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Effect of some Nutritional and Enviromental Factors on Production of Mono Sex Nile Tilapia (*Oreochromis niloticus)*

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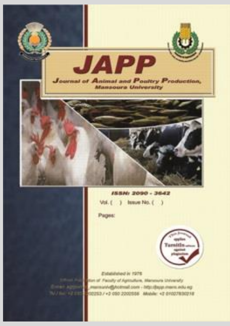

ABSTRACT

Two trials were conducted with Nile tilapia *(Oreochromis niloticus)* larvae and fry at the commercial fish hatchery in Fayoum Governorate during 2019. The first was conducted to study the effect of storage conditions for treated feeds with male hormone 17 α –methyltestosterone (17 α –MT), (light condition, store temperature. and time after preparation) on the rates of sex reversal, and growth performance. 5000 newly hatched fry were stocked in a hapa (2×1 m, and a water depth of 80 cm), and were fed with treated feed with 120 mg/kg (17α-MT) for 28 days. No significant difference among treated diets and the ability to sex-reverse fry. The highest sex reversal % was in the treated diets and refrigerated for use at 97.5% and prepared daily at 96.27%. The results of this trial indicate that the rates of male sexual transformation were affected by the method of preparing treated feed hormone, although this effect was limited. The second experiment was performed to study the effect of *Tribulus terrestris* powder on sexual transformation, compared to the male treated with 17α-MT in the same condition as the first experiment. A significant increase ($P \leq 0.05$) in percent male was observed with increasing *Tribulus terrestris* powder in the diets compared to control group. There are significant differences between treatments containing 17α-MT and the basal diet.The results of this trial refer to the recommendation that the *Tribulus terrestris* powder at 200 g/kg diet and 17α-MT at 150 mg/kg diet are the most effective for sex reversal in Nile tilapia larvae.

Keywords: Nile tilapia, sex reversal, *Tribulus terrestris*, growth performance, male hormone.

INTRODUCTION

There is no doubt that aquaculture has grown to be a significant industry in Egypt, owing to the vast quantities of fish produced, particularly from fish farming. Fish hatcheries play a significant role in supplying this industry with what it requires from the various fish fry, Nile tilapia is the most farmed fish (Mur, 2014; Soliman and Yacout, 2016; Kaleem and Bio Singou Sabi, 2021). The quest to secure monosex male production through sex reversal utilizing 17 α-methyl testosterone ((17α-MT)) led to the success of tilapia farming in Egypt. Nile tilapia, *Oreochromis niloticus*, has a number of advantages, including; reproductive cycles are brief, spawning is simple, growth is quick, feed conversion is good, high environmental tolerance, and feeding a variety of natural food organisms or low-cost artificial feeds, as well as firm flesh texture, neutral flavor, and marketability (Lind *et al.*, 2019).

Despite their many advantages, tilapias have early sexual maturity and unrestrained reproduction, which often results in an overloaded production system with young fish. Sexual maturity occurs at around 20 g weight. The unregulated reproduction of tilapia in the culture system results in little fish that can be sold, early sexual maturity in tilapia farming is a well-known issue (Bhujel and Suresh, 2012). When working with tilapia, management challenges include early maturation and frequent spawning. Because male tilapia develops faster, they are favored for culture. Sex reversal is the most widely utilized way for providing male tilapia for culture among the different techniques that have been explored (Ghosal *et al.*, 2015).

Monosex (all male) tilapia production played a significant role in meeting this challenge due to its rapid growth rates, tendency of a wide variety of environments, resistance to stress and diseases, greater energy conservation, lowered aggressiveness, and greater uniformity of size at harvest (El-Sayed, 2006; Kulwa *et al.*, 2021). It is feasible to intervene at this early stage of development and direct gonadal development in order to establish monosex populations. The culture of all male tilapia is one of several methods for controlling reproduction in mixed-sex populations (Cagauan *et al.*, 2004;Baroiller and Cotta, 2018; Gabriel, 2019). The most frequent androgen used to control tilapia sex is methyl testosterone. Various dose rate and treatment duration procedures have been examined. All of them are reliant on hormone treatment in sexually unequally differentiated fry.

Many researches have opted to use herbal plants rather than medicines in fish farms in recent years, because they are more secure and less expensive. Some plants used for this purpose include pawpaw *Carica papaya* seeds (Kareem *et al.*, 2016), *Azadirachta indica* leaves (Saadony *et al.*, 2020), *Moringa oleifera* (Gad *et al.*, 2019), Mangifera indica (Obaroh and Nzeh, 2013), Gokshura *Tribulus terrestris* (Turan and Cek, 2007). and maca (*Lepidium meyenii*) tuber meal (Lee *et al.*, 2004). The objective of the first experiment was to study the effect of storage conditions

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for treated feeds with the male hormone 17 α-methyltestosterone on the rates of sex reversal, survival%, growth performance and feed utilization parameters in Nile tilapia *O. niloticus* larvae and fry. The second study aimed to compare the use of 17α-MT, and Gokshura *(Tribulus terrestris)* powder as reproductive controller agents for *Oreochromis niloticus* in addition to tracking fry growth performance after sex reversal period.

MATERIALS AND METHODS

Experimental design

The first was to study the impact of storage conditions for treated feeds with male hormone 17α-MT on the rates of sex reversal and growth performance in Nile tilapia *O. niloticus* larvae. The second experiment was performed to study the impact of *Tribulus terrestris* powder on sexual transformation (sex reversal), compared to the male treated with 17α-MT in addition to tracking growth and feed utilization parameters; the following diagram shows the design of the first experiment:

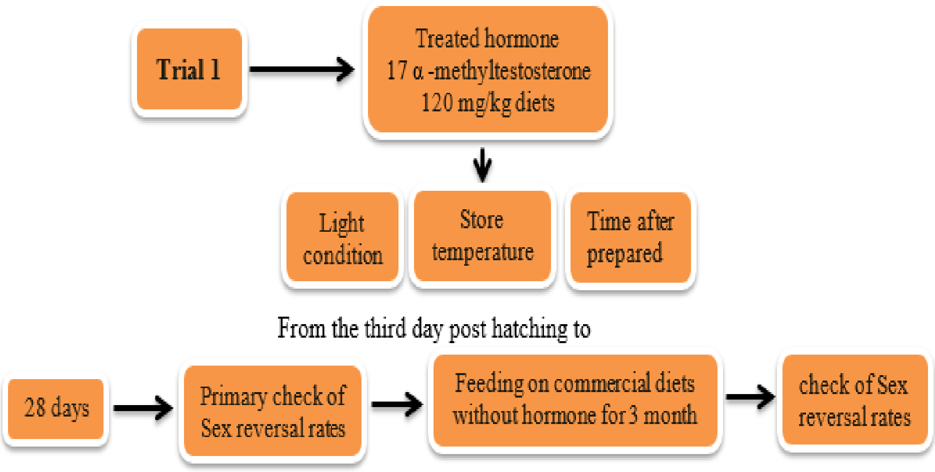

Fig 1. Design of the first experiment

Experimental Fish

Brood fish of *O. niloticus* were allowed to breed naturally in hapas (4×7m) with a water depth of about 80 cm. The hapas were placed in an earthen pond with an area of 4200 m^2, during 2019 with a sex ratio of 3 females to 1 male. Every 3-4 days, the eggs in the female's mouth were checked. The eggs were collected from hapas were transferred to the hatching laboratory, where a semi-industrial hatchery system was used. About 5000 of the newly hatched fry were stocked in a hapa (2×1 m, and a water depth of 80 cm), after the yolk sacs were absorbed per treatment.

Preparation, feeding treated feeds by hormone.

The experimental diet was prepared by mixing hormone at 120 mg/kg in the prepared diet (Table 2). There's a chance that the feed containing the masculinization hormone won't be completely absorbed, resulting in a lower male-to-female ratio than with conventional hormonal inversion techniques, an example of this when water is not clear. Hence, hormonal concentrations, superior to conventional protocols of sex reversal, should be tested (Costa e Silva *et al.*, 2022), the ethanol dissolved hormone was sprayed over used diet and mixed well and dried for 2 hours under shed. The treatment groups in trial one were as follows:

Fish naturalization

After the nursing period, a total of 10% of fish (samples) were collected and taken randomly from each replication for gonad inspection. The sex of hormone-treated tilapia was determined based on genital papilla. with a magnifying glass, then it was verified again after incubation. The male has two orifices just forward of the anal fin: anus and a urogenital opening, which normally forms a tiny papilla. In contrast, the female has three orifices: the anus, the transverse genital opening, and a small urine orifice that is hardly visible to the human eye. At the end of the experiments, fish in each hapa were netted, and counted for survival rate determination. Length and weight measurements were made by using a precision balance, magnifying glass and ruler.

Plant material and herbal meal preparation (Trial 2).

Tribulus (*Tribulus terrestris*) powdermeal was purchased from one of the shops selling extracts and medicinal plants in Fayoum governorate. The tubers were ground to a fine base to facilitate mixing with commercial feeds, in the second experiment, two doses of *Tribulus terrestris* meal was used to compare the doses of the hormone where 100 g (T_8) and 200g (T_9) of Tribulus was added per kg diet. These levels were chosen based on the studies conducted by some researchers and the percentage of extract, which many researchers were used the extract, not the dried. The extract of *T. terrestris* at a rate of 0, 3, 6, or 9 g/30 l water for 30 days was used by Turan and Cek (2007) for sex reversal of African catfish. The chemical composition of *Tribulus terrestris* according to Amirshekari *et al.* (2016); 16.7% for Crude protein , 2.7% for Crude fat and 7.8% for Crude ash.

Two different doses of the hormone were used; 100 mg/kg diet (T_{10}) and 150 mg/kg diet (T_{11}), each dose was mixed with the prepared feed in Table (1). Nile tilapia larvae at the stage of first feeding were randomly distributed into groups of 5000 fish per hapa, three hapa were assigned for each treatment category. The diet of 17 α-MT and herbal was applied from the third day post hatching to 28 days. After that, the feeding of the fish continued on the commercial diet. The feeding rate applied was 25 -12% (from first week to the fourth week), which was offered eight times a day with the same total amount of feed per day, while feeding rate during nursing period was 8-5% and 6-4 times a day.

Table 1. Treatments used in the first experiment

Treatment	Description
T_1	Basal diet(does not contain 17α-MT)
T_2	Treated diet with 17α-MT and preserved in clear vessels
T_3	Treated diet with 17α-MT and preserved in dark vessels
T_4	Treated diet with 17α-MT and kept at room temperature
T_5	Treated diet with 17α-MT and kept at refrigerated for further use
T_6	Treated diet with 17α-MT and prepared every few days
T_7	Treated diet with 17α-MT and prepared daily

Table 2. Formulation and composition of the artificial diet used for *O. niloticus* fry

Ingredients	%
Fish meal	41
Soy bean meal	27.5
Yellow corn	25.5
Bran	3
Fish oil	2.5
Minerals& Vitamines	0.5
Total	100
Crude protein (%)	45
ME(Kcal/kg feed)	2890
Diet used during fingerlings experiment (commercial diet[1])	
Crude protein (%)	32
ME(Kcal/kg feed)	2900

1: commercial diet was purchased from Skretting Egypt For fish Nutrition, El-Sharqâya, 44621. Egypt

Growth performance and feed utilization parameters

Growth parameters such as Survival (%),weight gain (g), total length (mm), specific growth rate (SGR, %/ day), feed conversion ratio (FCR), and protein efficiency ratio (PER) for different experimental groups at the end of 28 and 90 days using standard formulae as follow; Weight gain (mg) = Mean final fish weight- Mean initial fish weight. Specific Growth Rate (%/ day) = (ln W_2 - ln W_1 ×100)/T_2-T_1), where; W_1=the initial live body weight (g) at time T_1 (day) W_2= the final live body weight (g) at time T_2 (day). Survival (%) = Number of fry present on completion of experiment ×100/No. of fishes stocked. Protein efficiency ratio (PER) = Weight gain, g / Protein intake, g. Total length (mm) = Final length - Initial length. Length gain (%) = (Final length - Initial length) ÷ Initial length×100.

Determination of Antioxidant Activity of *Tribulus terrestris* (TT): Free Radical Scavenging by DPPH (it is a common antioxidant assay) was conducted according to Njoya, (2021); Sharma and Tej, (2009) and Srisailam *et al.* (2017).

Water quality parameters: During the rearing period, water quality parameters (temperature, dissolved oxygen (DO; mg/L), pH, total ammonia nitrogen (TAN;mg/L), salinity (ppt), alkalinity (ppm), nitrite (mg/L), turbidity and some minerals) between treatments were measured weekly up to the end of the experiment. Temperature and DO concentration were measured by the EcoSense DO200A Dissolved Oxygen Meter, pH by the EcoSense pH 100A, salinity by EcoSense EC300A, and turbidity by turbidity meter TU-2016 While total ammonia, nitrite and some minerals were mustered by the YSI 9300 Photometer.

Statistical analysis: The analysis of variance and means tests were analyzed using statistical package for social science Version 24 (SPSS Inc., Chicago, IL, USA). The significant differences at level P < .05 were determined. a one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used.

RESULTS AND DISCUSSION

Water quality during experiments

The water quality status monitored during the experimental period is summarized in Tables 3 and 4. The parameters such as temperature, dissolved oxygen, pH, ammonia, salinity, alkalinity, nitrite, turbidity and some minerals were measured and found congenial for the growth Nile tilapia, The DO and temperature levels stayed well within this range, which is favourable for tilapia growth. (Soto, 1992 and Phelps and Popma, 2000).

Table 3. Water quality values measured during trial one.

Treatment	Temp (c°)	DO (mg/L)	pH	TAN (mg/L)	Salinity ppt	Alkalinity ppm	Nitrite (mg/L)	PO_4	Fe	Cu	Zn	Turb Ntu
	During sex reversal period (28 days)											
T_1	26.8	6.21	7.35	0.121	1.44	165	0.027	1.66	0.21	0.022	0.09	28.32
T_2	26.8	5.90	7.33	0.111	1.44	171	0.026	1.62	0.14	0.024	0.09	28.51
T_3	26.9	6.11	7.52	0.113	1.42	171	0.027	1.63	0.15	0.024	0.08	28.44
T_4	26.7	6.07	7.21	0.131	1.41	3168	0.026	1.66	0.13	0.028	0.11	28.53
T_5	27.1	5.88	7.27	0.101	1.50	168	0.029	1.66	0.19	0.027	0.08	27.97
T_6	26.8	5.86	7.45	0.192	1.39	171	0.029	1.64	0.22	0.025	0.08	28.05
T_7	26.8	5.43	7.24	0.101	1.38	173	0.028	1.63	0.18	0.026	0.08	28.88
	During nursing period (90 days)											
T_1	28.81	5.81	7.11	0.351	2.11	181	0.045	1.72	0.20	0.020	0.091	29.93
T_2	28.55	5.33	7.81	0.311	2.31	181	0.049	1.70	0.18	0.020	0.092	29.72
T_3	28.92	5.88	6.79	0.353	2.13	179	0.058	1.78	0.21	0.021	0.089	29.39
T_4	29.02	5.77	7.01	0.333	2.25	182	0.048	1.80	0.17	0.020	0.187	29.59
T_5	28.94	6.15	7.33	0.381	2.18	181	0.048	1.83	0.21	0.021	0.089	30.08
T_6	29.05	6.41	7.35	0.299	2.30	186	0.049	1.78	0.23	0.020	0.082	30.19
T_7	29.11	6.03	7.11	0.307	2.26	183	0.048	1.76	0.19	0.023	0.084	30.18

Table 4. Water quality values measured during trial two.

Treatment	Temp (c°)	DO (mg/L)	pH	TAN (mg/L)	Salinity ppt	Alkalinity ppm	Nitrite (mg/L)	PO_4	Fe	Cu	Zn	Turb Ntu
	During sex reversal period (28 days)											
T_8	27.2	5.91	7.42	0.131	1.40	168	0.026	1.67	0.21	0.027	0.11	28.43
T_9	27.0	5.88	7.31	0.141	1.44	167	0.028	1.66	0.22	0.027	0.09	28.95
T_{10}	26.9	6.01	7.5	0.129	1.44	169	0.028	1.70	0.18	0.025	0.09	27.99
T_{11}	26.9	5.96	7.1	0.119	1.51	169	0.029	1.69	0.17	0.023	0.09	28.01
	During nursing period (90 days)											
T_8	28.14	6.11	7.20	0.351	2.32	181	0.046	1.81	0.20	0.025	0.098	29.88
T_9	28.84	5.43	7.53	0.344	2.33	179	0.049	1.82	0.21	0.020	0.093	29.96
T_{10}	28.59	5.21	7.61	0.327	2.18	178	0.051	1.76	0.24	0.021	0.092	30.06
T_{11}	28.77	5.33	7.58	0.331	2.26	179	0.051	1.78	0.27	0.021	0.090	30.08

Effect of environmental condition on sex reversal, growth performance and feed utilization of Nile tilapia.

Results presented in Table (5) showed the sex reversal rate of Nile tilapia larvae (%) as affected by light, temperature condition and time after preparing for treated feed by 17α-MT.

Light: Storage conditions for feeds of MT-treated regarding the effect of light were shown in Table (5). The results showed that ther was no significant difference between the stored MT-treated diets (clear and dark vessels) and ability to sex reverse fry, where the percentage of sex reversal of Nile tilapia males for the second and third treatments was 91% and 95.97% respectively. The Nile tilapia populations receiving non treated feed averaged 44.84% males, to give preference to keeping treated feed with hormone in black packages or under dark conditions, as a logical result, the value of the first treatment T_1 (free of MT hormone) came lower in the rate of male sex reversal.

A steroid's potency can be reduced during the feed making process, pure methyl testosterone, a stable, light-sensitive hormone with a melting point of 162 to 167°C, should be kept at room temperature in an amber bottle that is sealed and light-proof (Sigma Chemical Company, 1994). Varadaraj *et al.* (1994) also found that poor storage of hormones and hormone-treated feed can have a significant impact on their efficacy.

According to the results of Table (6 and 7), it is clear that the placement of the hormone treated feed in packages, whether clear or dark, had no significant influence on all growth parameters of the larvae during sex reversal period. However, significant differences were found between treatments during post transsexual (the nursery stage); weight gain, final weight, SGR and FCR in fish fed T_3 improved significantly ($P \leq 0.05$) compared to T_2 or the control group T_1. This may be due to the effect of light on the treated feed, although it does not negatively affect the sexually transformed larvae, but it may affect the fry resulting from those practices during the following months.

Store temperature: The effect of feeding on diets placed in the refrigerator or at room temperature on the sex reversal rate of Nile tilapia larvae is shown in Table (5). There was no significant difference between the stored MT-treated diets and the ability to sex reverse fry. The results of the T_5 were higher than the values of the T_4 in the rate of male sexual conversion. 97.57% male, 2.43 female. and 91% male, 9.0% female, respectively. but without significant differences between them at the same time. Significant differences were found between the treatments whose diets contained hormones compared with the basal diet (does not contain hormones), as the last was the lowest in the rate of sex reversal for males.

Phelps and Smith (2001) investigated the effect of storage conditions of feed on the growth and sex reversal efficacy of Nile tilapia. Fry were fed feed treated with 17α-MT or a non-treated feed for 28 days, and seven feed storage regimes were investigated. Their results indicated that there were no significant differences between treatments. whether with sex transformation to male or some growth performance parameters different at storage temperature.

The efficacy of sex reversal was unaffected by the feed storage conditions of MT-treated rations. With MT-treated feeds stored under any of the aforementioned storage regimes, Nile tilapia populations ranging from 91 % to 97.57 males were produced. According to Varadaraj *et al.* (1994) only 55 % of males were produced, when Mozambique tilapias fry were given MT-treated feed that was held at room temperature and exposed to sunlight and ambient air. According to the NRC (1981), feed held in a place with high temperatures, high moisture, or both will cause lipid peroxidation and vitamin deterioration.

Temperature and light sensitivity are two factors that influence MT deterioration (Varadaraj *et al.*, 1994). However, even though the feed was stored at varying light and temperature before use, these parameters had no effect on the MT concentration in the feed in this investigation. Depending on the temperature height and genetic background, temperature variations are expected to have varying effects on sex differentiation during rearing (Argue and Phelps, 1995).

The growth results (6 and 7) indicate that for treatments T_1, T_4 and T_5 no significant differences were observed during the nursery stage contrary to the stage of sexual transformation, which were weight gain, final weight and SGR the best in T_5 followed by T_1 and T_4 at the same time no differences were noted regarding FCR and survival % among all treatments.

Time after preparing: The effect of feeding on diets prepared daily or prepared every few days on the sex reversal rate of Nile tilapia larvae is shown in Table (5). Feed storage conditions of MT-treated had no impact on efficacy of sex reversal. The highest sex reversal % was in the treated diet with hormone and prepared daily 96.27% followed by T_6 at 93.6% males in populations receiving non-treated feed averaged 44.84% males. Populations greater than 96% of males were produced with MT-treated feeds that had been stored under all of the described storage time (daily and a few days). Feed that has been held at room temperature for more than 90 days is susceptible to the breakdown of oils, vitamins C and E, and other vitamins, as well as the peroxidation of the lipid component (NRC, 1981).

Varadaraj *et al.* (1994) reported that negative storage of hormones and hormone-treated feed can have a significant impact on their efficacy. The findings of this section are similar to those of different authors, such as Howerton *et al*. (1992) and Suprayudi *et al.* (2021), regarding the anabolic effect of MT in fish and all male culture of tilapia under condition storage and preparation management, also the previous authors suggested that the storage of treated feeds could have a major impact on treatment efficacy. Before being fed to tilapias, MT-treated feeds may have been stored in a variety of conditions and for different periods of time before being produced and distributed in commercial quantities.

Male conversion ratio is influenced by many environmental and genetic factors, according to Phelps and pompa (2000), these factors include water temperature, hormone solubility in the solvent, feeding protocol, salinity, photoperiod, stocking density, and hormone storage conditions.

Marwah *et al.* (2007) studied the stability of MT in fish feed before using in tilapia sex reversal. Prolonged storage for up to 6 months at 40 °C had no effect on the concentration of MT in fish feed. This suggests that MT-treated feed may be stored for at least six months, and possibly up to 18 months, in a standard freezer and still retain sufficient potency for sex reversal. Regarding storage experiments, MT concentrations in fish feed are stable and shows little or no decline when preserved at 4 °C or below.

As long as the differences are very small referring to the duration of preparation of the treated feeds, it is possible to refer to the economic factor and the cost of preparation per day compared to preparing in quantities sufficient for some days.

After 28 days of hormonal treatment, growth performance (Table 6) was affected by the preparation period for hormonally treated feed, where the growth efficiency decreased with basal diets. The fry resulting from these treatments were also affected during the nursery period (Table 7), and treatment T_7 was the best followed by T_6 and T_1 at a time when the survival rate between treatments was not affected.

Table 5. Effect of light, temperature condition, and time after preparing on sex reversal of Nile tilapia larvae.

Treat/	light condition				Temperature condition				Time after preparing			
parameter	T_1	T_2	T_3	SED	T_1	T_4	T_5	SED	T_1	T_6	T_7	SED
Male %	44.84^b	91.00^a	95.97^a	2.904	44.84^b	91.00^a	97.57^a	2.86	44.84^b	93.62^a	96.27^a	2.82
Female %	55.16^a	9.00^b	4.03^b	2.904	55.16^a	9.00^b	2.43^b	2.86	55.16^a	6.38^b	3.73^b	2.82

- (a, b) Average in the same row having different superscripts are differ significantly (P≤0.05).
- SED is the standard error of difference

Table 6. Effect of light, temperature condition, and time after preparing on growth performance and feed utilization during sex reversal and nursing stage of Nile tilapia.

Treat/	light condition			Temperature condition			Time after preparing		
parameter	T_1	T_2	T_3	T_1	T_4	T_5	T_1	T_6	T_7
Initial weight (g)/1000 fry	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4	26.4
Final weight (g)/1000 fry	211	212	209.5	211^ab	208.5^b	212.5^a	211^b	214.5^a	215^a
Weight gain (g)/1000 fry	184.6	185.6	183.1	184.6^{ab}	182.1^b	186.1^a	184.6^b	188.1^a	188.6^a
SGR (% day)	7.42	7.44	7.40	7.42^{ab}	7.38^b	7.45a	7.42^b	7.48^a	7.49^a
FCR	1.36	1.36	1.37	1.36	1.37	1.34	1.36	1.32	1.33
Survival rate %	82.63	81.26	80.68	82.63	80.07	82.35	82.63	81.12	80.91

- (a, b) Average in the same row having different superscripts are differ significantly (P≤0.05).

Table 7. Effect of light, temperature condition, and time after preparing on growth performance and feed utilization during nursing stage of Nile tilapia.

Treat/	light condition			Temperature condition			Time after preparing		
parameter	T_1	T_2	T_3	T_1	T_4	T_5	T_1	T_6	T_7
Initial weight(g)	0.211	0.211	.209	0.211	0.209	0.209	0.211	0.209	0.209
Final weight (g)	24.12^c	25.13^b	25.43^a	24.12	26.11	25.39	24.12^c	25.56^b	27.16^a
Weight gain (g)	23.90^c	24.92^b	25.23^a	23.90	25.90	25.18	23.90^c	25.35^b	26.95^a
Initial length (mm)	30	30	29	30	29	30	30	31	31
Final length (cm)	10.50^b	10.60^b	10.75^a	10.50	10.80	10.65	10.50^b	10.65^b	10.95^a
SGR (% day)	5.27^c	5.31^b	5.34^a	5.27	5.36	5.33	5.27^b	5.34^a	5.41^a
FCR	1.984^a	1.983^b	1.982^c	1.984	1.981	1.982	1.984^a	1.982^b	1.980^c
Survival rate %	92.57	92.825	91.67	92.57	90.975	90.74	92.57	93.78	90.57

- (a, b, c..) Average in the same row having different superscripts are differ significantly (P≤0.05).

Effect of adding *Tribulus terrestris* powder and 17α-methyl testosterone in the diets on sex reversal, growth performance, and feed utilization of Nile tilapia.

Chemical analysis for *Tribulus terrestris*

The data of the composition of tribulus powder are given in Table 8 and Fig (2). The total phenol was 62.74_{mg} as gallic acid/g and total flavonoid was 4.31 mg as catchin/ g, while the value of IC_{50} was 1.17_{mg} and 0.86 for antioxidant reducing power (ARP). The results showed that tribulus contains many compounds that show antioxidant and antimicrobial activity such as 2, 4-Di-tert-butylphenol, Octadecane and Heptacosane, in addition to many male stimulating compounds such as Heptacosane and Heptadecane.

Çömlekçioğlu and Çırak (2021) investigated chemical analysis of tribulus to know its content of fatty acids and main components. Oleic acid, palmitic acid, and linoleic acid are the three primary fatty acids found in plant extracts. The total phenolic substance value of plant extracts varies between 2.20-18-77 mg g-1, total flavonoid amount varies between 0.06-0.50 mg g-1 and DPPH value varies between 1.54-10.54 mg mL^{-1}. Also, Lan *et al.* (2009) isolated five steroidal saponins from the fruits of *Tribulus terrestris.* These natural compounds such as; tannins, flavonoids, alkaloids, steroids and terpenoids promote androgenic and anabolic processes as well as stimulation of digestion, immunity, and appetite and play an importance role in controlling unwanted reproduction in tilapia farms (Chakraborty *et al*., 2013; Mehrim *et al*., 2019).

Natural diets rich in phenolic and flavonoid compounds with antioxidant activity have attracted attention in nutrition and food science in recent decades (Lee *et al.*, 2015). Natural flavonoid and phenolic compounds are plant secondary metabolites that hold an aromatic ring containing one or more hydroxyl groups (Tungmunnithum *et al.*, 2018). Because their hydroxyl groups can directly contribute to antioxidant action, phenolic substances are effective electron donors (Bendary *et al.*, 2013). Furthermore, some of them stimulate the synthesis of endogenous antioxidant molecules in the cell (Côté *et al.*, 2010).

Numerous researches have demonstrated that *Tribulus terrestris* possesses, anti-oxidant properties, anti-inflammatory effects, anti-infertility, liver disease, supplemental activity, antibacterial activity, kidney disease, aphrodisiac activity, anti-stress oxidative stress, pro-sexual androgen enhancing effects, liver and kidney, hyperplasia, antioxidant, metal chelator activity, anti- androgenic activity, aphrodisiac properties, and hepatoprotective activity (Sandeep *et al*., 2015; Miraj, 2016). Thus, these studies support the ability of Tribulus to be used as a substitute or in conjunction with the synthetic 17α-MT hormone and also the latest healthy growth of tilapia larvae and fry.

Table 8. Concentration of the main compounds of the *Tribulus terrestris* powder

Analyte	Test Result	Unit
Total Phenol	62.74	mg as gallic acid / g
Total Flavonoid	4.31	mg as catchin/ g
IC_{50}	1.17	Mg
ARP	0.86	

The IC_{50} is the concentration of drug required for 50% inhibition.
ARP: antioxidant reducing power; is used to define antioxidant action of an antioxidant and it is defined as reciprocal of EC50.

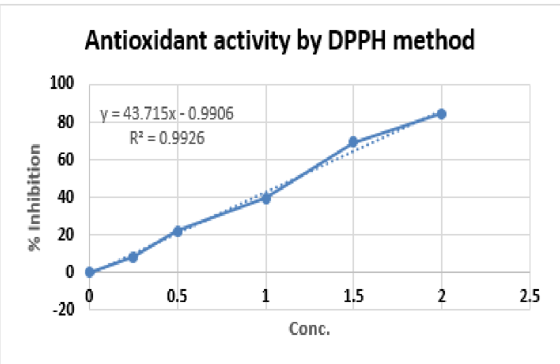

Fig 2. Antioxidant activity by DPPH method

Tribulus terrestris

The results of adding *Tribulus terrestris* powder in the diet on sex reversal % of Nile tilapia larvae showed in Table (9). The obtained results show that there are significant differences between the treatments, the highest male percentage of (83.50%) was achieved at the dose of 200 g/ kg diet *terrestris* powder (T_9) followed by T_8 (71.53%), the basal diet T_1 was the worst in terms of the rate of sex reversal for males of Nile tilapia.

Zaki *et al*. (2020) studied the effect of dietary inclusion of *Tribulus terrestris* and 17 α- methyltestosterone on sex reversal, growth performance, feed utilization, and survival rates of red tilapia, whereas, the treatment containing 2 g *T. terrestris* as extract/kg diet gave a good sex reversal rate for male red tilapia compare 1g *T. terrestris* as extract/kg diet. The studies conducted by Noor El Deen *et al.* (2020) using Aquatic extract of Tribulus terrestris at different levels (0, 1, 2 and 3 g/kg feed) showed that plant extract at a concentration of 2 g/kg feed resulted in the highest percentage of males (97.43±0.13) and may be considered the best concentration for monosex tilapia production with Tribulus terrestris extract among the concentrations tested.

T. terrestris incorporation in sexually undifferentiated tilapia fry resulted in high male percentages, which could be related to phytochemical substances such as steroidal saponin protodioscin that may be regarded as androgenic bioactive phytoconstituents that are reported to inhibit the biological synthesis and the action of estrogen. working as aromatase inhibitors and antagonists to nuclear estrogen receptors in gonad germ cells (Rempel and Schlenk, 2008). In another study, feeding *Tribulus terrestris* extract at a concentration of 2.5 g/kg basal diet to *O. niloticus* resulted in an 84 % male population (Omitoyin *et al.*, 2013). These results agreed with those of Kavitha and Subramanian, (2011); Gültepe *et al.* (2014); Omar *et al.* (2014).

Growth results (Table 10) show no significant differences between all parameters during 28 days. On the contrary of nursery stage, T_9 gave the highest weight gain, final weight, SGR, and best FCR, followed by T_8, while the basal diet was the worst. These results may agree with Yeganeh *et al*. (2017) in the superiority of the two doses of *T. terrestris* extract compared to the control diet, no significant differences were observed among doses of *T. terrestris* extract in FCR and survival. At the same time, they may differ in that the best effect came with the lowest dose (the highest WG and FW were obtained at 1g/kg^{-1} followed by 2 g/kg^{-1} *T. terrestris* extract). According to several studies, the improved growth of fish fry fed medicinal plants such as *T. terrestris* may be due to the stimulation of pancreatic enzyme secretion, absorption, and digestion of nutritional components (Dhas *et al*., 2015).

17α- methyl testosterone

Table (9) displays results related to adding methyl testosterone in increasing proportions in diets on the sex reversal % of Nile tilapia larvae. The highest male percentage was noted in 150 mg of 17α-MT /kg feed (T_{11}) with no significant differences observed in 100 mg/kg diet (T_{10}). The lower male percentage recorded 44.84 % in groups whose diets were without MT hormone (T_1).

Chidambaram *et al.* (2016) investigated the effects of 17 α-methyl testosterone on Nile tilapia sex reversal and growth at various levels of addition, the basal diet was devoid of 17α–MT. T_1, T_2, T_3 and T_4 were fed with feed containing 50, 60 and 100 mg kg-1 of 17α –MT, they showed that treated groups of (T_2) produced the biggest male population, with 93.3 % males. On the contrary, some researchers get results indicating that sex reversal rate improved with low doses of the hormone such as Zaki *et al*, (2020), where the decreased male percentage of 80 % in the fish group treated with 100 mg 17 α -MT/kg diet compared to the male percentage of 90 % in the fish group treated with 60 mg 17 α-MT / kg diet. Also Marjani *et al.* (2009) and Basavaraja and Raghavendra (2017) found lower male percentage in red tilapia fry using the 100 mg of 17α- MT/kg feed compared to other lower doses.

El-Griesy and El-Gamal (2012) used different doses of MT to study the degree of sex stability after one year of treatment, different doses of MT (40, 60 and 80 mg /kg feed) were orally administered to sexually undifferentiated fries up to the 28th day post hatching, their results showed that the highest survival % of the fry were recorded in the group treated with 60 mg MT/kg of feed compared other groups. The maximum sex reversal of males (95and 97%) was recorded at 60 mg of 17 α-MT/kg of diet after 75 days and one year of treatment respectively, many authors recommended 60 mg 17α- MT/kg diet as the best hormonal treatment (El-Griesy and El-Gamal, 2012 and Rodmongkoldee and Leelapat, 2017).

Regarding growth performance (Table 10), FCR and survival%, there are significant differences between the treatments (28 or 90 days); during the sexual transformation period , the highest number was T_{10} and T_1 (no significant differences) compared to T_{11}, while in the growing fish stage. The best growth parameters were obtained with treatments T_{11} followed by T_{10} while the last was T_1. According to Gauthaman and Ganesan (2008), increased testosterone levels are associated with improved growth performance. FCR values, on the other hand, were considerably higher in the hormonal treatments (30, 60, and 100 mg 17 α -MT/ kg diet) than in the control, with the best values coming from the 60 mg 17 α -MT dose (Zaki *et al*., 2020). As a result, increasing the hormone dose in Nile tilapia meals may improve the feed conversion ratio.

Table 9. Effect of different *T. terrestris* and 17-α MT levels on percentages of males and females of Nile tilapia fry.

	***T. terrestris* levels**	
Treatments	**Male %**	**Female %**
T_1	44.84^c	55.16^a
T_8	71.53^b	28.47^b
T_9	83.50^a	16.50^c
SED	3.08	3.08
	17- α MT levels	
Treatments	Male %	Female %
T_1	44.84^b	55.16^a
T_{10}	95.97^a	4.03^b
T_{11}	96.47^a	3.53^b
SED	2.800	2.800

- (a, b, c..) Average in the same row having different superscripts are differ significantly (P≤0.05).
- SED is the standard error of difference

Table 10. Effect of different *T. terrestris* and 17α-Methyl testosterone levels on growth performance and feed utilization during sex reversal and nursing stage of Nile tilapia.

Treat/ parameter	**Sex reversal period**					
	T_1	**T_8**	**T_9**	**T_1**	**T_{10}**	**T_{11}**
Initial weight (g)/1000 fry	26.4	26.4	26.4	26.4	26.4	26.4
Final weight (g)/1000 fry	211	211.5	209	211^a	211.5^a	205.5^b
Weight gain (g)/1000 fry	184.6	185.1	182.6	184.6^a	185.1^a	179.1^b
SGR (% day)	7.42	7.43	7.39	7.42^a	7.43^a	7.33^b
FCR	1.36	1.35	1.38	1.36	1.36	1.4
Survival rate %	82.63	81.75	81.39	82.63	79.58	81.09
	Nursing period					
Parameters	T_1	T_8	T_9	T_1	T_{10}	T_{11}
Initial weight(g)	0.211	0.209	0.209	0.211	0.209	0.209
Final weight (g)	24.12^c	27.04^b	30.37^a	24.12^c	30.06^b	31.13^a
Weight gain (g)	23.90^c	26.83^b	30.16^a	23.90^c	29.85^b	30.92^a
Initial length (mm)	30	30	29	30	30	28
Final length (cm)	10.50^c	10.95^b	11.45^a	10.50^b	11.40^a	11.65^a
SGR (% day)	5.27^c	5.40^b	5.53^a	5.27^c	5.52^b	5.56^a
FCR	1.984^a	1.980^b	1.977^c	1.984^a	1.977^b	1.976^c
Survival rate %	92.57	92.34	91.26	92.57	91.16	91.29

- (a, b, c..) Average in the same row having different superscripts are differ significantly (P≤0.05).

CONCLUSION

The results indicate that the male hormone is affected by some environmental factors with regard to its ability to sex reverse or the efficiency of resulting fry in growth and survival rate, although this effect is low. Results of second trial refer to the *Tribulus terrestris* powder at 200 g/kg diet and 17 α-methyl testosterone at 150 mg/kg diet are the most effective for sex reversal in Nile tilapia larvae under experimented condition.

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تأثير بعض المعاملات الغذائية والبيئية علي إنتاج البلطي النيلي وحيد الجنس

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أجريت تجربتان على يرقات وزريعة أسماك البلطي النيلي*Oreochromis niloticus* بأحد المفرخات التجارية بمحافظة الفيوم خلال عام 2019. تم إجراء الدراسة الأولى لدراسة تأثير بعض العوامل مثل (الضوء، درجة الحرارة وفترة إعداد وتحضير الأعلاف) في العلف المعامل بهرمون الذكوره 17 ألفاميثيل تستوستيرون(17 α –MT) بتركيز 120 ملجم/كجم علف على معدلات الإنعكاس الجنسي وآداء النمو. تم تخزين حوالي 5000 يرقة لكل هابة (2× 1م، وارتفاع الماء 80 سم) وغذيت اليرقات علي الأعلاف المعالجه لمدة 28 يوم. لم تلاحظ فروقاً معنوي بين المعاملات الغذائية المعالجة بالـ MT– α 17 والقدرة على عكس الجنس، وجاءت أعلى نسبة مئوية لإنعكاس الجنس(لذكور) في المعاملات الغذائية المضاف اليها الهرمون الذكري والمحفوظه بالثلاجه 97.5% وكذلك المحضرة يوميا 96.27٪. تشير نتائج هذه التجربة إلى أن معدلات التحول الجنسي للذكور في يرقات البلطي النيلي تأثرت بطريقة تحضير العلف المعالج هرمونيا بالرغم من أن هذا التأثير كان محدودًا. أجريت التجربة الثانية لدراسة تأثير إضافة مسحوق نبات الحسك الطبي المجفف (*Tribulus terrestris*)على التحول الجنسي مقارنة بالهرمون الذكري MT– α 17 تحت نفس تجهيزات التجربة الأولى. أظهرت النتائج وجود فروقاً معنوية بين المعاملات بزيادة مسحوق الحسك الطبي مقارنة بمعاملة الكنترول. وجدت أيضاً فروقاً معنويه بين المعاملات المضاف اليها هرمون الذكورة 17 ألفاميثيل تستوستيرون وعليقة الكنترول. يمكن التوصية بناءً علي نتائج هذه التجربة بإستخدام مجفف نبات الحسك الطبي بمعدل200 جم/كجم علف وهرمون 17 ألفا ميثيل تستوستيرون بمعدل 150 ملجم/كجم علف حيث تعتبر الأكثر فاعلية لعكس الجنس ليرقات أسماك البلطي النيلي.

الكلمات الداله: البلطي النيلي – التحول الجنسي – نبات الحسك – آداء النمو – الهرمون الذكري.