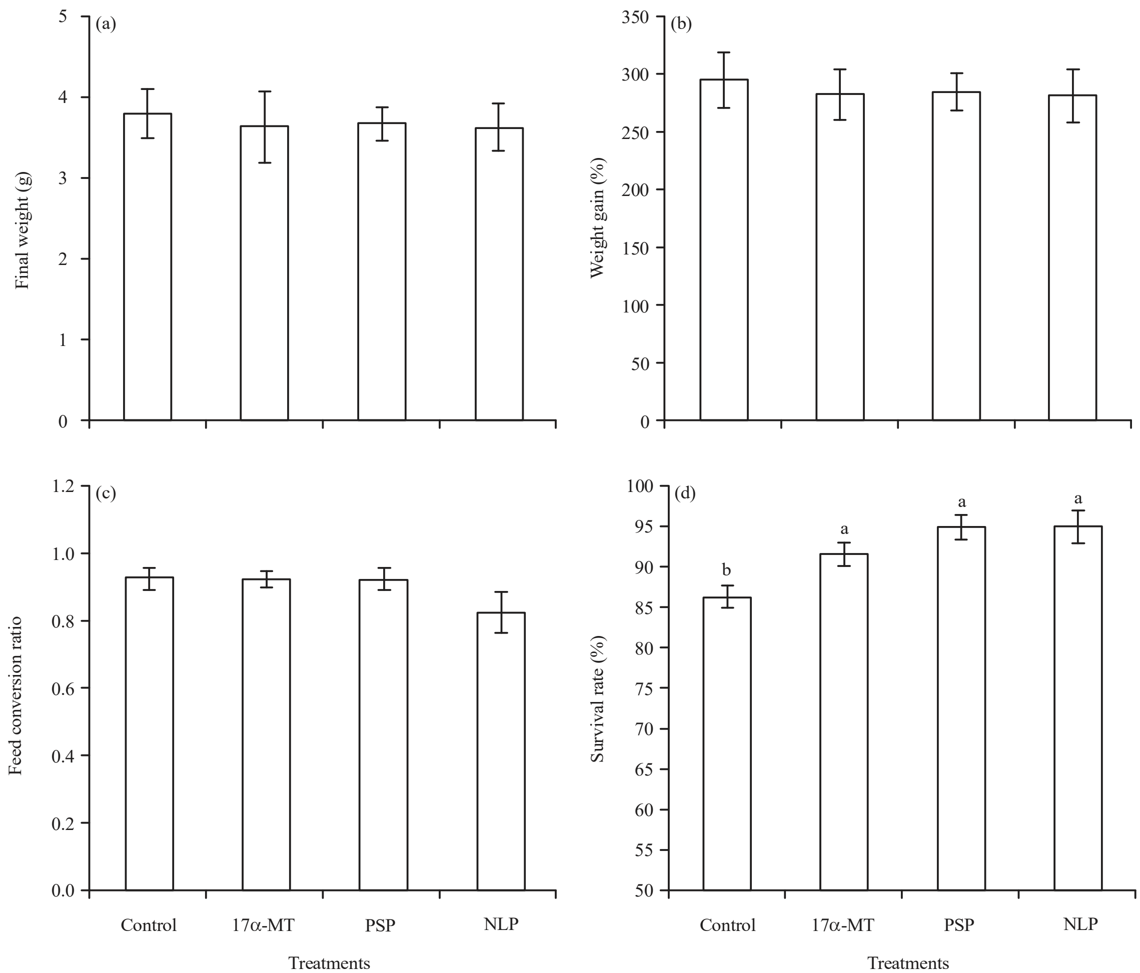

Fig. 1(a-d): Effect of dietary 17α-MT, PSP and NLP on (a) Final weight, (b) Weight gain, (c) Feed conversion ratio and (d) Survival rate of *O. niloticus* fingerlings at the end of the treating period

Vertical bars indicate standard error, different letters denotes significant differences between treatments ($p<0.05$), 17α-MT: 17α-methyltestosterone, PSP: Pawpaw seeds powder, NLP: Neem leaves powder

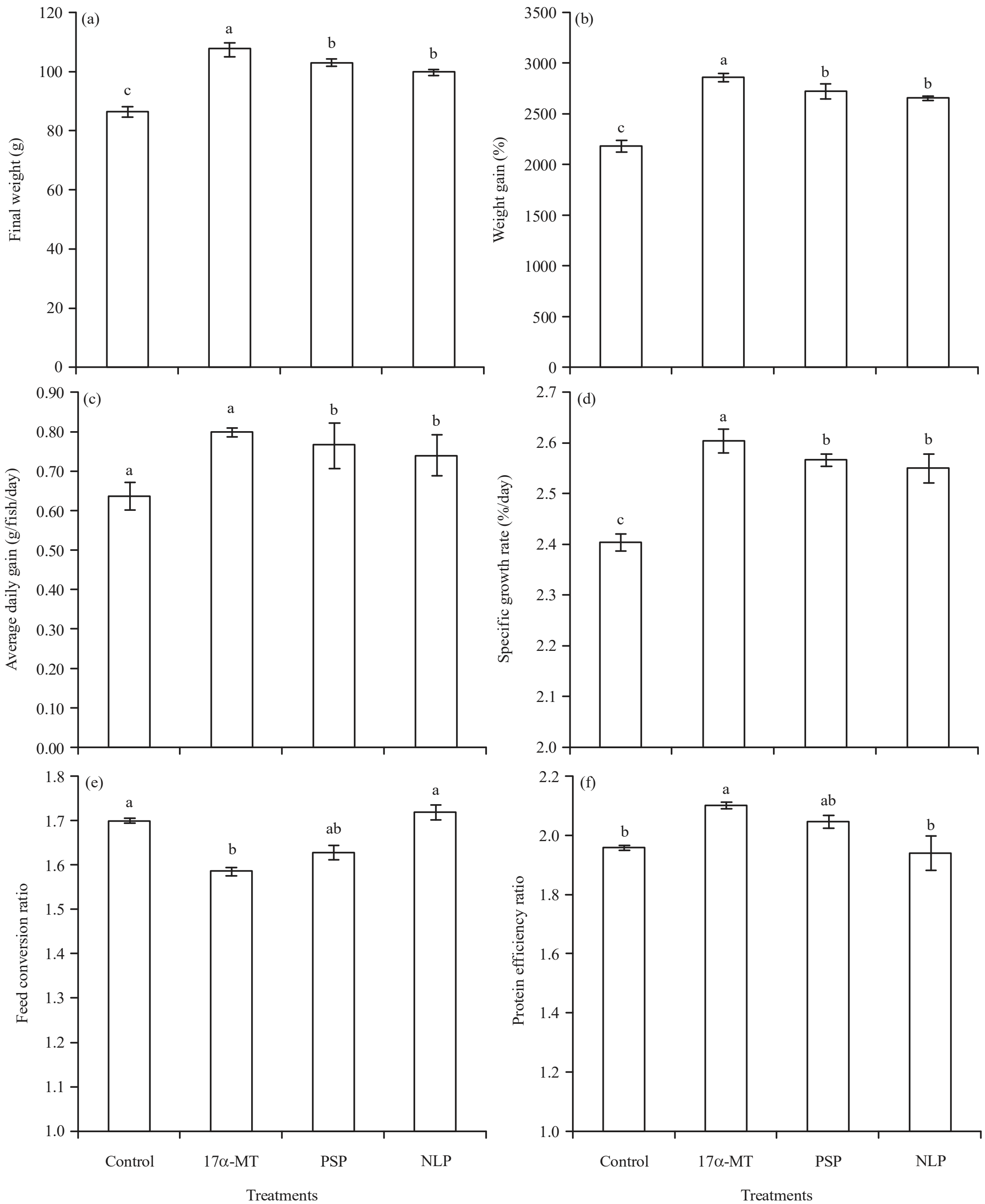

Fig. 2(a-f): Effect of dietary 17α-MT, PSP and NLP on growth performance parameters (a) Final weight, (b) Weight gain, (c) Average daily gain, (d) Specific growth rate, (e) Feed efficiency parameters (feed conversion ratio) and (f) Protein efficiency ratio of the adult *O. niloticus* at the end of the rearing period

Vertical bars indicate standard error, different letters denotes significant differences between treatments ($p<0.05$), 17α-MT: 17α-methyltestosterone, PSP: Pawpaw seeds powder, NLP: Neem leaves powder

Testosterone levels in serum and fish muscles: Testosterone levels in serum and its residual in fish muscles of adult *O. niloticus* male at the end of the rearing period are presented in Fig. 4. Serum testosterone levels significantly decrease in fish treated by PSP among other treatments, which gave the lowest values ($p<0.05$). Fish treated with 17α-MT had the highest testosterone level in serum among all treatments ($p<0.05$). The treatment with NLP gave the highest

Table 2: Effect of dietary 17α-MT, PSP and NLP on the whole body composition of the adult *O. niloticus* at the end of the rearing period (Mean±SE)

	Treatments			
Traits (%)	Control	17α-MT	PSP	NLP
Water content	76.51±0.17[b]	76.33±0.16[b]	73.05±0.11[c]	77.49±0.08[a]
Ash content	13.02±0.06[a]	12.55±0.14[ab]	11.47±0.24[c]	12.13±0.19[b]
Fat content	26.50±0.32[c]	27.43±0.35[c]	29.10±0.16[b]	30.29±0.29[a]
Protein content	60.47±0.26[a]	60.02±0.43[a]	59.44±0.14[a]	57.57±0.47[b]

Mean in the same row having different letters are significantly different at p<0.05, 17α-MT: 17α-methyltestosterone, PSP: Pawpaw seeds powder, NLP: Neem leaves powder

Table 3: Effect of dietary 17α-MT, PSP and NLP on K, GSI and sperm quality parameters of the adult *O. niloticus* males at the end of the rearing period (Mean±SE)

	Treatments			
Parameters	Control	17α-MT	PSP	NLP
K (%)	1.87±0.03[a]	1.70±0.05[b]	1.68±0.07[b]	1.63±0.05[b]
GSI (%)	1.35±0.14[b]	1.20±0.11[b]	1.91±0.11[a]	2.25±0.27[a]
Sperm count ($\times 10^9$ mL^{-1})	1.53±88.31[b]	1.60±101.4[b]	2.22±219.0[a]	2.18±110.60[a]
Motility (viability, %)	75.67±2.33[c]	85.00±0.00[a]	69.00±3.79[d]	80.00±0.00[b]
Forward	4.00±0.58[a]	4.67±0.33[a]	2.67±0.33[b]	4.00±0.00[a]
Motility duration (min. sec.)	12'36''±1.57[c]	25'23''±0.45[a]	17'65''±0.85[b]	12'62''±0.70[c]
Dead (%)	25.67±1.45[b]	17.67±0.88[c]	40.67±1.76[a]	21.33±1.33[bc]

Mean in the same row having different letters are significantly different at p<0.05, 17α-MT: 17α-methyltestosterone, PSP: Pawpaw seeds powder, NLP: Neem leaves powder, K: Condition factor, GSI: Gonadosomatic index

Table 4: Effect of dietary PSP and NLP on K, GSI, ovarian and fecundity parameters of the adult *O. niloticus* females at the end of the rearing period (Mean±SE)

	Treatments		
Parameters	Control	PSP	NLP
K (%)	1.78±0.08	1.82±0.06	1.69±0.05
GSI (%)	3.68±0.28	3.13±0.12	3.78±0.19
Ovary volume (cm^3)	3.333±0.67	3.633±0.13	4.667±0.33
Ovarian-specific gravity ($g\ cm^{-3}$)	0.977±0.06	0.953±0.05	1.027±0.009
Egg number (g^{-1})	263.3±12.02[c]	400.0±10.0[a]	343.3±8.82[b]
Egg diameters (mm)	1.71±0.04[a]	1.457±0.03[b]	1.600±0.09[ab]
Absolute fecundity (egg $female^{-1}$)	816.3±75.70[b]	1318±60.15[a]	1435±86.87[a]
Relative fecundity	11.75±0.72	11.28±0.21	12.04±0.968

Mean in the same row having different letters are significantly different at p<0.05, 17α-MT: 17α-methyltestosterone, PSP: Pawpaw seeds powder, NLP: Neem leaves powder, K: Condition factor, GSI: Gonadosomatic index

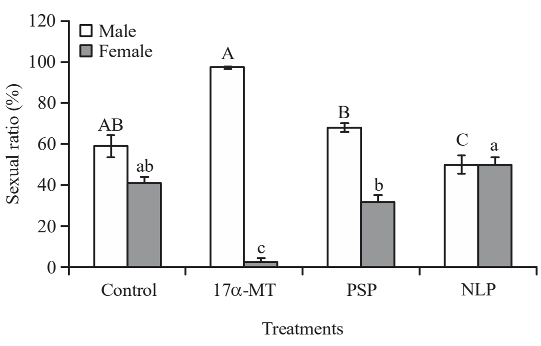

Fig. 3: Effect of dietary 17α-MT, PSP and NLP on sexual ratio of the adult *O. niloticus* at the end of the rearing period
Vertical bars indicate standard error, different capital and small letters denotes significant differences between treatments in male and female, respectively (p<0.05), 17α-MT: 17α-methyltestosterone, PSP: Pawpaw seeds powder, NLP: Neem leaves powder

values of the residual levels of testosterone in fish muscles, followed by17α-MT and then the control group compared to those treated by PSP (p<0.05).

Sperm quality parameters: The control group gave the highest significant in K compared to other treatments (p<0.05), without significant effects of fish treated with 17α-MT, PSP and NLP (p>0.05, Table 3). Fish treated with PSP or NLP appeared the significantly increase of GSI compared to the control and 17α-MT treatments (p<0.05). The sperm count and sperm dead percentage significantly increased in fish treated with PSP, while it gave the lowest values of motility and forward parameters compared to other treatments. Moreover, the fish treated with 17α-MT was the best treatment in motility, forward, motility duration and less dead percentage among all treatments (p<0.05).

Ovarian measurements and fish fecundity: No variations in K, GSI, ovary volume, ovarian-specific gravity and RF were detected among all treatments (p>0.05, Table 4). Furthermore, the fish treated with 17α-MT did not contain a sufficient number of females for sampling to measure ovarian measurements and fish fecundity. Fish treated with PSP had

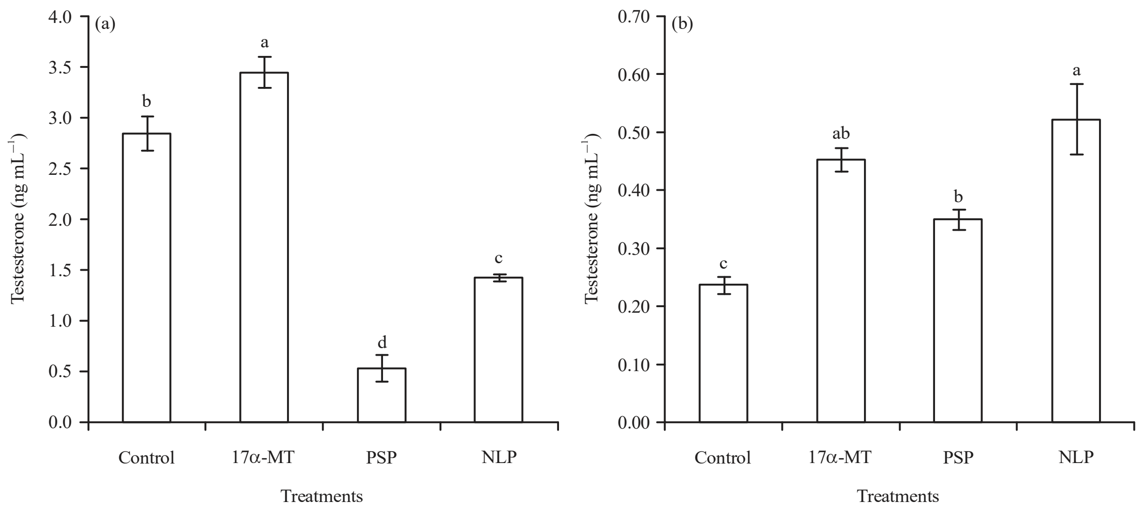

Fig. 4(a-b): Effect of dietary 17α-MT, PSP and NLP on (a) Testosterone levels in serum and (b) Muscles of the adult *O. niloticus* males at the end of the rearing period

Vertical bars indicate standard error, different letters denotes significant differences between treatments (p<0.05), 17α-MT: 17α-methyltestosterone, PSP: Pawpaw seeds powder, NLP: Neem leaves powder

Table 5: Effect of dietary 17α-MT, PSP and NLP on economic efficiency indicators of the adult *O. niloticus* during the rearing period (Mean±SE)

	Treatments			
Traits	Control	17α-MT	PSP	NLP
Total outputs[1]	15.03±0.22^{d}	20.18±0.04^{a}	19.31±0.22^{b}	18.69±0.19^{c}
Total food costs[2]	11.99±0.22^{b}	15.02±0.05^{a}	14.75±0.01^{a}	15.07±0.29^{a}
Net return[3]	3.03±0.00^{b}	5.17±0.09^{a}	4.56±0.21^{a}	3.62±0.49^{b}
Economic efficiency[4] (%)	25.31±0.48^{b}	34.41±0.76^{a}	30.90±1.37^{ab}	24.17±3.72^{b}

Mean in the same row having different letters are significantly different at p<0.05, 17α-MT: 17α-methyltestosterone, PSP: Pawpaw seeds powder, NLP: Neem leaves powder, [1]Total outputs/treatment (LE kg^{-1} fish) = Fish price×Total fish production, Total fish production/treatment = Final number of fish×Fish weight gain, [2]Total food costs/treatment (LE kg^{-1} diet) = Food costs/1 kg diet×Food intake, [3]Net return/treatment (LE) = Total outputs-total food costs, [4]Economic efficiency/treatment (%) = Net return/Total food costs×100

the highest and the lowest values of egg number/g of body weight and egg diameter among all treatments, respectively (p<0.05). Moreover, a significant increase in AF was observed in fish treated with NLP compared to other treatments (p<0.05, Table 4).

Indicators of economic efficiency: Data in Table 5 demonstrated that *O. niloticus* treated with 17α-MT was the highest significant values of total output, net return and relative economic efficiency, economic efficiency followed by PSP treatment compared to the control group (p<0.05). Meanwhile, no significantly (p>0.05) different were found between fish treated with 17α-MT or PSP.

DISCUSSION

This study exhibited that *O. niloticus* treated with 17α-MT gave highly significant values of all growth performance and food efficiency parameters, followed by fish treated with PSP. This improvement of growth performance and feed efficiency of *O. niloticus* fed 17α-MT and PSP may be related to the highest sexual ratio percentage 97 and 68% of males, respectively, whereas *O. niloticus* male has better growth than female[33,34]. A similar response has been reported by Abdelhamid *et al.*[35], Khalil *et al.*[36], Chakraborty *et al.*[37]. The superiority of growth in Nile tilapia male than female might be due to gonadal steroid hormones provoking direct and antagonistic effects on production of insulin-like growth factor[38] and enhance the release of growth hormone[39] as well as related to the lack of energy expenditure in egg production for mouth brooding by females and lower energy expenditure on courtship by males[40]. Additionally, Bhasin *et al.*[41] indicated that using17α-MT in sex reversal acts as a synergist for insulin, leading to muscle hypertrophy by growing muscle protein synthesis.

The results in the present study exhibited that the negative affects to add 17α-MT, PSP and NLPon K. This result varied with the improve of body weight of fish fed 17α-MT, PSP may be led to increase of fish body deep with increase the body length, which reflected the decline of K in these treatments. *O. niloticus* fed 17α-MT also gave the lowest significant values of GSI compared to other treatments. Similarly, with the obtained results herein, many studies reported that GSI of mono-sex *O. niloticus* males had significantly the lowest mean values compared to the mixed fish sex[13,35,36]. Regarding the *O. niloticus* sex ratios obtained in the present results, fish treated with 17α-MT gave the highest percentage of males (97.22%) compared to other treatments. This result is nearly similar to those reported by Chakraborty *et al.*[42]. However, the dietary addition of PSP gave 68% of *O. niloticus* males. These results are agreed with Ampofo-Yeboah[7] who revealed that *O. mossambicus* fed pawpaw seeds gave 65% male and 35% female. Meanwhile, *O. niloticus* treated with NLP showed an equal percentage of fish sex ratio, which means that NLP did not affect on sexing ratio of *O. niloticus* compared to other treatments.

Results in the current study indicated that the negative effect of PSP on sperm quality parameters (motility %, forward and percentage of dead sperm), whilst, fish treated with 17α-MT had a positive effect on all sperm quality parameters. These results are highly related to the present findings, where *O. niloticus* treated with 17α-MT had the highest value of serum testosterone level, while, dietary PSP had the lowest value of the serum testosterone. In males, testosterone peaks prior to the gonadotropins because the former hormone is more important for sperm development[43]. Furthermore, current findings regarding the decrease of sperm quality parameters of *O. niloticus* fed PSP agreed with those obtained by Verma and Chinoy[44], who found the aqueous papaya seed extract (PSE) caused a significant decrease of the sperm maturation and motility. PSE also caused a reduction in protein concentration of spermatozoa, which could be one of the causative factors for the reduction of sperm motility[45].

Results in the present study confirmed that fish treated with 17α-MT had a highly significant level of serum testosterone compared to other treatments. These results are agreed with Abdelhak *et al.*[13]. Inversely, Abdelhamid *et al.*[35], Rizkalla *et al.*[46] they found that plasma testosterone levels of treated *O. niloticus* with 17α-MT was lower than untreated fish and caused sterility. The previous indicators as the fish sexing ratio, dead sperm and serum testosterone level illustrated that the PSP had serious negative impacts on the reproductive responses of *O. niloticus*. These findings are more related to those recently obtained by Kareem *et al.*[14], who found that PSE was the most effective at delaying gonadal maturation of both male and female *O. niloticus*.

The rapid metabolism and excretion of 17α-MT by a fish treated early in its life history, combined with the extended period needed to produce a marketable size fish result in a safe consumer product[35]. In the present study, fish treated with NLP followed by 17α-MT appeared the highest of residues of testosterone in *O. niloticus* muscles compared to other treatments. These results are in accordance with those reported by Rizkalla *et al.*[46], who suggested that whole-body samples of non-hormone treated *O. niloticus* and those treated with 17α-MT for 28 days contained detectable amounts of testosterone only in the first 5 months after treated with 17α-MT.

Regarding the reproductive efficiency of females, dietary addition of PSP and NLP not significantly effects K, GSI, ovarian measurements and RF but they caused increasing the egg number/g and AF. Interestingly, the current findings showed that PSP led to the highest egg number/g and lowest egg diameter, which may be reflected in the maturity of ovary despite the high weight of fish at the end of the 2nd period. These results confirmed by the drastic histological alteration in the ovary of *O. niloticus* treated with PSP accordingly to Khalil *et al.*[19]. On the other side, fish treated with NLP did not have any effect on the reproductive performance parameters of *O. niloticus* females, although its improved growth performance parameters compared to the sex-mixed group. These obtained results did not agree with those reported by Refaey[20], who found that dietary NLP decreased the testosterone levels and increased the drastic histological alterations of testes and ovaries of *O. niloticus* by increasing the levels of NLP and exposure periods. More recently, Kapinga *et al.*[47] reported that both of 2 medicinal plants, Aspilia plant, *Aspilia mossambicensis* and Neem tree, *A. indica* leaf powders partially controlled prolific breeding of *O. niloticus*. These differences between the current findings and those obtained in the previous studies were may be related to the levels and exposure time of the dietary treatments, recovery period of fish, fish sexual maturation stage and fish size.

In the present study, fish treated with PSP appeared nearly economic efficiency compared to the fish treated with 17α-MT and superiority than other treatments. This improvement of economic efficiency due to an increase in growth performance, male sex ratio and enhance of FCR of both fish treated with PSP and 17α-MT. Similarly, with the obtained results herein, many attempts revealed that the sex-reversed tilapia grew better and economically than the non-sex-reversed fish[35,37], where tilapia males have better growth than females[34]. Moreover, Khalil *et al*.[36] demonstrated that using dietary 17α-MT has economically important effects in aquaculture.

CONCLUSION

From the preceding results in the present study, it could be conclude the superiority effects of dietary 6 g PSP kg^{-1}diet for 45 days for improving the growth performance, food utilization and economic efficiency, besides the controlling effects on the reproductive process of *O. niloticus*. These findings nearly seemed of those obtained by using dietary 17α-MT of *O. niloticus*. Consequently, it is recommend for using PSP as a natural reproductive inhibitor agent for *O. niloticus*, according to its environmentally friendly, economically and the safety effects on the fish and human health than using 17α-MT. Hence, advanced studies on using the different extracts of pawpaw seeds or other medicinal herbs as the reproductive controller agents for *O. niloticus* are consider necessary.

SIGNIFICANCE STATEMENT

This study discovers the effects of dietary pawpaw seeds powder (PSP) and neem leaves powder (NLP) as reproductive controller agents for *O. niloticus* instead of the synthetic hormone 17α-methyltestosterone (17α-MT), which help the farmers and researcher also to overcome the main problem (overpopulation) in the culture of *O. niloticus*. Thus, a new biological theory on this useful using of dietary PSP as a natural reproductive controller agent for *O. niloticus*, besides its prospective biologically, economically and safety effects on the fish environment, fish production and quality, as well as for human health than using 17α-MT may be arrive at.

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