Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 27(4): 95 – 110 (2023)
www.ejabf.journals.ekb.eg

Efficacy of Gokshura (*Tribulus terrestris*) on Sex Reversal and Growth Performance of the Nile Tilapia (*Orechromis niloticus*) Fries Reared in Prolonged Water Exchange

El-Sayed I. Attia, Abdel-Moneim M. Yones, Ataallah A. Metwalli, Hamed H.E. Saleh*

National Institute of Oceanography and Fisheries (NIOF), Aquaculture Division, Fish Hatching Lab., Cairo, Egypt

***Corresponding author: hhsaleh90@gmail.com**

ARTICLE INFO

Article History:
Received: March 11, 2023
Accepted: May 18, 2023
Online: July 12, 2023

Keywords:
Nile tilapia,
Fry,
Sex reversal,
Growth performance,
Tribulus terrestris,
Hormonal treatment

ABSTRACT

A total of 2000 fries of the Nile tilapia (*Oreochromis niloticus*) were obtained from private hatcheries to study the hormonal or gokshura extract and powder treatments on growth and sex reversal ratio under prolonged water exchange conditions. The 100- day experiment was applied using a completely randomized design. Fries were distributed in eight fiberglass tanks, with a volume of 1.5 m^3, fitting for a stocking density of 250 fries/ tank. The initial average weight was recorded as 24.25± 12.65 mg/ fry. The experiment was divided into two stages, the first one (Sex reversal) lasted for 28 days, while the second (Sex differentiation) extended for 72 days. Four experimental treatments were conducted as follows: T_1 was considered as a control diet without any addition; T_2; a diet with the addition of 100mg of 17α-methyltestosterone hormone/ kg of diet; T_3 formed the diet supplemented with 200g/kg of *Tribulus terrestris* plant powder, and T_4 diet which contained 2g/ kg extraction of *Tribulus terrestris* plant. Feeding for all experimental treatments with hormone and plant (extract and powder) lasted for a period of 28 days concerning the first stage, with a diet containing 40% of crude protein, followed by a seventy-two- day rearing, during which fish samples were fed on a diet containing 30% of crude protein without any addition. The results showed that T_4 gave the best performance, feed conversion ratio and survival rate, followed by T_3. However, less performance was obtained for T_2 and control. The highest male percentage was recorded for hormonal treatment (T_2), followed by T_4, T_3 and control. The results of sex reversal ratio for the Nile tilapia fries revealed that the use of 2g/ kg diet extract of *Tribulus terrestris* (T_4) showed no significant difference with the hormonal treatment using 17α-MT (T_2). The present results proved that the use of 2g/kg extract of *Tribulus terrestris* is an eco-friendly, safe and economical aquaculture technique replacing synthetic hormones in commercial hatcheries.

INTRODUCTION

The Nile tilapia is a widely farmed fish species due to its ability to grow under different conditions, gaining consumed acceptance with high marketability index **(Lind *et***

***al.*, 2019; Gewaily *et al.*, 2021)**. The Nile tilapia can withstand the stressful condition of zero water exchange (8 weeks) when fed probiotic-based diets **(Kord *et al.*, 2022)**.

Technology advancements including control of early maturity and prolific breeding have contributed to the expansion of tilapias' global production **(FAO, 2017)**. Masculinization of tilapia continues to be an important means for aquaculturists to prevent undesirable reproduction, which ultimately leads to higher fish growth; however, the popular means of all-male tilapia population via the use of synthetic hormones have been shrouded in criticisms by many stakeholders in the industry, based on the belief that the residual effects of such hormone has harmful side effects **(El-Sayed *et al.*, 2012)**.

Hormonal sex reversal using exogenous steroids, mainly 17α-methyl testosterone (MT), yields high success of masculinization **(Phelps, 2006; Homklin *et al.*, 2011)**. Therefore, MT is the common widely used method for the production of all individuals male in tilapia culture. Nevertheless, the carcinogenicity of MT and the related adverse effects on human health and aquatic ecosystems proceed to raise public concerns **(Mlalila *et al.*, 2015; Haitham, 2018)**. Protracted exposure to MT in the application process can cause hepatotoxicity and fetotoxicity **(Vick & Hayton, 2001)**. Additionally, MT poses a health risk to hatchery personnel engaged in tilapia seed production **(Megbowon & Mojekwu, 2014)**. Moreover, 30% of the administered hormone treated diet is unavailable to the fish during feeding **(Ramirez-Godinez *et al.*, 2013)**. Meanwhile, only 10% of the hormone in the consumed diet is utilized for sex inversion **(Ong *et al.*, 2012)**. As a result, hormone residues build-up in the closed environments as eitheractive metabolites excreted by the treated fish or leachates from uneaten food. Furthermore, synthetic hormones accumulate in the sediment water and aquatic biota while having extremely adverse effect on living organisms (including other fish) at a very low concentration **(Rezka *et al.*, 2015)**. The leakages of MT and its metabolites into the aquatic environment from uneaten or un-metabolized food have the potential to disrupt endocrine and reproductive systems of non-target aquatic organisms **(Ramirez-Godinez *et al.*, 2013; Rivero-Wendt *et al.*, 2013; Nian *et al.*, 2017)**.

The acceptance of plants for use in aquaculture is linked to the ease of access and being relatively safer for the environment and humans than synthetic hormones **(Chakraborty *et al.*, 2013; Reverter *et al.*, 2014)**. In terms of plant effect, several plant extracts are predominantly used to improve fish growth, enhance innate immune responses and control disease in aquaculture, compared to reproduction control **(Reverter *et al.*, 2014; Baluran *et al.*, 2018)**. On the other hand, the androgenic compounds present in some plant extracts could be used to control unwanted reproduction in tilapia production systems **(Gabriel *et al.*, 2017; Ghosal *et al.*, 2021)**. The phytoandrogens, for example, testosterone, rostenedione, dehydroepi and rosterone have been implicated in the fish sex reversal process **(Godwin *et al.*, 2003)**.

Gokshura, *Tribulus terrestris* is a herbaceous perennial yearling plant growing to a height of 10– 60cm. It belongs to order Zygophyllales and family Zygophyllaceae

(Yanala *et al.,* 2016). This plant is distributed in the Mediterranean, warm parts of Europe, Asia, USA, Africa and Australia **(Chen *et al.,* 2013)**. It is used in the traditional medicine in China, India, Iraq and Iran among others. Previous studies showed that *T. terrestris* contains steroids, flavonoids, unsaturated fatty acids, saponins, resins, alkaloids, aspartic acid, vitamins, tannins, glutamic acidnitrate and potassium **(Yan *et al.,* 1996)**. It has many medical effects, including antimicrobial, antibacterial, anti-toxic and anti-oxidant. In addition, this plant is used for the treatment of cardiovascular diseases, cancers, respiratory diseases and joint pain **(Kadry*et al.,* 2010)**. Additionally, *T. terrestris* extract increases body's ability to build muscle mass, strength and improves circulation and oxygen transfer **(Arsyad, 1996)**. Moreover, *T. terrestris* compounds increases libido and counteract of cold-natured infertility and menopausal disorders. Furthermore, it is used as a traditional medicine in India and China to treat sexual dysfunction and increase libido by increasing the level of testosterone and LH **(Martino-Andrade *et al.,* 2010)**. Among the several alternatives which can be considered to mitigate against the use of synthetic steroids for the sex reversal of tilapia is *Tribulus terrestris* extracts, explored as a potential sex reversal substitute for synthetic 17-α-methyltestosterone.

The potential of *T. terrestris* extracts trigger testosterone and 11-ketotetestosterone (11-KT) production, which transforms fish sex to males **(Gharaei *et al*., 2020; Ghosal & Chakraborty, 2020)** can be harnessed to control prolific spawning in tilapia production.

The present study was organized to evaluate different sex reversal and growth performances by using 17 α–methyltestosterone (MT) or gokshura (*Tribulus terrestris*) extract and powder in order to optimize seed production practices in the Nile tilapia (*Orechromis niloticus*) fries reared in prolonged water exchange.

MATERIALS AND METHODS

Ethical statement

This work was conducted with the strict recommendations and approval of the National Institute of Oceanography and Fisheries (NIOF, Egypt) Committee for ethical care and use of animals/aquatic animals (NIOF-IACUC, Code: NIOF-AQ5-F-23-R-024).

Experimental conditions

A total of 2000 fries of the Nile tilapia (*Orechromis niloticus*) were obtained from the private hatchery located in Fayoum Governorate, Egypt. Fish were transported to Fayoum Aquatic Research Station Lab. (National Institute of Oceanography and Fisheries). Fries were distributed among eight fiberglass tanks, with a volume of 1.5 m^3, a stocking density of 250 fries/ tank, an initial average weight of 24.25± 12.65 mg/ fry and and an initial total length of 9.50± 1.50mm/ fish. The water system includes two pumps and upstream sandy filter units at a point between the water source (Earthen pond) and tanks. The pumps drowned the water to the storage tanks and forced it through polyvinyl chloride (PVC) tubes into the rearing tanks. This experiment was conducted to study the hormonal or gokshura extract and powder treatments on growth and sex reversal ratio under prolonged water exchange conditions. During the experimental period (100 days), 10% of water tanks was changed. To avoid the rise of ammonia in tanks, 2 grams of yucca (*Yucca schidigera*) plant extraction and a probiotic were weekly added via dissolving it in 1 liter of water and distributing the outcome into the tank. Yucca (*Yucca schidigera*) can be added to water to improve its quality by reducing levels of ammonia **(Hassan *et al.*, 2017; Fayed *et al.*, 2019)**.

Experimental design

The experiment was applied using a completely randomized design. Four experimental treatments were used as follows: T_1 forming the control diet without any addition, T_2; diet (40% CP) supplemented with a 100mg of 17α-methyltestosterone hormone/ kg of diet, T_3; diet (40% CP) supported with adding 200g/ kg of *Tribulus terrestris* plant powder and T_4; diet (40% CP) which contained 2g/ kg of the extraction of *Tribulus terrestris* plant. Fish in the control were fed with a basal diet without any addition of hormone or plant powder and extraction.

Experimental fish were fed with a commercial diet containing 40% of crude protein for a period of 28 days concerning the 1st stage, which is considered the sex-reversal stage of fries. The second stage of fry rearing lasted for 72 days, during which the fries were fed a commercial diet of 30% crude protein to detect sex-differentiation of fries. Feeding for all experimental treatments with the hormone and plant (extract and powder) lasted for a period of 28 days concerning the first stage on a diet containing 40% crude protein, followed by a seventy-two- day rearing feeding on a diet with 30% crude protein without any addition. The experiment lasted for 100 days (28 days + 72 days).

Experimental management

Water quality parameters in experimental tanks were measured weekly according to (APHA, 2005) to ensure proper water quality for the experimental fish. Fish were fed 20% of their biomass in the first week, and the diets were offered 5 times/ day; then it was reduced to 15% in the second week and 10% in the third and fourth weeks. Fish were weighed every two weeks to adjust feed quantity of each diet. A fine commercial powder feed containing 40% crude protein (Skritting Egypt®) was used for the first 28 days of the experiment, while pelleted (1.2 mm) commercial fish feed containing 30% crude protein (Grand Aqua®) was used for the rest of the experiment, and fish were fed as 5% of total biomass during the sex-differentiation period.

Growth evaluation

Growth performance and diets efficiency were assessed via these equations:

- Body gain=[Final body mass-initial body mass].
- Specific growth rate (SGR, %/day)=100×(Ln final weight-Ln initial weight)/ time.
- Feed conversion ratio (FCR) = (feed given per fish)/ (weight gain per fish).

-Survival rate=100×(final number of fish/ initial number of fish).

Sex reversal measurements

At the end of the experiment (100 days), fish in each tank were netted and counted for survival rate determination. Sex ratio was determined through anesthetizing all fish in each tank, hence, visually examining the genital papilla of fish according to **Basavaraja (1991)**.

Statistical analyses

Statistical analyses were carried out using SPSS version 23, (2015) SPSS Institute, Cary, NC, USA). Fish performance data were tested for treatment effect using one-way analysis of variance (ANOVA). Significant differences ($P \leq 0.05$) between means were determined using Duncan test. The results are expressed as means ± standard error (SE).

RESULTS AND DISCUSSION

Chemical composition of gokshura (*Tribulus terrestris*)

The chemical composition of *Tribulus terrestris* includes 16.7% for crude protein, 2.7% for crude fat and 7.8% for crude ash according to **Amirshekari *et al.* (2016)**. The chemical composition of *Tribulus terrestris* leaves is composed of 65% for moisture, 21.33% for crude protein, 5% for crude lipid, 5% for crude ash, 13% for crude fiber and 55.67% for carbohydrates **(Hassan *et al.*, 2005)**. Whereas, the mineral composition of *Tribulus terrestris* leaves contains K (220 mg/ 100g), Ca (142 mg/ 100g), Mg (30 mg/ 100g), P (23.83 mg/ 100g), Na (5 mg/ 100g), Fe (2.80 mg/ 100g), Cu (1.28 mg/ 100g) and Zn (0.10 mg/ 100g) according to **Hassan *et al*. (2005)**. Moreover, it contains bioactive-component as steroids, flavonoids, unsaturated fatty acids, saponins, resins, alkaloids, aspartic acid, vitamins, tannins, glutamic acid nitrate and potassium **(Yan *et al.*, 1996)**.

Water quality

Table (1) shows that water quality parameters were maintained within the acceptable ranges as recorded in previous studies **(Boyd, 1979; Hassan *et al.*, 2013; Effendi *et al.*, 2020; Putra *et al.*, 2020)**. Water temperature, pH, dissolved oxygen and water ammonia were measured every week. Water temperature ranged from 28 to 30°C; dissolved oxygen ranged from 6.21 to 7.54mg/ l; water pH ranged from 7.6 to 7.8 mg/l; total ammonia ranged from 0.642 to 0.891 mg/l; nitrite fluctuated from 0.056 to 0.076 mg/l, and nitrate ranges were from 0.129 to 0.212 mg/l.

Table 1. Water quality parameters during the experiment

Parameter	Measurement range
Temperature; °C	28 to 30
pH	7.62 to 8.21
Dissolved oxygen, mg/l	6.21 to 7.54
Total ammonia, mg/l	0.642 to 0.891
Nitrite, mg/l	0.056 to 0.076
Nitrate, mg/l	0.129 to 0.212

Growth performance and survival rate of Nile tilapia fries

The growth performance in the length of fries in Table (2) shows an increase in T_4 and T_3, which were treated with an extract or powder of *Tribulus terrestris*, compared to other treatments. In the same manner, as presented in Table (3), the performance in weight of fries fed with *Tribulus terrestris* (T_4 and T_3) treatments significantly differ than other treatments.The results revealed that the highest weight gain (after 100 days) was obtained in T_4, with a weight of 18.65g followed by T_3, T_2 and T_1 (control). The highest survival rate (Fig. 1) was obtained in T_4 (84.6%), T_3 (83.6%), T_1 (82.2%, without significant difference between them, while recording significantly higher values, compared to T_2 (77%). The highest gain recorded with using *Tribulus terrestris* in the present study may be due to its containment of phytochemical compounds, such as flavonoids, steroidal sapogenins and alkaloids **(Gültepe *et al.*, 2014)**. These findings agree with the previous results by using *Tribulus terrestris* **(Omitoyin *et al.*, 2013; Gültepe *et al.*, 2014; Omar *et al.*, 2014; Sadiqin, 2021)**. Those compounds have been proved to possess a positive effect on fish growth performance.

Table 2. Growth performance in length of the Nile tilapia fries treated with different sex reversal methods for 28 days

Item	**Treatments**				***P*-value**
	T_1	**T_2**	**T_3**	**T_4**	
Initial length, mm/fish	9.50± 1.50	9.50± 1.50	9.50± 1.50	9.50± 1.50	1.00
Final length, mm/fish	21.50± 1.50^{c}	22.50± 0.50^{bc}	26.00± 1.00^{ab}	28.00± 1.00^{a}	0.035
Length gain, mm/fish	12.00± 0.00^{b}	13.00± 1.00^{b}	16.50± 0.50^{a}	18.50± 0.50^{a}	0.005
Daily length gain, mm/fish/day	0.43± 0.00^{b}	0.47± 0.04^{b}	0.59± 0.02^{a}	0.66± 0.02^{a}	0.006
SGR for length, %/day	2.95± 0.32	3.12± 0.49	3.64± 0.43	3.90± 0.44	0.450

Means sign by different superscript letters are significant ($P \leq 0.05$).
T_1: control diet without any addition.
T_2: fries treated with 100 mg 17α-methyl testosterone per kg diet.
T_3: fries treated with 200 g *Tribulus terrestris* powder per kg diet.
T_4: fries treated with 2 g *Tribulus terrestris* extract per kg diet.

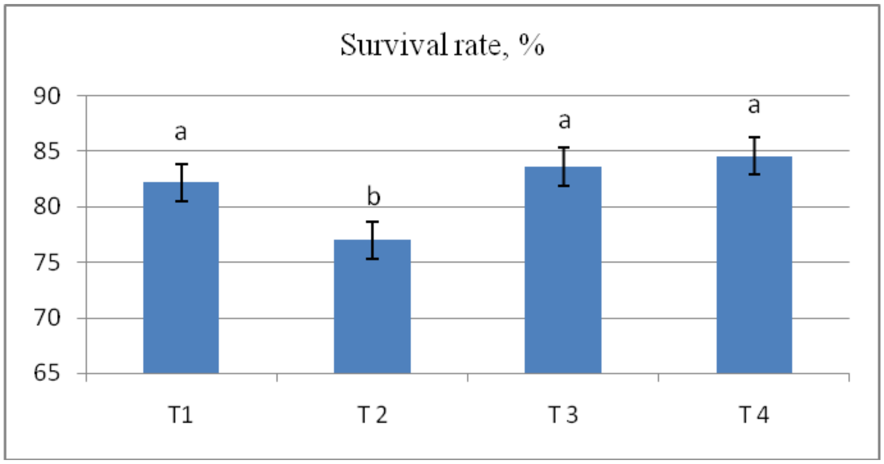
However, the high growth performance associated with T_2, which used 100mg 17α- MT/ kg diet compared to T_1 (control) coincides with the results of previous studies **(Khouraiba, 1997; Ekwu & Sikoki, 2001; Ajiboye *et al*., 2015)**. On the other hand, other research recorded that 60mg of 17α- MT/ kg diet was the best hormonal treatment in treated tilapia fries **(El-Greisy & El-Gamal, 2012; Rodmongkoldee & Leelapat, 2017)**. The enhancement in growth of tilapia fry treated with steroid as a result of the anabolic effect of MT and, in turn, inducing metabolic rates, and consequently improving growth indices **(Tveiten *et al*., 1998; Norbeck & Sheridan, 2011)**.

Table 3. Growth performance in weight of the Nile tilapia fries treated with different sex reversal methods for 28 days and after 100 days

Item	**Treatments**				***P*-value**
	T_1	**T_2**	**T_3**	**T_4**	
Sex reversal period (after 28 days)					
Initial body weight, mg/ fish	24.25± 12.65	24.25± 12.65	24.25± 12.65	24.25± 12.65	1.00
Final body weight, mg/ fish	158.00± 22.00^{c}	225.50± 45.50^{bc}	296.50± 21.50^{ab}	353.50± 27.50^{a}	0.039
Weight gain, mg/ fish	133.75± 9.35^{c}	201.25± 32.85^{bc}	272.25± 8.85^{ab}	329.25± 14.85^{a}	0.007
Daily weight gain, mg/fish/day	4.78± 0.34^{c}	7.19± 1.18^{bc}	9.73± 0.32^{ab}	11.76± 0.53^{a}	0.007
SGR, %/day	7.23± 1.57	8.46± 1.34	9.50± 1.81	10.13± 1.79	0.646
Sex-differentiation period (28th to 100 days)					
Final body weight, g/fish	14.44± 0.92^{b}	15.40± 1.56^{b}	17.84± 0.96^{ab}	19.00± 1.16^{a}	0.052
Weight gain, g/fish	14.28± 0.90^{b}	15.18± 1.52^{b}	17.55± 0.94^{ab}	18.65± 1.14^{a}	0.054
Daily weight gain, g/fish/day	0.204± 0.018^{b}	0.211± 0.022^{b}	0.244± 0.013^{ab}	0.259± 0.016^{a}	0.054
SGR, %/day	6.29± 0.11^{a}	5.89± 0.14^{b}	5.70± 0.03^{b}	5.54± 0.03^{b}	0.015

Means sign by different superscript letters are significant ($P \leq 0.05$).
T_1: control diet without any addition.
T_2: fries treated with 100 mg 17α-methyl testosterone per kg diet.
T_3: fries treated with 200 g *Tribulus terrestris* powder per kg diet.
T_4: fries treated with 2 g *Tribulus terrestris* extract per kg diet.

Fig. 1. Survival rate of the Nile tilapia fries after 100 days



(a and b) Average in having different superscripts differ significantly ($P \leq 0.05$)

T_1: control diet without any addition.

T_2: fries treated with 100 mg 17α-methyl testosterone per kg diet.

T_3: fries treated with 200 g *Tribulus terrestris* powder per kg diet.

T_4: fries treated with 2 g *Tribulus terrestris* extract per kg diet.

Feed conversion ratio (FCR) after 28 or 100 days

As presented in Table (4), FCR values show no significant difference among treatments. The best FCR values were obtained with the dietary of *T. terrestris* extract at 2g/kg and 200g/ kg powder (T_4 and T_3) after 28 or 100 days. The worst FCR was recorded in the control (T_1) that used a diet with no addition and T_2, which used hormonal concentration of 100 mg17-α MT/kg diet. The present results agree with those of **Omitoyin *et al.* (2013)**, **Gültepe *et al.* (2014)** and **Omar *et al.* (2014)**. The enhancement of feed utilization regarding FCR achieved from the inclusion of *T. terrestris* extract or powder to fish feeds can be attributed to their phytochemical compounds which improved feed digestion and nutrient absorbance **(Gültepe *et al.*, 2014)**. In the same manner, **Lone and Matty (1981)** concluded that using 17-α MT in fish fry diets improved feed utilization as detected in T_2.

Table 4. FCR of the Nile tilapia fries treated with different sex reversal methods for 28 days and after 100 days

Item	Treatments				*P*-value
	T_1	T_2	T_3	T_4	
FCR after 28 days	1.55± 0.19	1.48± 0.13	1.37± 0.01	1.32± 0.04	0.544
FCR after 100 days	1.87± 0.060	1.89± 0.055	1.93± 0.045	1.95± 0.040	0.689

T_1: control diet without any addition.

T_2: fries treated with 100 mg 17α-methyl testosterone per kg diet.

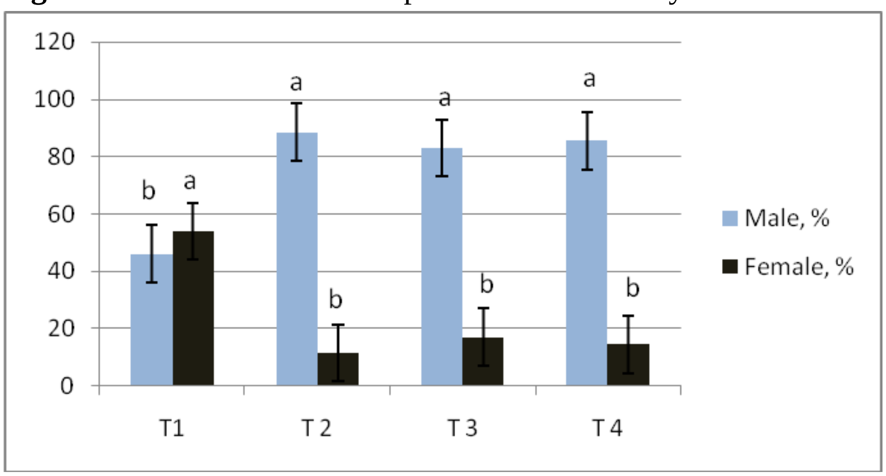
T_3: fries treated with 200 g *Tribulus terrestris* powder per kg diet.

T_4: fries treated with 2 g *Tribulus terrestris* extract per kg diet.

Sex ratio of the Nile tilapia fingerlings after 100 days

The effect of different experimental treatments on males and females of the Nile tilapia fingerlings after the whole experimental period (100 days) is presented in Fig. (2). The highest male percentage was recorded for T_2 (100 mg 17-α MT/ kg feed) treatment with 88.5%, followed by T_4 (85.5%) which was fed 2g of the extract of *T. terrestris*/ kg diet) and T_3 (83%) by using 200g of the powder of *T. terrestris*/ kg diet. However, the less significant male value was recorded with the control diet (46.0%). The present result obtained with the hormonal treatment is in line with the results of **Marjani *et al.* (2009)** and **Basavaraja and Raghavendra (2017)**. On the other hand, a higher value was recorded with hormonal treatment of 60mg of 17-α MT/ kg feed **(Ali *et al*., 2011; Celik *et al*., 2011; El-Greisy & El-Gamal, 2012)**. The current results are correlated with the results of **Marjani *et al.* (2009)** and **Basavaraja and Raghavendra (2017)**, reporting lower male percentage in red tilapia fry using the 100mg of 17α-MT/ kg feed, compared to other lower doses. The optimum concentration of 17 α-MT was important to avoid the problems related to overdoses of high concentration which led to sterility, as recorded in the study of **Goudie *et al.*(1983)**. In contrast, sub-optimal doses cause intersexes **(Popma & Green, 1990)**.

Fig. 2. Sex ratio of the Nile tilapia fries after 100 days



(a and b) Average in having different superscripts differ significantly ($P \leq 0.05$);
T_1: control diet without any addition;
T_2: fries treated with 100 mg 17α-methyl testosterone per kg diet;
T_3: fries treated with 200 g *Tribulus terrestris* powder per kg diet, and
T_4: fries treated with 2 g *Tribulus terrestris* extract per kg diet.

As shown in Fig. (2), the treated fry of the Nile tilapia showe a significant difference ($P \leq 0.05$) among fish groups. Fries of the Nile tilapia treated with *T. terrestris* which obtained highest male percentage agree with the use of the same plant value of 2g/ kg extract recorded in previous studies **(Ghosal & Chakraborty, 2020; Hassona *et al.*, 2020; Noor El Deen *et al.*, 2020; Sadiqin, 2021; Zaki *et al.*, 2021)**. The effect of *T.*

terrestris in the sexually undifferentiated tilapia fry may be attributed to some phytochemical compounds that inhibit the biological synthesis and the action of estrogen receptor in gonad germ cells **(Rempel & Schlenk, 2008)**.

In the same trend, some researchers have demonstrated the *Tribulus terrestris* anti-infertility and pro-sexual androgen enhancing effects **(Sandeep *et al*., 2015; Miraj, 2016)**. Consequently, the present results support the ability of *Tribulus terrestris* plant to be used as a substitute or in conjunction with the synthetic 17α-MT hormone and recorded the latest healthy growth of tilapia larvae and fry.

Male conversion ratio is influenced by many environmental and genetic factors, according to **Pandian and Varadaraj (1990)** and **Phelps and Pompa (2000)**. They stated that, male conversion ratio is influenced by many genetic and environmental factors (i.e. water temperature, the degree of hormone solubility in the solvent, feeding protocol, salinity, photoperiod, stocking density, conditions at the storage of the hormone or the plant extract and also storage conditions of treated feed and others).

CONCLUSION

The results emanating from this study might be implemented for the development of eco-friendly aquaculture technique, replacing synthetic hormones and chemotherapeutics with bio-degradable natural compounds. The result suggested that *Tribulus terrestris* might be regarded to be more potent for the induction of masculinization in the Nile tilapia as it produced higher percentage of males comparable to synthetic hormones. Dietary administration of *Tribulus terrestris* extract at a concentration of 2g/ kg feed resulted in high performance, feed conversion ratio and survival rate. It can also be used as safe and accepted for the production of sex reversed-male of the Nile tilapia. However, the highest percentage of males produced by *Tribulus terrestris* was found to be below the ideal requirement of 100% male population. Thus, further studies are required to establish an ideal treatment regime for the production of all-male tilapia population using the plant materials and provide conclusive evidence regarding their efficacy to be used as a sex-reversal agent in tilapia culture. Studies are also warranted for identification and isolation of the plant bioactive compound responsible for the androgenic property for potential commercial use.

REFERENCES

APHA (2005). Standard methods for the examination of water and wastewater, 21st ed. American Public Health Association; American Water Works Association; Water Environment Federation,Washington, DC.

Ajiboye, O.O.; Okonji, V.A. and Yakubu, A.F. (2015). Effect of testosterone induced sex reversal on the sex ratio, growth enhancement and survival of Nile tilapia

(*Oreochromis niloticus*) fed coppens and farm produced feed in a semi flow-through culture system. Fisheries and Aquaculture Journal, 6(2): 1.

Ali A.A.; Mahmoud Y.S. and Sovjak, R. (2011). Effect of hormonal treatment on sex reversal of Nile tilapia (*Oreochromis niloticus*) fry. Agricultura Tropicaet Subtropica (Czech Republic).

Amirshekari, T.; Ziaei, N.; Ghoreishi, S.M. and Esfandiarpour, E. (2016). The effects of adding aqueous extract and dried aerial part powder of *Tribulus terrestris* on productive performance and blood parameters of laying hens. J. Appl. Poult. Res., 25: 145-155.

Arsyad, K.M. (1996). Effect of protodioscin on the quantity and quality of spermatozoon from males with moderate idiopathic oligozoospermia. Medika, 22(8): 614-8, 1996.

Baluran, S.M.D.L.; Quiazon, K.M.A.; Garcia, G.G.; Fernando, S.I.D. and Velasco, R. R. (2018). Immunostimulatory effect of Benguet pine (Pinuskesiya) pollen on Nile tilapia (*Oreochromis niloticus* L.). International Journal of Biology Pharmacy and Allied Sciences, 7(9): 1652-1663.

Basavaraja, N. (1991). Effects of feeding high levels of 17α-methyl testosterone on the sex ratio and growth of two sizes of *Oreochromis mossambicus* (Peters). Indian Journal of Animal sciences, 61(7): 775-779.

Basavaraja, N. and Raghavendra, C.H. (2017). Hormonal sex reversal in red tilapia (*Oreochromis niloticus* and *Oreochromis mossambicus*) and inheritance of body colour in *O. mossambicus* and red tilapia: implications for commercialfarming. Aquaculture International, 25(3): 1317-1331.

Boyd, C.E. (1979). Water quality in warmwater fish ponds. Auburn: AL Alabama Agriculture Experiment Station, Auburn University. 482 pp.

Celik, I.; Guner, Y. and Celik, P. (2011). Effect of orally-administered 17α-methyl testosterone at different doses on the sex reversal of the Nile tilapia (*Oreochromis niloticus*, Linneaus 1758). Journal of Animal and Veterinary Advances, 10(7): 853-857.

Chakraborty, S.B.; Horn, P. and Hancz, C. (2013). Application of phytochemicals as growth-promoters and endocrine modulators in fish culture. Reviews in Aquaculture, 5: 1-19

Chen, G.; Su, L.; Feng, S.G.; Lu, X.; Wang, H. and Pei, Y. H. (2013). Furostanolsaponins from the fruits of *Tribulus terrestris*. Nat. Prod. Res., 27(13): 1186-1190.

Effendi, H.; Widyatmoko-Utomo B.A. and Pratiwi N.T.M. (2020). Ammonia and orthophosphate removal of tilapia cultivation wastewater with *Vetiveria zizanioides*. J. King Saud Univ. Sci., 32: 207-212.

El-Greisy, Z.A. and El-Gamal, A.E. (2012). Monosex production of tilapia, *Oreochromis niloticus* using different doses of 17α-methyltestosterone with respectto the degree of sex stability after one year of treatment. The Egyptian Journal of Aquatic Research, 38(1): 59-66.

Ekwu, A.O. and Sikoki, F.D. (2001). Comparison of hormonally induced sexdirection in two strains of *Oreochromis niloticus* (Trewavas). Journal of Aquatic Sciences, 16(2): 147-149.

El-Sayed, M.A.B.; Abdel-Aziz, H.E.S. and Abdel-Ghani, M.H. (2012). Effects of phytoestrogens on sex reversal of Nile tilapia (*Oreochromis niloticuss*) larvae treated with diets treated with 17α-methyltestosterone. Aquaculture, 360: 58-63.

FAO. (2017). Social and economic performance of tilapia farming in Africa. FAO Fisheries and Aquaculture Circular No. 1130. Food and Agriculture Organization of the United Nations Rome, Italy.

Fayed, W.M.A.; Khalil, R.H.; Sallam, G.R.; Mansour, A.T.; Elkhaya, B.K. and Omar, E.A. (2019). Estimating the effective level of *Yucca schidigera* extract for improvement of the survival, haematological parameters, immunological responses and water quality of European seabass juveniles (*Dicrntrarchus lubrax*) Aquaculture Reports 15, 100208.

Gabriel, N.N.; Qiang, J.; Ma, X.Y.; He, J.; Xu, P. and Omoregie, E. (2017). Sex reversal effect of dietary Aloe vera (*Liliaceae*) on genetically improved farmed Nile tilapia fry. North American Journal of Aquaculture, 79 (1): 100-105.

Gewaily, M.S.; Abdo, S.E.; Moustafa, E.M.; Abd El-kader, M.F.; Abd El-Razek, I.M.; El-Sharnouby, M.; Alkafafy, M.; Raza, S.H.; El Basuini, M.F.; Van-Doan, H. and Dawood, M.A.O. (2021). Dietary synbiotics can help relieve the impacts of deltamethrin toxicity of Nile tilapia reared at low temperatures. Animals, 11: 1790.

Gharaei, A.; Ebrahimi-Jorjani, H.; Mirdar-Harijani, J. and Kolangi-Miandare, H. (2020). Effects of *Tribullus terrestris* extract on masculinization, growth indices, sex determination reversal and steroid hormones level in Zebra fish (*Daniorerio*). International Aquatic Research, 12(1): 22-29.

Ghosal, I. and Chakraborty, S.B. (2020). Production of monosex all-male Nile tilapia using ethanol extract of *tribulus terrestris* seeds. In Proceedings of the Zoological Society, 73(2): 188-191.

Ghosal, I.; Mukherjee, D. and Chakraborty, S.B. (2021). The effects of four plant extracts on growth, sex reversal, immunological and haemato-biochemical parameters in Nile tilapia, *Oreochmomi sniloticus* (Linnaeus, 1758). Aquaculture Research, 52: 559-576.

Godwin, J.; Luckenbach, J.A. and Borski, R.J. (2003). Ecology meets endocrinology: Environmental sex determination infishes. Evolution & Development, 5(1): 40-49.

Goudie, C.A.; Redner, B.D.; Simco, B.A. and Davis, K.B. (1983). Feminization of channel catfish by oral administration of steroid sex hormones. Transactions of the American Fisheries Society, 112(5): 670-672.

Gültepe, N.; Acar, Ü.; Kesbiç, O.S.; Yilmaz, S.; Yildırım, Ö. and Türker, A. (2014). Effects of dietary *Tribulus terrestris* extract supplementation on growth, feed utilization, hematological, immunological, and biochemical variables of Nile tilapia *Oreochromis niloticus*. The Israeli Journal of Aquaculture-Bamidgeh, 66: 1-8.

Haitham, G.A. (2018). Hormones and fish monosex farming: A spotlight on immunity. Fish and Shellfish Immunology,72: 23-30.

Hassan, L.G.; Umar, K.J. and Usman, A. (2005). Nutrient content of the leaves of *Tribulus terrestris* (Tsaida), Journal of Tropical Biosciences, 5(2): 77-82.

Hassan, B.; El-Salhia, M.;Khalifa, A.; Assem, H.; Al Basomy, A. and El-Sayed, M. (2013). Environmental isotonicity improves cold tolerance of Nile tilapia, *Oreochromis niloticus*, in Egypt. Egypt. J. Aquat. Res., 39: 59-65.

Hassan, M.A.; Yusuf, M.S.; Badran, M.F.; Griesh, A.S. and Zidan, R.A. (2017). Effect of Yucca shidigera extract and or exogenous enzymes on nitrogenous compounds in Nile tilapia aquaculture. Int. J. Agr. Sci.Vet. Med., 5(1): 55-71.

Hassona, N.N.; Zayed, M.M.; Eltras, W.F. and Mohamed, R.A. (2020). Dietary supplementation of *Tribulus terrestris* extract improves growth and reproductive performances of the male Nile tilapia (*Oreochromis niloticus*). Aquaculture Research, 51(10): 4245-4254.

Homklin, S.; Ong, S.K. and Limpiyakorn, T. (2011). Biotransformation of 17α-methyltestosterone in sediment under differentelectron acceptor conditions. Chemosphere, 82: 1401-1407.

Kadry, H.; Abou-Basha, L.; El-Gindi, O. and Temraz, A. (2010). Antioxidant activity of aerial parts of *Tribulus terrestris* in rats. Pak. J. Pharm. Sci., 23(1): 59-62

Khouraiba, H.M. (1997). Effect of 17α-methyltestosterone on sex reversal and growth of Nile Tilapia, *Orechromis niloticus*. Zagazig Journal of Agricultural Research, 24: 753-767.

Kord, M.I.; Maulu, S.; Srour, T.M.; Omar, E.A.; Farag, A.A.; Nour, A.A.M.; Hasimuna, O.J.; Abdel-Tawwab, M. and Khalil, H.S. (2022). Impacts of water additives on water quality, production efficiency, intestinal morphology, gut microbiota, and immunological responses of Nile tilapia fingerlings under a zero-water-exchange system. Aquaculture, 547: 737503.

Lind, C.E.; Agyakwah, S.K.; Attipoe, F.Y.; Nugent, C.; Crooijmans, R.P.M.A. and Toguyeni, A. (2019). Genetic diversity of Nile tilapia (*Oreochromis niloticus*) throughout West Africa. Sci. Rep., 9:16767.

Lone, K.P. and Matty, A.J. (1981). The effect of feeding androgenic hormones on the proteolytic activity of the alimentary canal of carp, *Cyprinus carpio* L. Journal of Fish Biology, 18 (3): 353-358.

Marjani, M.; Jamili, S.; Mostafavi, P.G.; Ramin, M. and Mashinchian, A. (2009). Influence of 17-alpha methyl testosterone on masculinization and growthin tilapia (*Oreochromis mossambicus*). Journal of Fisheries and Aquatic Science, 4(1): 71-74.

Martino-Andrade, A.J.; Morais, R.N.; Spercoski, K.M.; Rossi, S.C.; Vechi, M.F.; Golin, M.; Lombardi, N.F.; Greca, C.S. and Dalsenter, P.R. (2010). Effects of *Tribulus terrestris* on endocrine sensitive organs in male and female Wistar rats. J. Ethnopharmacol., 127(1): 165-170.

Megbowon, I. and Mojekwu, T. (2014). Tilapia sex reversal using methyl testosterone (MT) and its effect on fish, man and environment. Biotechnology, 13: 213-216.

Miraj, S. (2016). *Tribulus terrestris*: Chemistry andpharmacological properties. Der Pharma Chemica, 8(17): 142-147.

Mlalila, N.; Mahika, C.; Kalombo, L.; Swai, H. and Hilonga, A. (2015). Human food safety and environmental hazards associated with the use of methyltestosterone and other steroids in production of all-male tilapia. Environmental Science & Pollution Research, 22(7): 4922-4931.

Nian, C.T.; Tumbokon, B.L.M. and Serrano-Jr, E.A. (2017). Pinustabulaeformis pollen as replacement for17α–methyltestosterone in the diet of *Oreochromis niloticus* larvae for sex reversal and growth. The Israeli Journal of Aquaculture – Bamidgeh, 69: 1-9.

Norbeck, L.A. and Sheridan, M.A. (2011). An in vitro model for evaluating peripheral regulation of growth in fish: effects of 17 β-estradiol and testosterone onthe expression of growth hormone receptors, insulin-like growth factors, and insulin-like growth factor type 1 receptors in rainbow trout (*Oncorhynchus mykiss*). General and Comparative Endocrinology, 173(2): 270-280.

Noor-El-Deen, A.I.; Zaki, M.S. and Shafi, M.E. (2020). Assessment *Tribulus terrestris* on sex-reverse on Nile tilapia with respect to its chemical composition. Researcher Journal, 12 (3): 6-11.

Omar, E.A.; Yousef, M.I.; Srour, T.M. and Mansour, A.T. (2014). Effect of dietary natural phytochemicals on sex-reversal, growth performance, feedutilization and body composition of Nile tilapia (*Oreochromis niloticus*) fry. Journal of Advance Agricultural Research, 19: 428-441.

Omitoyin, B.O.; Ajani, E.K. and Sadiq, H.O. (2013). Preliminary investigation of *Tribulus terrestris* (Linn., 1753) extracts as natural sex reversal agent in *Oreochromis niloticus* (Linn., 1758) larvae. International Journal of Aquaculture, 3: 74-76.

Ong, S.K.; Chotisukarn, P. and Limpiyakorn, T. (2012). Sorption of 17α–methyl testosterone onto soils and sediment. Water Air & Soil Pollution, 223: 3869-3875.

Pandian, T.J. and Varadaraj, K. (1990). Techniques to produce 100 percent maletilapia. Naga, The ICLARM Quarterly, 13(3): 3-5.

Phelps, R.P. (2006). Hormone manipulation of sex. In C.E. Lim and C.D. Webster (Eds.), Tilapia- biology, culture and nutrition (pp. 211–252). New York, London: Food Products Press. An imprint of the Haworth Press, Inc

Phelps, R.P. and Popma, T.J. (2000). Sex reversal of tilapia. Tilapia aquaculture in the Americas, 2: 34-59. Publisher, World Aquaculture Society. City, Baton Rouge.

Popma, T.J. and Green, B.W. (1990). Sex reversal of tilapia in earthen ponds. International conference for Aquaculture and Aquatic Environments Res. And Development. Series No.35, Auburn University, Alabama, pp: 15.

Putra, I.; Effendi, I.; Lukistyowati, I.; Tang, U.M.; Fauzi, M.; Suharman, I. and Muchlisin, Z.A. (2020). Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia *Oreochromis niloticus* system. F1000 Research 9 add issue and page numbers.

Ramirez-Godinez, J.; Beltran-Hernandez, R.I.; Coronel-Olivares, C.; Contreras-Lopez, E.; Quezada-Cruz, M. and Vazquez-Rodriguez, G. (2013). Recirculating systems for pollution prevention in aquaculture facilities. Journal of Water Resource & Protection, 5: 5-9.

Rempel, M.A. and Schlenk, D. (2008). Effects of environmental estrogens and antiandrogens on endocrine function, gene regulation, and health infish. International Review of Cell and Molecular Biology, 267: 207-252.

Rezka, P.; Balcerzak, W. and Kryłów, M. (2015). Occurrence of synthetic and natural estrogenic hormones in the aquatic environment. *Technical transactions Environmental engineering* DOI: 10.4467/2353737XCT.15.359.4824, 8p.

Reverter, M.; Bontemps, N.; Lecchini, D.; Banaigs, B. and Sasal, P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture, 433: 50-60.

Rivero-Wendt, C.; Miranda-Vilela, A.; Ferreira, M.; Borges, A. and Grisolia, C. (2013). Cytogenetic toxicity and gonadal effects of 17 α-methyltestosterone in *Astyanaxbim aculatus* (Characidae) and *Oreochromis niloticus* (Cichlidae). Genetics & Molecular Research, 12(3): 3862-3870.

Rodmongkoldee, M. and Leelapat, W. (2017). Effect of feed type on growth performance and sex reversal of Nile tilapia (*Oreochromis niloticus*). Burapha Science Journal, 22(1): 14-23.

Sandeep, P.M.; Bovee, T.F. and Sreejith, K. (2015). Antiandrogenic activity of nardostachys jatamansi DC and *Tribulus terrestris L*. and their beneficial effects on polycystic ovary syndrome-induced Rat Models. Metab Syndr Relat Disord. Aug, 13(6): 248-54. doi:10.1089/met.2014.0136. Epub Apr 28. PMID: 25919204.

Sadiqin, H.O. (2021). Sex reversal of Nile Tilapia. *Oreochromis niloticus* (Linneaus, 1758) using differently processed *Tribulus terrestris* L. extracts. Ph.D 219pp. Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Oyo state, Nigeria.

SPSS (2015). Statistical Package For Social Science (for Windows). Release 23 Copyright (C), SPSS Inc., Chicago, USA.

Tveiten, H.; Mayer, I.; Johnsen, H.K. and Jobling, M. (1998). Sex steroids, growth and condition of *Arctic charr* broodstock during an annual cycle. Journal of Fish Biology, 53 (4): 714-727.

Vick, A.M. and Hayton, W.L. (2001). Methyl testosterone pharmacolinetics and oral bioavailability in rainbow trout (*Oncorhynchus mykiss*). Aquatic Ecotoxicology, 52: 177-188.

Yan, W.; Ohtani, K. and Kasai, R. (1996). Yamasaki, K. Steroidal saponins from fruits of *Tribulus terrestris*. Phytochemistry, 42(5): 1417-22.

Yanala, R.S.; Sathyanarayana, D. and Kannan, K. (2016). A recent phytochemical review – Fruits of *Tribulus terrestris* Linn. Journal of Pharmaceutical Science and Research, 8(3): 132-140.

Zaki, F.M.; Said, M.M.;Tahoun, A.-Z. and Amer, M. (2021). Evaluation of different sex reversal treatments in red tilapia hybrid. Egyptian Journal of Aquatic Biology & Fisheries, 25(1): 279-292.