Effect of Adding *Tribulus Terrestris* Extract to the Diets of Nile Tilapia *Oreochromis Niloticus* Larvae on Sex Ratio and Growth Performance.

M. A. El-Kady, M.S. Hussein, M. F. Abd-Elghany, and M. M. Mabrouk *

Department of Fish Production, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.

* Corresponding author E-mail: mabrouk3m@azhar.edu.eg . (M. Mabrouk)

ABSTRACT

Our study was conducted to obtain the effect of *Tribulus terrestris* extract (TTE) on growth performance, feed utilization, fish body composition, some blood parameters, sex-ratio and sexual hormones of Nile tilapia larvae. Nile tilapia (*Oreochromis niloticus*) is fed dietary with TTE (0, 0.5, 1, 1.5, 2) g TTE/Kg diet and 17 α-methyl testosterone (17- α MT) hormone (60 mg 17- α MT/kg diet). The experiment consisted of six treatments (Control, $TTE_{0.5}$, TTE_{1}, $TTE_{1.5}$, TTE_{2} and 17- α MT). The treatments with TTE and 17- α MT were stopped after four weeks, and then the growth experiment continued for 14 weeks. Results indicated that TTE_{2} treatment showed better growth performance and feed utilization compared to 17- α MT treatment, as well as fish chemical composition. A significant improvement ($p<0.05$) was detected in total protein, Globulin and Triglycerides in all treatments compared to control. While there were non-significant differences ($p>0.05$) in albumin and GPT between all treatments. As for the ratio of mono-sex male, 17- α MT and TTE_{2} treatments recorded the highest ($p<0.05$) percentage of male, followed by $TTE_{1.5}$, TTE_{1} and $TTE_{0.5}$ treatments, respectively compared to the control. As for the sexual hormones, a significant increase ($p<0.05$) was showed in $TTE_{0.5}$, TTE_{1}, $TTE_{1.5}$ and TTE_{2} treatments, respectively compared to the 17- α MT treatment. In short, this study recommends adding TTE to the diets of Nile tilapia fry to obtain a mono-sex male, in addition,it improves the growth performance.

KEYWORDS: Nile tilapia, sex ratio and *Tribulus terrestris*.

INTRODUCTION

The sector of aquaculture has expanded rapidly as a result of the growing population in the world, with the recognition that products of aquaculture are of value to human nutrition and health (Ghosal *et al.,* 2016; Ashour *et al.,* 2018 and Paray *et al.,* 2021). Recently, The Nile tilapia is extensively farmed and fast increasing. Tilapia is the second most important farmed fish in the world and the most famous farmed fish in the current century (Prabu *et al.,* 2019). As Tilapia grows fast, it has tolerance to low water quality, relatively has lower FCR, resists disease, is easy to breed and is accepted for customers, tilapia became a suitable fish for farming (El-Sayed, 2019). Female Nile tilapia has high fertility while male tilapia shows faster growth performance and mono sex males are preferred for farming (El-Griesy and El-Gamal, 2012). With the continuous increase in the indiscriminate use of synthetic steroid 17-α MT, it may have negative effects on the aquatic environment (Papoulias *et al.,* 2000). Therefore, many studies have been conducted to try to find safe alternative such as plant extracts for monosexual tilapia production (Celik *et al.,* 2011 and Mukherjee *et al.* 2018). For instance, sex-reversed fish shows a higher growth rate as compared with fish control (Ghosal & Chakraborty, 2017).

Recently, botanical additives have been widely used as additives for monosexual tilapia production due to their relatively safe natural ingredients and to increase the growth rate of fish (Yeganeh *et al.,* 2017). The ingredients of additives of plants have various favorable effects on the fish health which may contain improving growth rate, feed consumption, stimulation of immunity, enhance the efficiency of antioxidants and antimicrobial vitality (Citarasu, 2010). It also improves health condition, sexual desire, sexual hormones secretion and count of sperm and activity in humans and fishes (Bucci, 2000 and Kavitha *et al.,* 2012). *Tribulus terrestris* is stated to be effective for mono-sex production of *Poecilia latipinna* population (Kavitha and Subramanian 2011). In addition, it was observed that *Tribulus terrestris* extract increased sexual activity (Salgado *et al.,* 2017). Also, *T. terrestris* extracts improved growth, immunity, appetite and antimicrobial activity of farmed fish due to the extract containing many important substances such as alkaloids, sterols, phenols, flavonoids and fatty acids (Chakraborty *et al.,* 2015). The addition of *T. terrestris* extracts contains an important substance known as saponins (Dhas *et al.,* 2015). Saponin in *T. terrestris* has an important role in protodioscin production (Ganzera *et al.,* 2001) as a result of elevated testosterone levels (Neychev & Mitev, 2005). (Cek, *et al.,* 2007a)

note that adding *Tribulus terrestris* to African catfish feed increased the number of males. In addition, (Cek *et al.,* 2007b) observed an increase sex reversal (male) in newborn guppies when immersed in *Tribulus terrestris* solution once a week. Also, the addition of exogenous testosterone increases spermatogenesis in immature animals and increases the level of testosterone in the testis as well as the size of the testis (Kumar *et al.,* 2006; Fekrazad *et al.,* 2014; Gültepe *et al.,* 2014; and Zahran *et al.,* 2014). Hassona *et al.* (2020) showed that *T. terrestris* extract had an effect on the growth and differentiation of genital organs due to the androgenic effect, which led to an increase in the growth rate.

Therefore the aim of this work was to study the effect of *T. terrestris* extract compared with 17- α MT on growth performance, feed utilization, body chemical composition, physiological parameters, sex hormones and sex ratio of Nile tilapia larvae.

MATERIALS AND METHODS

Experimental Fish samples

One-day-old Nile tilapia fry is transferred from a private hatchery in Bahr al-Baqar area, Port Said governorate in plastic bags full of oxygen. Initial mean weight and length of experimental fish were 0.013 g and 0.9 cm, respectively. The experimental fish were placed in earthen ponds in a fish farm located in the same location as previously mentioned, with a total area of 8400 m^2. The experimental units were installed with 18 net enclosures (Habes) (each of 15 m^3, 3 m × 5 m × 0.9 m). The fish density in each Habe was 500 fries/m^3 (a total of 7500 fish / treatment). Three replicates were assigned to each treatment. The Habes were fixed on wooden poles fixed to the bottom of the earthen pond. Habes were cleaned twice weekly during the experimental period. The rate of water change during the trial period was 15% per day. This experiment lasted for 18 weeks, starting from the 1st of June, 2019 to the 4th of October, 2018.

Experimental Design

Nile tilapia (*Oreochromis niloticus*) fed dietary with *Tribulus terrestris* extract TTE (0, 0.5, 1, 1.5, 2 g) TTE/Kg diet and methyl testosterone diet contained 60 mg 17 α MT /kg diet. The experiment consisted of six treatments included Control, $TTE_{0.5}$, TTE_1, $TTE_{1.5}$, TTE_2 and17- α MT. Fish in the control were fed with a commercial diet without any addition of hormone or *T. terrestris* extract. Moreover, feeding for all experimental treatments with the 17- α MT and *T. terrestris* extract lasted for a period of 4 weeks, followed by a 14-weeks- rearing fed on a basal diet without any additives.

Experimental management

All experimental enclosures were aerated using airline attached to air blower which compress air in the enclosures through air stones all day. Water quality parameters were measured weekly to ensure proper water quality for the experimental fish. Fish were fed seven times a day at the beginning of the experiment which was then reduced to five times daily. Each treatment was fed with 15% of their biomass for the first 14 days of the experiment and then the percentage was reduced to 10% for the rest of the experiment. The fish were completely weighed in all treatments weekly and accordingly the feeding rate is adjusted according to the weight of the fish. Commercial fine powder diets containing 48% crude protein (Aller Aqua Group®) with *T. terrestris* extract or 17- α MT were used for the first four weeks of the experiment. While in the second stage, granules (1.2 mm) of commercial fish diet containing 30% crude protein (Aller Aqua Group®) were used in the rest of the experiment without adding *TTE* or 17- α MT. Water quality standards were maintained within acceptable ranges.. The temperature of water ranged from 25.5 to 29.7 degrees Celsius and dissolved oxygen ranged from 5.8 to 6.4 mg/L.

Growth indices

The growth rate and feed consumption were estimated as following:

Weight gain (WG) = final weight (g) – initial weight (g)

Daily weight gain (DWG) = body weight gain (BWG) / period (day).

Condition factor (CF) = FW / FL^3 x 100

Where:

FW = Final weight (g)

FL = Final length (cm)

Specific growth rate (SGR)(% / day) = (L_n final weight– L_n initial weight / number of days) x 100.

Feed conversion ratio (FCR) = feed intake (g) / body weight gain (g)

Feed efficiency ratio (FER) = body weight gain (g) / feed intake (g)

Protein efficiency ratio (PER) = body weight gain (g) / protein intake (g)

Protein productive value (PPV) % = 100 X body protein gain / protein intake.

Survival and sex reversal measurements:

At the end of our trial, fish in each enclosure were netted, and counted for survival rate determination. Sex ratio was determined through dissecting selecting 100 fish from each enclosure randomly, and hence, visually examining their gonads.

Survival rate (SR) % = (number of fish survived at the end of the experiment / total fish number at the beginning) ×100.

Experimental analyses

A sample of fish (1000 larvae) was taken at the beginning of the experiment for chemical analysis, and three fish were taken from each treatment at the end of the experiment to analyze the final chemical composition of the whole body to estimate crude protein (CP), ether extract (EE), and Nitrogen free extract (NFE). Fish were collected from Habes, oven dried, minced, and kept at −20°C in the freezer for analysis. Chemical analysis of fish samples was analyzed according to (AOAC 2003). Gross energy (GE) contents of diets were calculated according to (Jobling. 1983) by the values of 5.65, 9.45, and 4 for CP, EE and NFE respectively. The digestible energy was calculated by applying the coefficient of 75% from estimated Gross energy values according to (Jobling, 1983).

Blood samples and analysis

Blood samples were obtained at the end of the experiment from the veins and arteries close to the heart of five fish from each treatment. Blood samples were centrifuged for 15 min to obtain serum. Serum samples were kept at -80°C until required analyzes were performed. Total protein and albumin were estimated according to Lowry *et al.,* (1951) and Wotton & Freeman (1982), respectively. As for the Globulin estimation, it was done by subtracting the albumin value from the total protein value. Triglycerides and Glucose were estimated according to Henry (1964) and MGowan *et al.,* (1983), respectively. In addition, the level of Glutamat pyruvic transaminase (GPT) was determined using kits of Biomericux, France, according to the instructions of the producer. The samples of stored plasma were analyzed for LH and FSH by Automated Enzyme Immunoassay system (AIA-360) called immulite/immulite 1000 system based on the methods mentioned by Beitens, (1976) and testosterone estimated by Enzyme-linked immune sorbent assay (ELISA) according to (Abraham, 1977). As for the sexual analyzes LH and FSH, they were performed by enzyme immunoassay system (AIA-360) based on the methods mentioned by Beitens, (1976) and for testosterone it was estimated by enzyme-linked immunosorbent assay (ELISA). According to (Abraham, 1977).

The statistical study

Raw data were analyzed using SPSS version 22 (2014). One-way ANOVA in SPSS was used to analyze the raw data of growth performance and feed utilization. Fish chemical composition blood parameters, Sex ratio and sexual hormones were analyzed using chi-square (Basavaraja & Raghavendra, 2017).

RESULTS AND DISCUSSION

Growth performance

Average values of final weight (FW), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR) and survival rate (SR) of Nile tilapia fed different levels of TTE and 17- α MT are shown in Table **(**1**)**. Initial weight was the same in all treatments with nonsignificant differences ($P > 0.05$). In our study, means of body weight at the start of the experimental time indicate that the distribution of individual fish between the experimental treatment was completely random. FW, WG, DWG and SGR were increased significantly ($P<0.01$) by TTE_2 followed by 17- α MT, then $TTE_{1.5}$, TTE_1 and $TTE_{0.5}$ treatments, respectively, while the control treatment recorded the lowest values.

Condition factor (CF) recorded its highest value in control treatment, while the lowest CF value was reported in 17- α MT treatment (Table 1). As for the survival rate (SR), it recorded its highest values ($P<0.01$) in TTE_2 followed by 17- α MT, $TTE_{1.5}$, TTE_1 and $TTE_{0.5}$, respectively, while the control treatment recorded the lowest value ($P<0.01$). In Table (2), fishes fed the TTE_2 diet reported a significantly higher ($P<0.01$) in feed intake (FI) and protein intake(PI)followed by TTE_2 and $TTE_{1.5}$, respectively whereas the lowest FI and PI was noticed in fish treated with the control. Fish fed on TTE_2, 17- α MT and $TTE_{1.5}$ diets, respectively had the lowest value ($P<0.01$) of FCR, while the worst FCR values were achieved by control treatment. As for FER, PER and PPV recorded the highest values ($P<0.01$) in TTE_2 followed by 17- α MT, $TTE_{1.5}$,

TTE_1 and $TTE_{0.5}$, respectively. While the control treatment recorded the lowest value (P<0.01).

Fish chemical analysis (CP, EE, and ash) of Nile tilapia was performed at the end of the experimental period, as described in Table (3). The highest significant (P < 0.05) CP value was observed in the TTE_2 treatment followed by $TTE_{1.5}$, 17- α MT, TTE_1 and $TTE_{0.5}$, respectively. While the lowest significant (P < 0.05) CP value was noticed in control treatment. The results showed that control treatment recorded the highest significance (P < 0.05) in ash and EE, while the lowest significance (P < 0.05) was showed in TTE_2 treatment.

These results are consistent with the results of Hassona *et al.*, (2020) who showed that *T. terrestris* extract had an effect on the growth and differentiation of genital organs due to the androgenic effect, which led to an increase in the growth rate. And also, Yeganeh *et al.*, (2017) which showed that *T. terrestris* extract of C. *nigrofasciatum* enhanced growth rate and feed conversion ratio. Moreover, the administration of *T. terrestris* raised the food utilization due to male hormones that stimulate its influence (Donaldson, 1979; Mukhopadhyay *et al.*, 1986 and Jayaprakas & Sindhu, 1996). Moreover, Gültepe *et al.*, (2014) found that the use of *T. terrestris* extract improves the growth performance of Nile tilapia. The improvement in growth performance as a result of *T. terrestris* extract addition in the current work may be due to the phytochemical compounds, mainly the *T. terrestris* extract such as flavonoids, steroidal sapogenins, and alkaloids which have, antitumor, anti-inflammatory and immunomodulatory activities, adding to *Gokshur* extract which also have hepatoprotective properties and antioxidant (Kumar *et al.*, 2006). Those compounds have been proved to possess a positive effect on fish growth performance (Omitoyin *et al.*, 2013 and Omar *et al.*, 2014), related that impact to increasing testosterone level (Gauthaman & Ganesan, 2008) which may be attributed to the improvement in growth performance using the extract.

As for survival rate (SR), its highest values (P<0.01) record in TTE_2 treatment followed by 17- α MT, $TTE_{1.5}$, TTE_1, $TTE_{0.5}$, treatments respectively (Table 1). While the control treatment recorded the lowest value (P<0.01). The higher survival rates in *T.terrestris* extract treatments may be due to the therapeutic properties of the plant extract and several compounds, as vitamins A, C, E, fatty acids and essential amino acid contents (Gharaei *et al.*, 2020). Also, Yeganeh *et al.*, (2017) observed that the survival rate was 100% in *Cichlasoma nigrofasciatum* when they were fed diets containing *T. Terrestris* powder.

On the other hand, these results are correlated with those of (Marjani *et al.* 2009; El-Griesy & El-Gamal, 2012) who observed that treating diets of Nile tilapia fry with 17 α -MT resulted in significantly (P<0.01) higher survival rates when compared to the control and other hormonal doses. Other studies reported that the inclusion of 17 α -MT in tilapia fry did not affect survival rates (Cruz & Mair, 1994 and Ajiboye *et al.*, 2015).

Physiological parameters

The results in Table (4) showed that all treatments with TTE and 17- α MT achieved high significant (P < 0.05) total protein and globulin, but the lowest significant (P < 0.05) values were recorded in the control group. As for albumin, its higher values were recorded in 17- α MT, TTE_2, $TTE_{1.5}$ and TTE_1 treatments, respectively. While the lowest significant values were showed in $TTE_{0.5}$ and control treatments, respectively. The best significant (P < 0.05) triglycerides value was showed in the $TTE_{0.5}$ treatment compared to all treatments while triglycerides decreased (P < 0.05) by control treatment. As for GE and DE, no-significant differences were observed between the treatments.

These results are in agreement with several trails in this segment that presented that *T. terrestris* contains various materials including saponin, flavonoids and alkaloids (Kumar *et al.*, 2006). Kavitha & Subramanian (2011) explained that this has an effect on enhanced the health status of Nile tilapia. Moreover, *T. terrestris* extract possesses a stronger immune response in Nile tilapia with raised levels of immune enzymes such as globulin and albumin (Wiegertjes, *et al.*, 1996). In addition, it has been stated that *T. terrestris* increased immunological and blood parameters of Nile tilapia (Gültepe *et al.*, 2014). Hassona *et al.* (2020) found that the addition of *T. terrestris* extract the health status without any negative effect on quality of water. The bio-active components of phyto-biotics have many positive effects on fish health that may improve immune stimulation, antioxidant capacity, antimicrobial activity and digestive enzyme secretion (Citarasu, 2010). The activities of this *T. terrestris* extract in improving the immunity of the farmed fish probably because of the existence of bio-active ingredients for example essential fatty acids,

phenolic, steroids, alkaloids and flavonoids (Chakraborty *et al.,* 2015).

Sexual ratio of Nile tilapia

Mono sex male was increased significantly (P<0.01) in 17- α MT and TTE_2 followed by $TTE_{1.5}$, TTE_1, $TTE_{0.5}$ treatments, respectively. While the control treatment recorded the lowest values (Table 5). The results from our study are supported by the findings of Ghosal and Chakraborty (2020) who reported that feed supplementation with the *T. terrestris* extract at the 2 g/kg diet conducted the best percentage of mono-sex (all male), which was high significant (P>0.05) compared to all treatments. The administration of the *T. terrestris* extract at (2g/kg) in the diets of sexually undifferentiated tilapia fry was also found to achieve higher male percentages 91:97% (Noor El Deen *et al.*, 2020 and Ghosal & Chakraborty, 2020). The high male percentages obtained from the inclusion of *T. terrestris* extract in the sexually undifferentiated tilapia fry may be attributed to some phytochemical compounds in the plant extract as steroidal saponin protodioscin that may be regarded as androgenic bioactive phytoconstituent that are reported to inhibit the biological synthesis and the action of estrogen working as antagonists and aromatase inhibitors to receptor of nuclear estrogen in cells of the gonad (Rempel & Schlenk, 2008). Adding of *T. terrestris* to the feed of Catfish raised percentage of male (Cek, *et al.,* 2007)a. Administration of *T. terrestris* in rabbits, rats and fish has also been conducted to raise androgen levels (El-Tantawy *et al.,* 2007).

Moreover, much higher male percentages were conducted in Nile tilapia fry fed 17-α MT at 60 mg/kg diet (Ali *et al.,* 2011; Celik *et al.,* 2011 and El-Greisy & El-Gamal, 2012). Hormone suppresses the oogenesis resulting in an inhibitory effect on the oogenesis process which, in return, relies on the concentration of 17 α -Methyltestosterone administrated in fish diets (Wolf *et al.,* 2004). On the other hand, Omar *et al.* (2014) recorded a significantly higher percentage male conversion rate in the 60 mg 17- α MT/ kg diet treatment compared to other *T. terrestris* extract treatments and the control group.

Sexual hormones

The highest significant (P< 0.05) FSH, LH, Total Testosterone and Free Testosterone were observed with TTE_2, $TTE_{1.5}$ and TTE_1, respectively followed by 17- α MT and $TTE_{0.5}$ treatments. While the lowest levels ($P \leq 0.05$) were showed with the control treatment (Table 6). Matching data were noticed in our study showed by Antonio *et al.* (2000). The effect of the addition of Tribulus on the diameter of the seminiferous tubules led to the efficiency of spermatozoa (Çiftçi, 2004). Hassona *et al.,* (2020) that adding *T. terrestris* extract to male Nile tilapia enhances testicular function, reproductive efficiency and semen quality. *T terrestris* extract contains Protodioscin, which has an important role in the conversion of testosterone into dihydrotestosterone, which has an important role in the manifestation of male sexual characteristics (Salgado *et al.,* 2017). Cek *et al.* (2007a) observed an improvement in sexual differentiation and spermatogenesis when *T. terrestris* was added to *C. gariepinus* diets. Moreover, it has been reported to enhance sexual desire, secretion of sexual hormones (testosterone and LH), health condition and sperm count (Bucci, 2000) and *Poecilia latipinna* male (Kavitha *et al.,* 2012). The *T. terrestris* is conducted to be effective for producing mono-sex (all male) *Poecilia latipinna* number (Kavitha and Subramanian 2011). Also, *T. terrestris* contains a type of important ingredients known as saponins (Dhas *et al.,* 2015). Cek *et al.,* (2007) determined that the addition of *T. terrestris* in catfish diets improved male sexual organs. Likewise, (Kumar *et al.,* 2006; Fekrazad *et al.,* 2014; Gültepe *et al.,* 2014 and Zahran *et al.,* 2014) showed that the addition of exogenous testosterone enhanced sperm formation and increased testicular volume as well as the level of hormones in the testicles.

CONCLUSION

In conclusion, the addition of *Tribulus terrestris* extract at the rates of 1.5 and 2 g/kg to Nile tilapia fry enhances the growth performance, chemical composition, blood parameters, sex ratio and sexual hormones without any negative effects on fish compare with 17 α-methyl testosterone hormone.

ACKNOWLEDGMENTS

We are immensely thankful to all of the staff in the Department of Fish Production, Faculty of Agriculture, Al-Azar University, Cairo, Egypt, for providing us facilities and support to do this experiment.

REFERENCES

Abraham, G.E. 1977: Handbook of Radioimmunoassay, Ed. Marcel Dekker, New York, NY, USA.

Ajiboye, O.O., Okonji, V.A., 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., Sovjak, R. 2011: Effect of hormonal treatment on sex reversal of Nile Tilapia (*Oreochromis niloticus*) fry. Agricultura Tropica et Subtropica (Czech Republic).

Antonio, J., Uelmen, J., Rodriguez, R., Earnest, C. 2000: The effects of *Tribulus terrestris* on body composition and exercise performance in resistance-trained males. International Journal of Sport Nutrition and Exercise Metabolism, 10(2), 208–215. https://doi.org/10.1123/ ijsnem.10.2.208

AOAC. 2003: Official methods of analysis of the Association of Official Analytical Chemists, 17th edn. Association of Official Analytical Chemists, Arlington, Virginia

Ashour, M., Abo-Taleb, H., Abou-Mahmoud, M., El-Feky, M. 2018: Effect of the integration between plankton natural productivity and environmental assessment of irrigation water, El-Mahmoudia Canal, on aquaculture potential of *Oreochromis niloticus*. Turkish Journal of Fisheries and Aquatic Sciences, 18(10), 1163–1175. https:// doi.org/10.4194/1303-2712-v18_10_03.

Basavaraja, N., 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 commercial farming. Aquaculture international, 25 (3): 1317-1331.

Bashir, A., Tahir, M., Samee, W., Munir, B. 2009: Effects of *Tribulus terrestris* on testicular development of immature albino rats. Biomedica, 25(1), 63–68.

Beitens, I. 1976: Gonadotropin determinations in timed 3-hour urine collections during the menstrual cycle and LHrh testing. Journal of Clinical Endocrinology and Metabolism, 43: 46-55

Bucci, L.R. 2000: Selected herbals and human exercise performance. American Journal of Clinical Nutrition, 72(2), 624S–636S. https://doi. org/10.1093/ajcn/72.2.624S

Çek, Ş., Turan, F., Atik, E. 2007: Masculinization of convict cichlid (*Cichlasoma nigrofasciatum*) by immersion in *Tribulus terrestris* extract. Aquaculture International, 15(2), 109–119. https://doi.org/10.1007/ s10499-006-9071-0

Cek, S., Turan, F., Atik, E. 2007: The effects of Gokshura, Tribulus terrestris on sex reversal of guppy, *Poecilia reticulata*. Pakistan Journal of Biological Sciences: PJBS, 10(5), 718–725. https://doi.org/10.3923/ pjbs.2007.718.725

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

Chakraborty, S.B., Molnár, T., Ardó, L., Jeney, G., Hancz, C. 2015: Oral administration of Basella alba leaf methanol extract and genistein enhances the growth and non-specific immune responses of *Oreochromis niloticus*. Turkish Journal of Fisheries and Aquatic Sciences, 15(1), 167–173.

Çiftçi, H.B. 2004: Injecting the prepubertal laboratory mice with a mixture of serine and threonine and its effect on spermatogenic function. SÜ Ziraat Fakültesi Dergisi, 18(34), 41–45.

Citarasu, T. 2010: Herbal biomedicines: A new opportunity for aquaculture industry. Aquaculture International, 18(3), 403–414. https://doi. org/10.1007/s10499-009-9253-7

Citarasu, T. 2010: Herbal biomedicines: A new opportunity for aquaculture industry. Aquaculture International, 18(3), 403–414. https://doi. org/10.1007/s10499-009-9253-7

Cruz, E.M.V., Mair, G.C. 1994: Conditions for effective androgen sex reversal in *Oreochromis niloticus* (L.). Aquaculture, 122 (2-3): 237-248.

Dhas, S.A., Selvaraj, T., Citarasu, T., Punitha, S.M.J., Babu, M.M. 2015: Effect of supplemented diet with maturation plant extract on reproductive performance of *Etroplus suratansis*. Aquaculture Reports, 2, 58–62. https://doi.org/10.1016/j.aqrep.2015.08.005

Donaldson, E.M. 1979: Hormonal enhancement of growth. Fish Physiology, 8, 455–597

El-Griesy, Z.A., El-Gamal, E.A. 2012: Mono-sex production of tilapia, *Oreochromis niloticus* using different doses of 17amethyltestosterone with respect to the degree of sex stability after one year of treatment. The Egyptian Journal of Aquatic Research 38(1): 59–66

El-Sayed, A.F.M. 2019: Tilapia culture. Cambridge, MA: Academic Press.

El-Tantawy, W.H., Temraz, A., El-Gindi, O.D. 2007: Free serum testosterone level in male rats treated with *Tribulus alatus* extracts. International Braz J Urol, 33(4), 554–559. https://doi.org/10.1590/ S1677-55382007000400015

Fekrazad, E., Keyhan, H., Fekrazad, R., Tajik, A. 2014: Effect of diode lasers on human sperm motility. Academic Research International, 5(5), 21.

Ganzera, M., Bedir, E., Khan, I. 2001: Determination of steroidal saponins in *Tribulus terrestris* by reversed-phase high-performance liquid chromatography and evaporative light

scattering detection. Journal of Pharmaceutical Sciences, 90(11), 1752–1758. https://doi. org/10.1002/jps.1124

Gauthaman, K., Ganesan, A.P. 2008: The hormonal effects of *Tribulus terrestris* and its role in the management of male erectile dysfunction–an evaluation using primates, rabbit and rat. Phytomedicine, 15 (1-2): 44-54

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

Ghosal, I., Chakraborty, S.B. 2020: Production of mono-sex 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., Hancz, C., Chakraborty, S.B. 2015: Efficacy of Basella alba and Tribulus terrestris extracts for production of mono-sex Nile tilapia, *Oreochromis niloticus*. Journal of Applied Pharmaceutical Science, 5 (8): 152-158

Ghosal, I., Chakraborty, S.B. 2017: Production of mono-sex all male Nile Tilapia using ethanol extract of *Tribulus terrestris* seeds. Proceedings of the Zoological Society, 73(2), 188–191. https://doi. org/10.1007/s12595-017-0254-7

Ghosal, I., Mukherjee, D., Hancz, C., Chakraborty, S.B. 2016: Production of monosex Nile Tilapia, *Oreochromis niloticus* by dietary and immersion treatment with Basella alba leaves and *Tribulus terrestris* seeds. International Journal of Fisheries and Aquatic Studies 4(1): 358–363

Gültepe, N., Acar, Ü., Kesbiç, O.S., Yılmaz, S., Yıldırım, Ö., Türker, A. 2014: Effects of dietary *Tribulus terrestris* extract supplementation on growth, feed utilization, hematological, immunological and biochemical variables of Niletilapia *Oreochromis niloticus.* The Israeli Journal of Aquaculture-Bamidgeh, 66, 1–8.

Hassona, N.N., Zayed, M.M., Eltras, W.F., 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.

Henry, R.J. 1964: Clinical chemistry, principles and techniques. Harper and Row Publishers, New York (USA)

Jayaprakas, V., Sindhu, B. 1996: Effect of hormones on growth and food utilization of the Indian major carp, *Cirrhinus mrigala*. Fishery Technology, 33(1), 21–27.

Jobling, M. 1983: A short review and critique of methodology used in fish growth and nutrition studies. J Fish Biol 23:685–703

Kavitha, P., Subramanian, P. 2011: Influence of Tribulus terrestris on testicular enzyme in fresh water ornamental fish *Poecilia latipinna*. Fish Physiology and Biochemistry, 37(4), 801–807. https://doi. org/10.1007/s10695-011-9478-z

Kavitha, P., Ramesh, R., Subramanian, P. 2012: Histopathological changes in *Poecilia latipinna* male gonad due to Tribulus terrestris administration. Vitro Cellular & Developmental Biology-Animal, 48(5), 306–312. https://doi.org/10.1007/s11626-012-9517-9

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.

Kumar, M., Soni, A.K., Shukla, S., Kumar, A. 2006: Chemopreventive potential of *Tribulus terrestris* against 7, 12-dimethylbenz (a) anthracene induced skin papillomagenesis in mice. Asian Pacific Journal of Cancer Prevention, 7(2), 289.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951: Protein measurement with Folin phenol reagent. J Biol Chem 193:265–275

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

MGowan, M.W., Artiss, J.D., Standbergh, D.R., Zak, B. 1983: A peroxidasecoupled method for colorimetric determination of serum triglycerides. Clin Chem 29:538–552

Mukherjee, D., Ghosal, I., Hancz, C., Chakraborty, S.B. 2018: Dietary administration of plant extracts for production of mono-sex tilapia: Searching a suitable alternative to synthetic steroids in tilapia culture. Turkish Journal of Fisheries and Aquatic Sciences 18(2): 267–275.

Mukhopadhyay, P., Venkatesh, B., Das, P. 1986: Growth and some biochemical changes in *Clarias batrachus* due to methyl testosterone. Indian Journal of Fisheries, 33(3), 262–269

Neychev, V.K., Mitev, V.I. 2005: The aphrodisiac herb *Tribulus terrestris* does not influence the androgen production in young men. Journal of Ethnopharmacology, 101(1–3), 319–323. https://doi. org/10.1016/j.jep.2005.05.017

Noor El Deen, A.I., Zaki, M.S., 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., Mansour, A.T. 2014: Effect of dietary natural phytochemicals on sex-reversal, growth performance, feed utilization and body composition of Nile tilapia (*Oreochromis*

niloticus) fry. Journal of Advance Agricultural Research, 19: 428-441.

Omitoyin, B.O., Ajani, E.K., 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.

Papoulias, D.M., Noltie, D.B., Tillitt, D.E. 2000: Effects of methyl-testosterone exposure on sexual differentiation in medaka, Oryziaslatipes. Marine Environmental Research 50: 181–184.

Paray, B.A., El-Basuini, M.F., Alagawany, M., Albeshr, M.F., Farah, M.A., Dawood, M.A.O. 2021: *Yucca schidigera* usage for healthy aquatic animals: Potential roles for sustainability. Animals, 11(1), 93. https://doi.org/10.3390/ani11010093.

Prabu, E., Rajagopalsamy, C., Ahilan, B., Jeevagan, I.J.M.A., Renuhadevi, M. 2019: Tilapia–An excellent candidate species for world aquaculture: A review. Annual Research & Review in Biology, 31(3), 1–14. https://doi.org/10.9734/arrb/2019/v31i330052

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

Rodmongkoldee, M., 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.

Salgado, R.M., Marques-Silva, M.H., Goncalves, E., Mathias, A.C., Aguiar, J.G., Wolff, P. 2017: Effect of dietary supplementation of *Tribulus terrestris* extract on semen quality and body fat index of infertile men. Andrologia, 49(5), e12655. https://doi.org/10.1111/ and.12655

Turan, F., Cek, S. 2007: Masculinization of African catfish (*Clarias gariepinus*) treated with gokshura (*Tribulus terrestris*). The Israeli Journal of Aquaculture – Bamidgeh, 59(4), 224–229.

Wiegertjes, G.F., Stet, R.M., Parmentier, H.K., van Muiswinkel, W.B. 1996: Immunogenetics of disease resistance in fish: A comparative approach. Developmental & Comparative Immunology, 20(6), 365–381. https://doi.org/10.1016/S0145-305X(96)00032-8

Wolf, C.J., LeBlanc, G.A., Gray Jr, L.E. 2004: Interactive effects of vinclozolin and testosterone propionate on pregnancy and sexual differentiation of the male and female SD rat. Toxicological Sciences, 78 (1): 135-143.

Wotton, I.D., Freeman, H. 1982: Micro-analysis in medical biochemistry. Churchill, New York, USA

Yeganeh, S., Sotoudeh, A., Movaffagh, A.N. 2017: Effects of Tribulus terrestris extract on growth and reproductive performance of male convict cichlid (*Cichlasoma nigrofasciatum*). Turkish Journal of Fisheries and Aquatic Sciences, 17(5), 1003–1007. https://doi. org/10.4194/1303-2712-v17_5_15

Zahran, E., Risha, E., AbdelHamid, F., Mahgoub, H.A., Ibrahim, T. 2014: Effects of dietary Astragalus polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology, 38(1), 149–157. https://doi.org/10.1016/j. fsi.2014.03.002

Table 1: Effect of adding *T. terrestris* extract to Nile tilapia fry diets on growth performance.

Items	Control	$TTE_{0.5}$	TTE_{1}	$TTE_{1.5}$	TTE_{2}	17- α MT
I W	0.013	0.013	0.013	0.013	0.013	0.013
F W	166.7 ± 5.4^{e}	183.1 ± 2.1^{d}	188.3 ± 1.2^{cd}	192.1 ± 1.2^{bc}	203.3 ± 0.9^{a}	197.3 ± 1.5^{ab}
WG	166.6 ± 5.4^{e}	182.9 ± 2.1^{d}	188.3 ± 1.2^{cd}	191.9 ± 1.2^{bc}	203.3 ± 0.9^{a}	197.3 ± 1.5^{ab}
DWG	1.32 ± 0.044^{e}	1.45 ± 0.017^{d}	1.49 ± 0.009^{cd}	1.52 ± 0.009^{bc}	1.61 ± 0.007^{a}	1.56 ± 0.015^{ab}
CF	1.95 ± 0.007^{a}	1.95 ± 0.023^{a}	1.88 ± 0.035^{b}	1.82 ± 0.013^{bc}	1.87 ± 0.028^{b}	1.77 ± 0.006^{c}
SGR%	3.25 ± 0.011^{d}	3.29 ± 0.003^{c}	3.30 ± 0.002^{bc}	3.30 ± 0.002^{bc}	3.32 ± 0.001^{a}	3.31 ± 0.002^{ab}
SR%	83.2 ± 2.1^{c}	85.7 ± 1.7^{b}	86.5 ± 1.9^{b}	86.4 ± 1.3^{b}	91.5 ± 1.8^{a}	89.3 ± 1.9^{a}

Values in the same row with different Letters are significantly different at $p<0.05$. IW= Initial weight, FW = Final weight, WG = weight gain, DWG = Daily weight gain, CF = Condition factor, SGR = Specific Growth rate and SR%= survival rate.

Table 2: Effect of adding *T. terrestris* extract to Nile tilapia fry diets on feed utilization.

Items	Control	$TTE_{0.5}$	TTE_{1}	$TTE_{1.5}$	TTE_{2}	17- α MT
FI	273.7±2.91^{ab}	263.6±2.14^{c}	266.7±1.08^{bc}	267.9±0.33^{abc}	270.9±4.06^{abc}	274.7±1.24^{a}
PI	82.1±0.87^{ab}	79.1±0.64^{c}	80.0±0.32^{bc}	80.4±0.09^{abc}	81.3±1.21^{ab}	82.4±0.37^{a}
FCR	1.64±0.051^{a}	1.44±0.008^{b}	1.42±0.012^{b}	1.40±0.008^{bc}	1.33±0.021^{c}	1.39±0.015^{bc}
FER	0.608±0.019^{c}	0.694±0.004^{b}	0.706±0.006^{b}	0.716±0.004^{b}	0.751±0.012^{a}	0.718±0.007b
PER	2.03±0.064^{c}	2.31±0.014^{b}	2.35±0.021^{b}	2.39±0.014^{b}	2.50±0.039^{a}	2.39±0.026^{b}
PPV%	27.40±0.96^{d}	32.81±0.54^{c}	35.60±0.10^{b}	37.37±0.90^{b}	39.79±0.71^{a}	36.30±0.93^{b}

Values in the same row with different Letters are significantly different at $p<0.05$. FI= feed intake, PI= protein intake, FCR = feed conversion ratio, FER= feed efficiency ratio, PER= protein efficiency ratio and PPV = protein productive value

Table 3: Effect of adding *T. terrestris* extract to Nile tilapia fry diets on the chemical composition of fish.

Items	Control	$TTE_{0.5}$	TTE_{1}	$TTE_{1.5}$	TTE_{2}	17- α MT
DM%	21.83±0.18	21.93±0.32	22.03±0.22	22.27±0.24	22.10±0.17	21.70±0.31
CP%	61.83±0.44^{e}	64.67±0.51^{d}	68.67±0.32^{c}	70.27±0.61^{ab}	71.97±0.41^{a}	69.83±0.24^{b}
EE%	17.33±0.49^{a}	15.67±0.89^{b}	15.36±0.07^{b}	13.63±0.35^{cd}	12.80±0.17^{d}	14.10±0.29^{c}
ASH%	20.83±0.38^{a}	19.67±0.58^{a}	15.97±0.32^{b}	16.10±0.40^{b}	15.23±0.24^{b}	16.07±0.42^{b}
GE[1]	5131±34^{b}	5134±35^{b}	5331±17^{a}	5257±22^{a}	5275±7^{a}	5277±33^{a}
DE[2]	3848±26^{b}	3850±27^{b}	3998±14^{a}	3943±17^{a}	3956±6^{a}	3958±25^{a}

Values in the same row with different Letters are significantly different at $p<0.05$.

Table 4: Effect of adding *T. terrestris* extract to Nile tilapia fry diets on some physiological parameters.

Items	Control	$TTE_{0.5}$	TTE_{1}	$TTE_{1.5}$	TTE_{2}	17- α MT
T P (g/dl)	5.23±0.22^{b}	6.27±0.23^{a}	6.07±0.09^{a}	6.53±0.09^{a}	6.30±0.26^{a}	6.43±0.19^{a}
Albu. (g/dl)	3.93±0.27^{b}	3.93±0.38^{b}	4.20±0.26^{a}	4.23±0.18^{a}	4.23±0.29^{a}	4.47±0.33^{a}
Glob. (g/dl)	1.30±0.11^{b}	2.33±0.19^{a}	1.87±0.18^{a}	2.30±0.11^{a}	2.07±0.09^{a}	1.97±0.31^{a}
Trig. (mg/dl)	171.3±2.6^{c}	211.3±4.6^{a}	203.7±3.5^{b}	190.7±2.1^{b}	191.7±2.2^{b}	191.3±4.4^{b}
GPT (U/ml)	28.67±3.3	25.67±1.5	29.00±3.1	34.33±1.5	32.33±2.4	27.33±3.4
Gluc (mg%)	109.7±4.3	106.0±4.0	109.3±3.1	105.7±1.5	104.0±1.7	104.3±2.9

Values in the same row with different Letters are significantly different at $p<0.05$. TP=Total protein, Albu.= Albumin, Glob.= Globulin, Trig = Triglyceride, Gluc. =Glucose, GPT= Glutamat pyruvic transaminase.

Table 5: The effect of adding *Tribulus terrestris* in fish diets on the sexual ratio of Nile tilapia fry

Items	Control	$TTE_{0.5}$	TTE_{1}	$TTE_{1.5}$	TTE_{2}	17- α MT
Male	54±1.2^{e}	65.33±1.5^{d}	72.33±2.3^{c}	81.67±2.1^{b}	92.33±1.2^{a}	93.33±1.2^{a}
Female	46±2.0^{a}	34.67±2.5^{b}	27.67±4.1^{c}	18.33±3.5^{d}	7.67±2.1^{e}	6.67±2.1^{e}

Values in the same row with different Letters are significantly different at $p<0.05$.

Table 6: Effect of adding *Tribulus terrestris* to fish diets on some sex hormones of Nile tilapia

Items	Control	$TTE_{0.5}$	TTE_{1}	$TTE_{1.5}$	TTE_{2}	17- α MT
FSH (IU/L)	2.70±0.36^{c}	4.97±0.14^{b}	5.95±0.55^{a}	6.48±0.41^{a}	6.98±0.17^{a}	3.70±0.36^{c}
LH (IU/L)	148±0.07^{c}	289±0.34^{b}	368±0.55^{a}	354±0.62^{a}	417±0.25^{a}	235±0.30^{b}
T. Tests. (ng/ml)	4.83±0.17^{c}	6.87±0.52^{b}	7.50±0.35^{a}	8.10±0.40^{a}	8.27±0.12^{a}	6.83±0.41^{b}
F. Tests. (pg/ml)	105±2.9^{c}	175±8.7^{b}	205±5.8^{a}	211±8.3^{a}	211±4.4^{a}	176.±8.8^{b}

Values in the same row with different Letters are significantly different at $p<0.05$. T. Testes. = Total Testesterone, F. Testes. = Free Testesterone

تأثير إضافة مستخلص تريبولوس تيريستريس إلى علائق يرقات البلطي النيلي علي أداء النمو والنسبة الجنسية والهرمونات الجنسية

محمد علي القاضي، محسن صالح حسين، محمد فتحي عبد الغني، محمد محمود مبروك *

قسم الإنتاج السمكي، كلية الزراعة، جامعة الأزهر، القاهرة ، مصر.

* البريد الإلكتروني للباحث الرئيسي: mabrouk3m@azhar.eg.edu

الملخص العربي

أجريت هذه الدراسة لمعرفة تأثير مستخلص نبات تريبولوس تيريستريس (TTE) على أداء النمو والاستفادة الغذائية وتركيب جسم الأسماك وبعض مقاييس الدم والنسبة الجنسية والهرمونات الجنسية وذلك ليرقات البلطي النيلي. تمت تغذية زريعة البلطي النيلي (*Oreochromis niloticus*)على عليقة تحتوي على TTE (0 ، 0.5 ، 1 ، 1.5 ، 2) جرام من TTE / كجم علف ، وعلى 17 ألفا ميثيل تستوستيرون (60 ملجم / كجم علف (17- α MT). وبالتالي كانت التجربة عبارة عن ستة معاملات (عليقة ضابطة، $TTE_{0.5}$، TTE_1، $TTE_{1.5}$، TTE_2، 17- α MT). تم إيقاف التغذية بـ TTE و 17- α MT بعد أربعة أسابيع، ثم استمرت تجربة النمو لمدة 14 أسبوعًا. أشارت النتائج إلى أن الأسماك في معاملة TTE_2 أظهرت أداء نمو واستفادة أفضل للأعلاف مقارنة بالمعاملة 17- α MT وكذلك التركيب الكيميائي للأسماك. بالنظر إلى المتغيرات الفسيولوجية ، لوحظ تحسن معنوي ($p < 0.05$) في البروتين الكلي والجلوبيولين والدهون الثلاثية في جميع المعاملات مقارنة مع الكنترول. بينما لا توجد فروق معنوية ($p > 0.05$) في الألبومين و GPT بين جميع المعاملات. أما بالنسبة لنسبة الذكور وحيد الجنس فقد سجلت معاملة 17- α MT و TTE_2 أعلى نسبة ($p < 0.05$) للذكور تليها معاملات $TTE_{1.5}$ و TTE_1 و $TTE_{0.5}$ مقارنة بمعاملة الكنترول. بالنسبة للهرمونات الجنسية لوحظ زيادة معنوية ($p < 0.05$) في معاملات $TTE_{0.5}$ و TTE_1 و $TTE_{1.5}$ و TTE_2 مقارنةً المعاملة 17- α MT. باختصار توصي هذه الدراسة بإضافة TTE إلى علائق زريعة البلطي النيلي للحصول على ذكور وحيدة الجنس بالإضافة إلى أنه يحسن أداء النمو.

الكلمات الاسترشادية: البلطى النيلى، وحيد الجنس، تريبولوس تيرستريس.