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RESEARCH PAPER

Effects of Tribulus Terrestris Extract on Growth and Reproductive Performance of Male Convict Cichlid (*Cichlasoma nigrofasciatum*)

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Abstract

This study evaluated the effects of diet containing 1 and 2 g kg^{-1} *T. terrestris* extract on growth and reproductive performance of male Convict cichlid (*C. nigrofasciatum*). 117 male *C. nigrofasciatum* with an average initial weight of 2.30 ± 0.04 g were distributed into glass aquariums (13 fish/tank) in 3 replicates. The highest weight gain was obtained in 1 g kg^{-1} (123.83 ± 6.41 g) followed by 2 g kg^{-1} extract (121.13 ± 8.09 g) and control (80.10 ± 11.71 g) group ($P< 0.05$). In groups that received extract, specific growth rate increased significantly ($P< 0.05$). Comparison of feed conversion ratio and condition factor in different treatments indicates no significant differences ($P> 0.05$). Protein levels of dried body decreased after feeding the fish with extract ($P< 0.05$). Fish fed with extract showed higher lipid and moisture content than fish fed by control diet ($P< 0.05$). The highest belly diameter was observed in treatment containing 1 g kg^{-1} extract (9.93 ± 0.23 cm, $P< 0.05$). Fertilization (%) decreased significantly in 2 g kg^{-1} extract when compared with control and other group ($P< 0.05$). The highest amount of hatching rate (%) was observed in 1 g kg^{-1} (94.24 ± 3.01) and its least amount was found in 2 g kg^{-1} extract (72.65 ± 0.23, $P> 0.05$).

Keywords: *Cichlasoma nigrofasciatum*, growth, reproductive performance, *Tribulus terrestris*

Introduction

In recent year, herbal supplements have been tested in aquaculture as an alternative to chemicals. Using of plant-based additives in aquaculture is one of the methods used to improve weight gain and feed efficiency in cultured fish (Dada, 2015). Plants are natural source of safer and cheaper chemicals. Beneficial effects of bioactive plant substances in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune responses and antibacterial, antiviral and antioxidant actions (Citarasu, 2010). Herbal supplements are efficient and more environmentally friendly in disease management that enhances immunity and resistance to pathogens (Harikrishnan, Balasundaram, & Heo, 2010). *T. terrestris* L. (Zygophyllaceae), also called gokshura, is a medicinal herb used in Bulgaria, eastern Europe, the Far East, China, and India to treat sexual deficiencies (Bucci, 2000). *T. terrestris* has been reported to enhance libido sexualis, increase testosterone and LH levels, improve athletic performance, and increase the number and motility of spermatozoa (Bucci, 2000; Gauthaman, Adaikan, & Prasad, 2002). *T. terrestris* contains a number of substances known as steroidal saponins (Dhas, Selvaraj, Citarasu, Punitha, & Babu, 2015).

Saponin in *T. terrestris* thought to be responsible for its effect on testosterone levels is known as protodioscin (Ganzera, Bedir, & Khan, 2001). It is believed that *T. terrestris* affects androgen metabolism, significantly increasing testosterone or testosterone precursor levels (Neychev & Mitev, 2005).

The effects of *T. terrestris* preparation on human males and experimental animals are well known (Kistanova, Zlatev, Karcheva, & Kolev, 2005). Oral administration of *T. terrestris* increased male populations of African Catfish (*Clarias gariepinus*; Turan & Cek, 2007). New-born guppies (*Poecilia reticulata*) treated with *T. terrestris* were sex reversed, underwent spermatogenesis, and had a better growth rate than untreated progeny (Cek, Turan, & Atik, 2007a). Administration of *T. terrestris* extract to the zebra cichlid (*C. nigrofasciatum*) also produced successful sex reversal (Cek, Turan, & Atik, 2007b).

The aim of our investigation was to study the

effect of the *T. terrestris* extract on growth and reproductive performances of the ornamental fish, male convict cichlid (*C. nigrofasciatum*).

Materials and Methods

T. terrestris seeds were prepared from the local plant market, washed in sterile distilled water, air-dried in shade and powdered. 250 g of powder extracted with 500 ml 70% ethanol (Kavitha & Subramanian, 2011). The prepared crude extract was used for the entire experiment as a stock solution.

Commercial food on the market (manufactured by Biomar France) was used to prepare the diets. Proximate composition of the diets was 42.88 ± 0.55 % protein, 28.25 ± 0.58% lipid, 5 ± 0 % fiber, 8.27 ± 1% moisture and 9.67 ± 0.18 % ash. 2 diets containing *T. terrestris* extract with amounts of 1 and 2 g kg^{-1} of diet and a control diet (without *T. terrestris* extract) were prepared. The experiment was carried out in triplicate for each diet. Male and female were separated from initial stock of immature Convict cichlid after sex determination (the male has a larger size with a bulge on forehead and the female has orange spot on the abdomen). 9 aquariums (80 l) were stocked with 117 male *C. nigrofasciatum* with an average initial weight of 2.30 ± 0.04 g (13 fish/aquarium). The fish were fed four times daily (08.00, 10.00, 14.00 and 16.00 h) for 45 days (3% of their body weight). The female fish with an average initial weight of 1.65 ± 0.00 g (15 fish/aquarium) were stocked in 3 aquarium (80-l) and fed only with commercial diet without *T. terrestris* extract (3% of their body weight; four times daily) until they were used for reproduction. Biometry of the groups was done every 15 days for feeding regulation. During the experiment, water was exchanged daily at a rate of ~20% of the total volume. Water temperature was determined daily (26.22 ± 2ºC) .The average pH, dissolved oxygen, TDS (Total Dissolved Solids), salinity and electrical conductivity were 8.64 ± 0.05, 7.75 ± 0.21 ppm, 825.61 ± 59 ppm, 0.8 ± 0 ppt and 1649.93 ± 119 µs/cm, respectively.

Growth Performance and Body Composition

Growth performance and feed utilization were calculated according to the following formulae:

(1) Weight Gain (WG) = 100 × [(final fish weight – initial fish weight) / initial fish weight]

(2) Specific Growth Rate (SGR) = 100 × [(ln final fish weight) – (ln initial fish weight) / experimental days]

(3) Feed Conversion Ratio (FCR) = feed intake / weight gain

(4) Condition Factor (CF) = [weight of the fish / (length of the fish)^3]

(5) Survival Rate (SR) = [(total number of fish at the end of trial / total number of fish at the start of trial) × 100]

Proximate analyses of the diets were performed using standard methods (AOAC 2000). Dry matter was analyzed by drying at 50 °C for 48 h in oven to a constant weight, crude lipid was analyzed by ether extraction, crude protein was analyzed by the Kjeldahl method, and crude ash was analyzed by incineration at 550 °C for 6 h. Samples of fish (3 fish) in each treatment were weighed before putting them in an oven for 24 h at 105 °C, and then reweighed to estimate the moisture content. Other factors of body composition (crude lipid, protein and ash) were determined according to that of mentioned above.

Reproductive Performance

To measure the gonadosomatic index (GSI), at the end of period, 3 male fish were randomly selected from each aquarium and their testes were removed and weighed. For reproduction of fish, 3 male were randomly selected from each aquarium. 20-liter aquarium tank was used for the reproduction of fish (one male and 3 females in each tank; after mating, two other female fish were removed from the tank). To stimulate the spawning of fish, water temperature was gradually increased to 29 ± 1°C.

Reproductive parameters were calculated using the following formulae:

(1) GonadoSomaticIndex (GSI, %) = (Gonad weight / Body weight) × 100

(2) Relative Fecundity (RF) = Total eggs / Total weight of fish

(3) Hatching (%)= (Number of hatched larvae / Number of eggs eyed) × 100

(4) Fertilization (%)= (Number of fertilize eggs / Total number of eggs) × 100

Statistical Analysis

The effects of treatment on different parameters were analyzed by one-way analysis of variance (ANOVA) after checking for normality distribution of data by Kolmogorov-Smirnov test. Wherever significant differences were found, a Duncan's multiple range tests was performed for mean differences. Statistical analysis was performed using SPSS ver. 19. Data are expressed as mean ± SD.

Results

Growth Performance

Data on growth performance and survival rate of *C. nigrofasciatum* is presented in Table 1. There was a significant difference ($P<0.05$) for final weight (FW), WG and SGR Between the groups. The highest WG and FW were obtained in 1 g kg^{-1} followed by 2 g kg^{-1} *T. terrestris* extract and control. In groups that received *T. terrestris* extract, SGR increased significantly ($P<0.05$). Comparison of FCR and CF in

different treatments indicates no significant differences (P>0.05). Survival rate of fish in all groups was 100%.

Body Composition

Results of protein, moisture, lipid and whole body ash content are presented in Table 2. There were no significant differences between ash content of fish in all groups (P>0.05). Fish fed on *T. terrestris* extract had a significantly (P<0.05) higher lipid and moisture content than fish fed the control diet. As Compared to controls, protein levels of fish body decreased after feeding the fish with *T. terrestris* extract (P<0.05).

Reproductive Performance

Results of reproductive parameters are presented in Table 3. There were no significant differences between GSI in all groups (P>0.05). The highest belly diameter was observed in 1 g kg^{-1} *T. terrestris* extract which showed significant difference with the control and 2 g kg^{-1} group (P<0.05). Fertilization (%) decreased significantly in 2 g kg^{-1} *T. terrestris* extract (83.12 ± 1.45) when compared with control and other supplemented group (P<0.05). The highest amount of hatching rate (%) was observed in 1 g kg^{-1} and its least amount was found in 2 g kg^{-1} *T. terrestris* extract; There was no significant difference between groups fed with dietary supplementation of *T. terrestris* extract and control group in hatching rate (%).

Discussions

Growth Performance

The purpose of this study was to investigate the impact of *T. terrestris* extract as supplementary diet on growth and body composition of *C. nigrofasciatum*. According to the results, *T. terrestris* extract supplementation at 1 and 2 g kg^{-1} significantly increased WG and SGR as compared to control group in this study. Different levels of *T. terrestris* extract

Table 1. Growth performance of C. nigrofasciatum fed diets containing different levels of T. terrestris extract for 45 days*

Growth parameters	Control	1 gkg-1	2 gkg-1
IW (g)	2.36 ± 0.03a	2.28 ± 0.03a	2.27 ± 0.03a
FW (g)	4.25 ± 0.22b	5.10 ± 0.10a	5.04 ± 0.26a
WG (%)	80.10 ± 11.71b	123.83 ± 6.41a	121.13 ± 8.09a
SGR (%)	1.30 ± 0.14b	1.76 ± 0.10a	1.79 ± 0.07a
FCR	1.652 ± 0.21a	1.27 ± 0.12a	1.22 ± 0.05a
CF	3.51 ± 0.03a	3.87 ± 0.15a	3.79 ± 0.24a
SR (%)	100.00 ± 0.00a	100.00 ± 0.00a	100.00 ± 0.00a

* Values (mean ± SEM) superscripted by different alphabets within the same line are significantly different (P<0.05).

Table 2. Body Composition (as dry basis) of *C. nigrofasciatum* fed diets containing different levels of *T. terrestris* extract for 45 days*

Body Composition	**Control**	**1 gkg^{-1}**	**2 gkg^{-1}**
Moisture	60.49 ± 0.87^{b}	66.09 ± 0.07^{a}	66.26 ± 0.39^{a}
Crude protein	54.79 ± 1.31^{a}	50.20 ± 0.94^{b}	40.28 ± 0.80^{c}
Crude lipid	28.34 ± 0.65^{c}	35.53 ± 0.47^{b}	44.60 ± 0.40^{a}
Crude ash	16.86 ± 1.13^{a}	14.27 ± 0.81^{a}	15.12 ± 0.69^{a}

*Values (mean ± SEM) superscripted by different alphabets within the same line are significantly different (P<0.05).

Table 3. Reproductive performance of *C. nigrofasciatum* (♂) fed with *T. terrestris* extract and *C. nigrofasciatum* (♀) not fed *T. terrestris* extract *

Reproductive parameters	Control	1 gkg^{-1}	2 gkg^{-1}
Fish Weight(g) ♂	7.27 ± 0.45^{a}	8.20 ± 0.20^{a}	8.05 ± 0.05^{a}
Fish Lenght(cm) ♂	5.80 ± 0.11^{a}	5.96 ± 0.03^{a}	6.03 ± 0.03^{a}
Fish Weight (g) ♀	2.99 ± 0.06^{a}	3.23 ± 0.13^{a}	3.05 ± 0.02^{a}
Fish Lenght (cm) ♀	5.16 ± 0.16^{a}	4.43 ± 0.16^{a}	4.73 ± 0.44^{a}
GonadoSomaticIndex (GSI, %) ♂	0.73 ± 0.06^{a}	1.14 ± 0.13^{a}	0.99 ± 0.23^{a}
Belly Diameter ♂ (cm)	8.80 ± 0.09^{b}	9.93 ± 0.23^{a}	8.95 ± 0.05^{b}
GonadoSomaticIndex (GSI, %) ♀	9.20 ± 1.11^{a}	10.21 ± 0.10^{a}	9.94 ± 0.19^{a}
Relative Fecundity	76.96 ± 17.14^{a}	84.76 ± 4.80^{a}	62.04 ± 6.83^{a}
Fertilization (%)	96.57 ± 0.89^{a}	96.01 ± 1.40^{a}	83.12 ± 1.45^{b}
Hatching (%)	86.28 ± 6.81^{ab}	94.24 ± 3.01^{a}	72.65 ± 0.23^{b}

*Values (mean ± SEM) superscripted by different alphabets within the same line are significantly different (P<0.05).

(200, 400 and 600 mg kg^{-1}) in diets enhanced growth performance in *Oreochromis niloticus* (Gultepe, Acar, Kesbic, Yılmaz, Yıldırım, & Turker, 2014). Similar results were reported by Cek *et al*., (2007a,b) who used the *T. terrestris* extract (0.05, 0.1, 0.15, 0.2 and 0.3 g l^{-1}) for masculinization of guppy *P. reticulata* and *C. nigrofasciatum* (Cek *et al*., 2007 a, b). Feed additives were used to accede better production results in farm animals; previous studies showed that herbs stimulate secretion of pancreatic enzymes, absorption and digestion of nutrient compounds (Dhas *et al*., 2015). Fallahpour, Banaee, & Javadzade, (2014) suggested that unknown factors in various medicinal herbs led to favourable results in fish growth trials.

Body Composition

The present study showed changes in body composition except for ash in fish that fed with diet supplemented by *T. terrestris* extract when compared to the control. Gültepe *et al*., (2014) reported that body composition of the fish was not influenced by dietary *T. terrestris* extract treatment and there were no significant differences between groups (P>0.05). In present study, control sample revealed the highest level of crude protein, there was a significant difference between treatment groups and control (P<0.05). The highest amount of lipid was observed in groups that containing 2 g kg^{-1} extract (P<0.05); also moisture percentage in 1 and 2 g kg^{-1} was more than control (P<0.05). It was reported that pumpkin (*Telfairia occidentalis*) leaf powder (5, 10, 15 and 20 g kg^{-1}) in diet of African catfish (*C. gariepinus*) did not affect the contents of fish carcass moisture, protein and ash among treatments (Dada, 2015). Fallahpour *et al*., (2014) evaluated the effects of different concentration (0.25, 0.50 and 1%) of marshmallow (*Althaea officinalis* L.) extract on carcass composition of Common carp (*Cyprinus carpio*); their results showed that slight changes in body composition of the fish fed with diet supplemented by marshmallow extract as compared to control (P>0.05). *T. terrestris* is a natural herb which helps enhance testosterone levels in human and animals (Cek *et al*., 2007b) and testosterone affects the basal metabolic rate in animals (Welle, Jozefowicz, Forbes, & Griggs, 1992). So in this study, it is possible that changes in the composition of fish carcasses due to changes in the testosterone levels in the fish.

Reproductive Performance

Elahi, Asl & Shahian (2013) validate the potential of *T. terrestris* extract as a safe therapeutic alternative to current modalities for the management of sexual dysfunction in males. Protodioscin, the main phytochemical agent of the Tribulus genus, This component is known to convert testosterone into dihydrotestosterone, which plays important roles in male attributes (Salgado, Marques-Silva, Goncalves, Mathias, Aguiar, & Wolff, 2016).

A strong relationship between sperm motility and reproduction were reported so that an increased motility results into increased reproduction. Reproductive capacity is the most conclusive way of testing sperm motility (Olaniyi , Adebowale, & Mustapha, 2016).

In this study, the lowest fertilization and hatching rate were observed in 2 g kg^{-1} extract (P<0.05). It can be caused by use of *T. terrestris* extract in high dose. In this regard, it was reported that higher inclusion (15%) of mulberry *(Morus nigra)* in the diet had negative influence on male African cat fish (*C. gariepinus*) because it can lead to a serious problem in the organ (Olaniyi *et al*., 2016).

According to our search, any reports were found about negative effects of *T. terrestris* on fish gonads; so the effects of *T. terrestris* on fish gonads and reproductive performance in high dosage need more investigations.

The highest hatching rate was observed in 1 g kg^{-1} extract (P<0.05); it had no significant difference with control group (P>0.05).

Olaniyi *et al*., (2016) reported that dietary supplementation with Mulberry *(M. nigra)* leaf meal (5, 10 and 15%) improved hatchability and sperm count of *C. gariepinus*. Dhas *et al*., (2015) observed an increased in GSI, fertilization and hatching of *Etroplus suratansis* broodstock treated with herbal maturation diet prepared from *Mucuna pruriens*: *Withania somnifera*: *Moringa oleifera* (150:300:150 mg Kg^{-1}).

There were no significant differences among treatments in GSI (P>0.05); although belly diameter in fish fed with 1 g of *T. terrestris* extract significantly increased (P<0.05). In the study of Yılmaz, Cek, & Mazlum, (2013) that investigated the effects of *T. terrestris* extract (50 and 100 mg kg^{-1}) on GSI of *Oncorhynchus mykiss* did not observe any significant differences between different groups. It is possible that aphrodisiac plant enhances sexual behaviors via an increase in the testosterone (Cek *et al*., 2007a). Trisomboon, Watanabe, Wetchasit, & Taya, (2007) showed that, serum testosterone levels significantly increase in immature rats orally treated with dry *Kaempferia parviflora* powder (1 g kg^{-1}) in water for 5 days. Also, it has been reported that *T. terrestris* enhance testosterone levels in human and animals (Cek *et al*., 2007a). Although in this study, was not observed significant improvement in GSI, fertilization and hatching rate of fish fed with *T. terrestris* extract Compared to control; but, it may be that achieve more favorable results by using lower dosage of *T. terrestris* extract in fish diets.

Conclusion

The present results suggests that supplemented

T. terrestris extract in feeds for *C. nigrofasciatum* improves growth performance and hatching rate; we conclude that *T. Terrestris* extract in lower dosage has the potential to be used as a supplement in fish diets. The use of medicinal plants in fish will be an efficient tool to achieve sustainable, economical, and safe fish production.

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References

AOAC (Association of Official Analytical Chemists), 2000. *Official methods of analysis of AOAC International*: AOAC International. Arlington, VA, USA.

Bucci, L. R. (2000). Selected herbals and human exercise performance. *The American journal of clinical nutrition*, 72(2), 624s-636s.

Çek, 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. http://dx.doi.org/ 10.3923/pjbs.2007.718.725

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. http://dx.doi.org/ 10.1007/s10499-006-9071-0

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

Dada, A. A. (2015). Use of fluted pumpkin (*Telfairia occidentalis*) leaf powder as feed additive in African catfish (*Clarias gariepinus*) fingerlings. *International Journal of Biological and Chemical Sciences*, 9(1), 301-307. http://dx.doi.org/10.4314/ijbcs.v9i1.27

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. http://dx.doi.org/ 10.1016/j.aqrep.2015.08.005

Elahi, R. K., Asl, S., & Shahian, F. (2013). Study on the effects of various doses of *Tribulus terrestris* extract on epididymal sperm morphology and count in rat. *Glob Veterin*, 10, 13-17. http://dx.doi.org/10.5829/idosi.gv.2013.10.1.7158

Fallahpour, F., Banaee, M., & Javadzade, N. (2014). Effects of dietary marshmallow (*Althaea officinalis* L.) extract on growth performance and body composition of common carp (*Cyprinus carpio*). *International Journal of Advanced Biological and Biomedical* Research, 2(8), 2453-2460. http://dx.doi.org/10.18869/IJABBR

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 science*, 90(11), 1752-1758. http://dx.doi.org/10.1002/jps.1124

Gauthaman, K., Adaikan, P., & Prasad, R. (2002). Aphrodisiac properties of *Tribulus Terrestris* extract (Protodioscin) in normal and castrated rats. *Life Sciences*, 71(12), 1385-1396. http://dx.doi.org/10.1016/S0024-3205(02)01858-1

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 Nile Tilapia *Oreochromis niloticus*. *The Israeli Journal of Aquaculture-Bamidgeh*, 66, 1-8. http://hdl.handle.net/10524/49098

Harikrishnan, R., Balasundaram, C., & Heo, M.S. (2010). Herbal supplementation diets on hematology and innate immunity in goldfish against *Aeromonas hydrophila*. *Fish & shellfish immunology*, 28(2), 354-361. http://dx.doi.org/10.1016/j.fsi.2009.11.013

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. http://dx.doi.org/10.1007/s10695-011-9478-z

Kistanova, E., Zlatev, H., Karcheva, V., & Kolev, A. (2005). Effect of plant *Tribulus terrestris* extract on reproductive performances of rams. *Biotechnology in Animal Husbandry*, 21(1-2), 55-63.

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), 319-323. http://dx.doi.org/ 10.1016/j.jep.2005.05.017

Olaniyi, C.O., Adebowale, T.T., & Mustapha, R.O. (2016). Effect of mulberry leaf meal on growth and reproductive performance of African catfish. *Journal of Zoology*, 5(2), 20-24. http://www.cibtech.org/cjz.htm

Salgado, R., Marques-Silva, M., Gonçalves, E., Mathias, A., Aguiar, J., & Wolff, P. (2016). Effect of oral administration of *Tribulus terrestris* extract on semen quality and body fat index of infertile men. *International Journal of Andrology*, 48, 1-6. http://dx.doi.org/ 10.1111/and.12655

Trisomboon, H., Watanabe, G., Wetchasit, P., & Taya, K. (2007). Effect of daily treatment with Thai herb, *Kaempferia parviflora*, in Hershberger assay using castrated immature rats. *Journal of Reproduction and Development*, 53, 351-356. http://doi.org/10.1262/jrd.18092

Turan, F., & Cek, S. (2007). Masculinization of African catfish (*Clarias gariepinus*) treated with gokshura (*Tribulus terrestris*). *The Israeli Journal of Aquaculture - Bamidgeh*, 59, 224-229. http://hdl.handle.net/10524/19235

Welle, S., Jozefowicz, R., Forbes, G., & Griggs, R. C. (1992). Effect of testosterone on metabolic rate and body composition in normal men and men with muscular dystrophy. *The Journal of Clinical Endocrinology & Metabolism*, 74(2), 332-335. http://doi.org/10.1210/jcem.74.2.1730811

Yılmaz, E., Çek, Ş., & Mazlum, Y. (2013). Effects of synthetic and natural steroids on the growth, sex reversal and gonadal development of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Aquatic Science*, 30, 123-131.