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ISSN: 2594-3146

Journal of Agriculture and Forestry University

Volume 2 2018

Agriculture and Forestry University
Rampur, Chitwan

ISSN: 2594-3146

Journal of Agriculture and Forestry University

Volume 2 **2018**

Frequency of Publication	Annual
Editorial Policy	A medium of publishing original scientific papers
Official Language	English
ISSN	2594-3146
Subject of Interest	Plant Science, Animal Science, Veterinary Science, Forestry, and Social Science

Subscription

Category	**Rate**
SAARC countries	US$ 10.00 postage extra
Other countries	US$ 15.00 postage extra
AFU faculty	NRs. 200.00
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Mode of Payment	By Bank Draft or Cheque on Bank of Kathmandu, Narayangarh, Chitwan, Nepal. It should be addressed to AFU-Directorate of Research and Extension (Exp), Rampur, Chitwan, Nepal
Correspondence	JAFU Secretariat Agriculture and Forestry University, Rampur, Chitwan, Nepal E-mail: dor@afu.edu.np

Correct citation: Authors detail with surname of first author, first name, followed by first name and surname of other authors in sequence (2018). Title of the article, Journal of AFU (Volume 2): pages, Agriculture and Forestry University, Chitwan, Nepal.

Agriculture and Forestry University
Rampur, Chitwan, Nepal

Journal of Agriculture and Forestry University (JAFU)

Volume 2 **2018**

Review Articles

Research Articles

Research Article

EFFICACY OF TAMOXIFEN ON SEX REVERSAL OF NILE TILAPIA (*Oreochromis niloticus*)

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ABSTRACT

The efficacy of tamoxifen, a non-steroidal aromatase inhibitor, for inducing masculinization in Nile tilapia was assessed. Nine days post hatch tilapia fry were reared in fine meshed nylon hapas, suspended in a green cemented pond, and fed with tamoxifen (Cytotam 20) @ 150, 200, 250, 300, 350, 500, and 1000 mg/kg diet for 30 and 40 days. In 30 days treatment group, the percentage of male fed with tamoxifen @ 150, 200, 250, 300, 350, 500 and 1000 mg/kg diet were 67.6±0.5, 72.4±2.7, 84.5±1.8, 87.5±1.4, 89.7±1.0, 90.0±1.3 and 96.7±1.2, respectively, while control group had 50.8±0.9 to 50.9±1.4% males. Although the treatment with tamoxifen in lower concentrations (150-500 mg/kg diet) significantly increased the male proportion compared to control diet, the percentage of male was significantly highest in treatment with 1000 mg/kg diet. There were no significant differences in percentage of male between 30 and 40 days of treatment durations. Likewise there was no significant difference in the survival of Nile tilapia fries among control and treatment groups. The findings revealed that tamoxifen has potential for production of all-male Nile tilapia. The best dose of tamoxifen was 1000 mg/kg feed. However, increased dose of tamoxifen should be tested to induce 100% masculinization.

Key words: Cytotam 20; non-steroidal aromatase inhibitor; masculinization

INTRODUCTION

Nile tilapia is an important aquaculture species which is ranked in the second most produced fish with an annual world production of 5.6 million tons per annum (Fitzsimmons, 2016). Monosex culture of male tilapia is preferred to the mixed sex system due to sexual size dimorphism, males being substantially larger than females (Beardmore et al., 2001). In addition, precocious reproduction of this species also leads to low growth performance of stocked fish as a consequence of over-crowding and feed competition in production ponds. Currently male monosex populations are produced mainly by androgen (17-α methyltestosterone) treatment. However, the use of steroids in aquacultures is not desirable and it is avoided in many countries, on account of its adverse environmental effects (Baroiller et al., 2009). Furthermore, the 17-α methyltestosterone is quite expensive and not easily available in many developing countries like Nepal. As an alternative, the non-steroidal aromatase inhibitors (AIs) might be useful tools for sex reversal in fish.

Many studies reported the possibility of using non-steroidal AIs in sex reversal of fishes. For example, fadrozole and exemestane have been used for the masculinization of genetic females in many species of gonochoristic fish (Kitano et al., 2000; Kwon et al., 2000; Afonso et al., 2001; Uchida et al., 2004; Ruksana et al., 2010; Pandit and Nakamura, 2015) and sex-changing protogynous species (Nakamura et al., 2003; Bhandari et al., 2004; Alam et al., 2006). Another non-steroidal AI, tamoxifen, has also been used for successful masculinization of Japanese medaka (Chikae et al., 2004), Japanese flounder (Kitano et al., 2007), bagrid catfish (Park et al., 2003), guppy (Chakraborty et al., 2012) and Nile tilapia (Singh et al., 2012). The aromatase enzyme (P450arom) is the key enzyme for biosynthesis of estradiol-17β (E_2) from testosterone. The principle behind masculinization in fishes using AIs is that they block P450 aromatase enzyme activity, leading to reductions in the production of estrogen (Steele et al., 1987). The ability of AI to induce sex reversal in fish makes this class of chemical a valuable tool for analyzing the role of estrogen in the processes of sex differentiation and sex change.

Tamoxifen has been used in the treatment of breast and ovarian cancer in postmenopausal women (Nazarali and Narod, 2014). It is cheaper than 17-α methyltestosterone and easily available in Nepal. Although few studies reported the possibility of using tamoxifen in successful masculinization of fishes, there are no reports about the use of tamoxifen for mass production of monosex male tilapia fry in commercial scale. If this technology can be successfully applied in commercial scale, it will be a good alternative of 17-α methyltestosterone to produce all-male tilapia fry in easier and cheaper way. Thus, the main objective of

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this study was to determine the efficacy, dose and duration of tamoxifen treatment for mass production of monosex male population in Nile tilapia. The other objective was to determine the effect of tamoxifen feeding on growth and survival of tilapia fry.

MATERIALS AND METHODS

This experiment was conducted at the Department of Aquaculture and Fisheries, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal. The experiment was conducted in two phases. In the first phase, three low doses (150, 200 and 250 mg/kg feed) of tamoxifen was used. Based on the results of first phase experiment, a second phase experiment was conducted using four high doses (300, 350, 500 and 1000 mg/kg feed) of tamoxifen. For both experiments, rearing of Nile tilapia broods was done in hapa placed in a cemented pond and were allowed to breed naturally in hapa. Fertilized eggs were collected from the mouth of several females, randomly mixed, counted and placed in jar incubation system (Ranjan et al., 2015).

In the first phase experiment, a total of 3000 individuals at 8 days after hatching (dah) were used, with 250 fries in each of 12 nylon hapa of 0.5 m^3 (0.5m x 0.5m x 1.0 m) size. The hapa were placed in a green water cemented pond (4.9 m x 4.5m x 1.25 m). The experiment was conducted in a completely randomized design (CRD). There were four treatments with three replications of each treatment. The treatments were: (1) Feed without tamoxifen (control; only fishmeal); (2) Tamoxifen at the rate of 150 mg/kg feed; (3) Tamoxifen at the rate of 200 mg/kg feed; and (4) Tamoxifen at the rate of 250 mg/kg feed. In the second phase experiment, a total of 3750 individuals at 8 days after hatching (dah) were used, with 250 fries in each of 15 nylon hapa of 0.5 m^3 (0.5m x 0.5m x 1.0 m) size. The hapa were placed in a green water cemented pond (4.9 m x 4.5m x 1.25 m). The second phase experiment was also conducted in a completely randomized design (CRD). There were five treatments with three replications of each treatment. The treatments were: (1) Feed without tamoxifen (control; only fishmeal); (2) Tamoxifen at the rate of 300 mg/kg feed; (3) Tamoxifen at the rate of 350 mg/kg feed; (4) Tamoxifen at the rate of 500 mg/kg feed; and (5) Tamoxifen at the rate of 1000 mg/kg feed.

To prepare the treatment diet, Tamoxifen Citrate Tablets IP 20 mg (Cytotam 20; Cipla Ltd., India) was dissolved in 100% ethanol and mixed to the dry fish meal powder, which was then dried overnight at room temperature to completely evaporate the ethanol (Ruksana et al., 2010). For experimental treatments, fish were fed with tamoxifen containing feed from 9 days after hatch for 30 and 40 days, whereas control groups were fed with normal dry fishmeal feed. Fish were fed four times daily (7.00 am, 11.00 am, 2.00 pm and 5.00 pm) @ 20%, 15% and 10% of the total body weight per day in first week, second week and rest of the time, respectively. Water temperature, dissolved oxygen, pH and transparency were measured weekly at 7.00-8.00 am.

In the first phase experiment, after 30 days of treatment, half of the fishes from all treatments were taken and cultured in another hapa for 45 days feeding with normal feed, while the remaining half of fishes were continued to feeding tamoxifen until 40 days. After 40 days of treatment, fish were reared for additional 35 days feeding with normal diet. Fish growth and survival were calculated at the end of treatment and experimental period. Gonadal status of fish was observed by scarifying all fishes from each group anaesthetizing with clove oil. The gonads were excised and squashed with aceto-carmine to determine the sex of fish (Guerrero and Shelton, 1974). In the second phase experiment, after 30 days of treatment, fish were reared for additional 30 days feeding with normal diet and then growth survival and gonadal status was observed as described above for the first phase experiment. Data were analyzed statistically by analysis of variance (ANOVA) using SPSS (version 21.0) statistical software package (SPSS Inc., Chicago). All means were given with ± standard error (S.E.).

RESULTS

Phase-I

In 30 days treatment group, the percentage of male fed with tamoxifen @ 150, 200 and 250 mg/kg diet were 67.6±0.5, 72.4±2.7 and 84.5±1.8, respectively, while control group showed 50.8±0.9% males (Table 1). Similarly, in 40 days treatment group, the percentage of male fed with tamoxifen @ 150, 200 and 250 mg/kg diet were 68.6±3.1, 74.7±1.6 and 84.8±0.8, respectively, while control group showed 51.5±0.3% males (Table 1). In both treatment durations, the percentages for males in tamoxifen @ 250 mg/kg diet were significantly higher than control and lower dose treatments ($p<0.05$). There was no significant difference in percentage of male between 30 and 40 days of treatment durations ($p>0.05$).

Table 1. Effects of tamoxifen treatment at different doses for 30 and 40 days on sex ratio of Nile tilapia during gonadal sex differentiation

Treatments	Male (%)	
	30 days treatment	40 days treatment
Control feed	50.8±0.9[a]	51.5±0.3[a]
Tamoxifen @ 150 mg/kg diet	67.6±0.5[b]	68.6±3.1[b]
Tamoxifen @ 200 mg/kg diet	72.4±2.7[c]	74.7±1.6[c]
Tamoxifen @ 250 mg/kg diet	84.5±1.8[d]	84.8±0.8[d]

Mean values with different superscript in the same column are significantly different ($p<0.05$)

At the end of experiment, the survival percentage of 30 days treatment group fed with tamoxifen @ 150, 200 and 250 mg/kg diet were 92.8±4.4, 88.0±3.2 and 84.8±4.2, respectively, while control group showed 92.4±4.6% survival (Table 2). There was no significant difference in the survival of Nile tilapia fries among control and treatment groups ($p>0.05$). Similarly, the survival percentage of 40 days treatment group fed with tamoxifen @ 150, 200 and 250 mg/kg diet were 91.0±4.2, 87.2±3.2 and 84.6±4.1, respectively, while control group showed 91.7±4.5% survival (Table 2). There was no significant difference in the survival of Nile tilapia fries among control and various treatment categories ($p>0.05$). There was no significant difference in the weight of fish among control and treatment groups ($p>0.05$) (Table 2).

Table 2. Effects of tamoxifen treatment on growth and survival of Nile tilapia fry at the end of treatment period of 30 and 40 days

Treatments	30 days		40 days	
	Weight (g/fish)	Survival (%)	Weight (g/fish)	Survival (%)
Control feed	0.6±0.0	92.4±4.6	0.7±0.0	91.7±4.5
Tamoxifen @ 150 mg/kg diet	0.5±0.0	92.8±4.4	0.7±0.1	91.0±4.2
Tamoxifen @ 200 mg/kg diet	0.6±0.1	88.0±3.2	0.7±0.1	87.2±3.2
Tamoxifen @ 250 mg/kg diet	0.5±0.0	84.8±4.2	0.6±0.0	84.6±4.1

The weekly average water temperature, dissolved oxygen, pH and Secchi disk depth of the cemented tank where the experimental hapa were set were 29.8±0.5 °C, 5.7±0.2mg/L, 7.6 and 29.3±1.1 cm, respectively (Table 3).

Table 3. Weekly mean and range of water quality parameters of experimental pond. Values in the parenthesis are range values

Parameters	Mean±SE
Water temperature (°C)	29.8±0.5 (27.5-31.2)
Dissolved oxygen (mg/L)	5.7±0.2 (4.2-6.9)
pH	7.6 (7.3-7.9)
Secchi disk depth (cm)	29.3±1.1 (25.5-34.2)

Phase-II

The first phase experiment showed that there was no significant difference in percentage of male between 30 and 40 days of treatment durations. Thus, in the second phase fish were treated only for 30 days. The percentage of male fed with tamoxifen @ 300, 350, 500 and 1000 mg/kg diet were 87.5±1.4,

89.7±1.0, 90.0±1.3 and 96.7±1.2, respectively, while control group showed 50.9±1.4% males (Table 4). The percentages for males in tamoxifen @ 1000 mg/kg diet were significantly higher than control and other treatments ($p<0.05$). There was no significant difference in percentage of male among 300, 350 and 500 mg/kg diet treatments ($p>0.05$; Table 4).

Table 4. Effects of Tamoxifen treatment at different doses for 30 days on sex ratio of Nile tilapia during gonadal sex differentiation

Treatments	Male (%)
Control feed	50.9±1.4[a]
Tamoxifen @ 300 mg/kg diet	87.5±1.4[b]
Tamoxifen @ 350 mg/kg diet	89.7±1.0[b]
Tamoxifen @ 500 mg/kg diet	90.0±1.3[b]
Tamoxifen @ 1000 mg/kg diet	96.7±1.2[c]

Mean values with different superscript in the same column are significantly different ($p<0.05$)

At the end of experiment, the survival percentage of treatment group fed with tamoxifen @ 300, 350, 500 and 1000 mg/kg diet were 90.8±2.7, 86.0±1.0, 83.5±3.1 and 83.9±4.7, respectively, while control group showed 90.5±3.6% survival (Table 5). There was no significant difference in the survival of Nile tilapia fries among control and various treatment groups ($p>0.05$; Table 5).

Table 5. Effects of Tamoxifen treatment on body weight and survival of Nile tilapia at the end of treatment period of 30 days

Treatments	Weight (g/fish)	Survival (%)
Control feed	0.6±0.1	90.5±3.6
Tamoxifen @ 300 mg/kg diet	0.5±0.0	90.8±2.7
Tamoxifen @ 350 mg/kg diet	0.5±0.1	86.0±1.0
Tamoxifen @ 500 mg/kg diet	0.6±0.0	83.5±3.1
Tamoxifen @ 1000 mg/kg diet	0.6±0.1	83.9±4.7

The weekly average water temperature, dissolved oxygen, pH and Secchi disk depth of the cemented tank where the experimental hapa were set were 26.5±0.4 °C, 5.9±0.4mg/L, 7.7 and 33.3±2.1 cm, respectively (Table 6).

Table 6. Weekly mean and range of water quality parameters of experimental pond. Values in the parenthesis are range values

Parameters	Mean±SE
Water temperature (°C)	26.5±0.4 (24.7-28.0)
Dissolved oxygen (mg/L)	5.9±0.4 (4.0-7.0)
pH	7.7 (7.4-8.0)
Secchi disk depth (cm)	33.3±2.1 (27.4-38.2)

DISCUSSION

The present study examined the efficacy of tamoxifen, a non-steroidal aromatase inhibitor, on sex reversal of Nile tilapia. We found that dietary administration of tamoxifen dose-dependently induced masculinization of the sexually undifferentiated Nile tilapia. Although the treatment with tamoxifen in

lower concentrations (150-500 mg/kg diet) significantly increased the male proportion compared to control, the percentage of males was significantly higher in high dose treatment (1000 mg/kg diet) than lower dose treatments. After 30 days of treatment, the percentage of male fed with tamoxifen @ 150, 200, 250, 300, 350, 500 and 1000 mg/kg diet were 67.6±0.5, 72.4±2.7, 84.5±1.8, 87.5±1.4, 89.7±1.0, 90.0±1.3 and 96.7±1.2, respectively. The finding of the present study is similar to many previous studies in different fish species. A dose-dependent increase in percentage of males was observed in bagrid catfish fed diets treated with tamoxifen where the highest dose of 200 ppm produced 90% males (Park et al., 2003). On the other hand, dietary administration of tamoxifen at a dose of 2 mg/g diet to 8 days post hatch Nile tilapia fry for 150 days have resulted in gonads with both testicular and ovarian tissue (Nakamura et al., 2004). In an immersion experiment, tamoxifen treatment of Nile tilapia juveniles with 200 µg/L for 60 days produced 90% male (Singh et al., 2012). Moreover, at high concentration tamoxifen has been found to inhibit the normal vitellogenin induction in female medaka (*Oryzias latipes*) during oral administration (Chikae et al., 2004) and immersion experiments (Sun et al., 2007). Such masculinizing effect of tamoxifen was associated with blockage of estrogen function as it competes with endogenous estradiol for binding with estrogen receptor (Liu et al., 2010), and suppression of cyp19a expression (Kitano et al., 2007). In contrast to the present finding, Guiguen et al. (1999) reported that in rainbow trout (*Oncorhynchus mykiss*) and tilapia (*Oreochromis niloticus*), tamoxifen does not induce the masculinization in the gonadal sex differentiation. These results show that there is a difference of sensitivity for tamoxifen in gonadal sex differentiation among several fish species.

Some previous studies applied longer treatment duration (more than 60 days treatment) of tamoxifen and obtained slightly higher percentage of male than the present experiment (Singh et al., 2012). In contrast to these results, the present study showed there were no significant differences in percentage of male between 30 and 40 days of treatment durations. This indicates that 30 days of treatment duration is sufficient for tamoxifen treatment.

The present experiment showed that tamoxifen feeding does not significantly affect the growth and survival of fry. However, tamoxifen fed group showed slightly higher mortality than the control diet. Similar high mortality was observed in Nile tilapia after dietary treatment with tamoxifen (Nakamura et al., 2003; Chikae et al., 2004). A dose-dependent cumulative mortality was observed in *Pseudobagrus fulvidraco* fed diets treated with tamoxifen (Park et al., 2003). In *Oryzias latipes* as well, the hatchability of fertilized eggs and time of hatching were significantly delayed after exposure to high concentration of tamoxifen (Sun et al., 2007).

CONCLUSION

The study showed the possibility of using tamoxifen as an alternative of 17-α methyltestosterone for monosex male production of Nile tilapia. In Nepalese context, the tamoxifen is about eight times cheaper than 17-α methyltestosterone as well as it is easily available in the country. The best dose of tamoxifen among treated treatments was 1000 mg/kg feed. However, increased dose of tamoxifen should be tested to induce 100% masculinization. More efforts are required to investigate the effects of tamoxifen on the sex-determining mechanisms, and on environments and ecology. How to minimize the use of exogenous chemicals to enhance the aquaculture production is still a challenge to biologists, ecologists, and environmental scientists.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the help of the Aquaculture and Fisheries Program, Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal for providing research and laboratory facilities. This work was financially supported by the Directorate of Research and Extesion (AFU) and Aqua-Fish Innovation Lab Project.

REFERENCES

Afonso, L.O.B., Wassermann, G.J. & Oliveira, R.T.D. (2001). Sex reversal in Nile tilapia *Oreochromis niloticus* using a non-steroidal aromatase inhibitor. *Journal of Experimental Zoology 290*, 177–181.

Alam, M.A., Bhandari, R.K., Kobayashi,Y., Soyano, K.& Nakamura, M. (2006). Induction of sex change within two full moons during breeding season and spawning in grouper. *Aquaculture 255*, 532–535.

Baroiller, J.F., D'Cotta, H., Bezault,E., Wessels, S.& Hoerstgen-Schwark, G. (2009). Tilapia sex determination: Where temperature and genetics meet. *Comparative Biochemistry and Physiology Part A: Molecular &Integrative Physiology 153*, 30-38.

Beardmore, J.A., Mair, G.C.& Lewis, R.I. (2001). Monosex male production in finfish as exemplified by tilapia: applications, problems, and prospects. *Aquaculture 197*, 283–301.

Bhandari, R.K., Higa, M., Nakamura, S.& Nakamura, M. (2004). Aromatase inhibitor induces complete sex in protogynous honeycomb grouper, *Epinephelus merra*. *Molecular Reproduction and Development 67*, 303–307.

Chakraborty, S.B., Molnár, T. & Hancz, C. (2012). Effects of methyltestosterone, tamoxifen, genistein and *Basella alba* extract on masculinization of guppy (*Poecilia reticulata*). *Journal of Applied Pharmaceutical Science 2(12)*, 48-52.

Chikae M., Ikeda, R., Hasan, Q., Morita, Y.& Tamiya, E. (2004). Effects of tamoxifen, 17α-ethynylestradiol, flutamide, and methyltestosterone on plasma vitellogenin levels of male and female Japanese medaka (*Oryzias latipes*). *Environmental Toxicology and Pharmacology17*, 29-33.

Fitzsimmons, K. 2016. Supply and demand in global tilapia markets 2015. World AquacultureConference, February, 2016, Book of Abstracts, World Aquaculture 2016, Las Vegas, USA.

Guerrero, R.D. & Shelton, W.L. (1974). An aceto-carmine squash method for sexing juvenile fishes. *Progressive Fish-Culturist 36*, 56.

Guiguen, Y., Baroiller, J.F., Ricordel, M.J., Iseki, K., McMeel, O.M., Martin,S.A.M. & Fostier, A. (1999). Involvement of estrogens in the process of sexdifferentiation in two fish species: The rainbow trout (*Oncorhynchus mykiss*) and a tilapia (*Oreochromis niloticus*). *Molecular Reproduction and Development 54*, 154–162.

Kitano T., Yoshinaga, N., Shiraishi, E., Koyanagi, T. & Abe, S. (2007). Tamoxifen induces masculinization of genetic females and regulates P450 aromatase and Müllerian inhibiting substance mRNA expression in Japanese flounder (*Paralichthys olivaceus*). *Molecular Reproduction and Development 74*, 1171-1177.

Kitano, T., Takamune, K., Nagahama, Y.& Abe, S.-I. (2000). Aromatase inhibitor and 17α-methyltestosterone cause sex-reversal from genetical females to phenotypic males and suppression of P450 aromatase in Japanese flounder *Paralichthys olivaceus*. *Molecular Reproduction and Development 56*, 1–5.

Kwon, J.Y., Haghpanah, V., Kogson-Hurtado, L.M., McAndrew, B.J. & Penman, D.J. (2000). Masculinization of genetic female Nile tilapia *Oreochromis niloticus* by dietary administration of an aromatase inhibitor during sexual differentiation. *Journal of Experimental Zoology 287*, 46–53.

Liu, Z.H., Zhang, Y.G. & Wang, D.S. (2010). Studies on feminization, sex determination, and differentiation of the Southern catfish, *Silurus meridionalis*: a review. *Fish Physiology and Biochemistry 36*, 223-235.

Nakamura, M., Bhandari, R.K. & Higa, M. (2003). The role estrogens play in sex differentiation and sex changes of fish. *Fish Physiology and Biochemistry 28*, 113-117.

Nazarali S.A. & Narod S.A. (2014). Tamoxifen for women at high risk of breast cancer. *Breast Cancer 6*, 29-36.

Pandit, N.P. & Nakamura, M. (2015). Effect of Aromatase inhibitor on gonadal development and growth of female Nile tilapia (*Oreochromis niloticus*). *Nepalese Journal of Aquaculture and Fisheries 2*, 40-47.

Park, I.S., Oh, H.S. & Koo, J.G. (2003). Effect of oral tamoxifen on growth and survival in the bagrid catfish *Pseudobagrus fulvidraco*. *Aquaculture Research 34*, 1471-1474.

Ranjan, R., Shrestha, M.K., Pandit, N.P. & Khanal, N.B. (2015). Efficacy of common carp (*Cyprinus carpio*) testis on sex reversal of Nile tilapia (*Oreochromis niloticus*) fry. *Nepalese Journal of Aquaculture and Fisheries 2*, 92-100.

Ruksana, S., Pandit, N. P. & Nakamura, M. (2010). Efficacy of Exemestane, a new generation of aromatase inhibitor, on sex differentiation in a gonochoristic fish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 152*, 69-74.

Singh R., Singh, A.K. & Tripathi, M. (2012). Effect of a non-steroidal tamoxifen on the gonad and sex differentiation in Nile tilapia, *Oreochromis niloticus*. *Journal of Environmental Biology 33*, 799-803.

Steele, R.E., Mellor, L.B., Sawyer, W.K., Wasvary, J.M. & Browne, L.J. (1987). In vitro and in vivo studies demonstrating potent and selective estrogen inhibition by the nonsteroidal aromatase inhibitor CGS16949A. *Steroids 50*, 147–161.

Sun L., Zha, J., Spear, P.A. & Wang, Z. (2007). Tamoxifen effects on the early life stages and reproduction of Japanese medaka (*Oryzias latipes*). *Environmental Toxicology and Pharmacology 24*, 23–29.

Uchida, D., Yamashita, M., Kitano, T., & Iguchi, T. (2004). An aromatase inhibitor or high water temperature induce oocyte apoptosis and depletion of P450 aromatase activity in the gonads of genetic female zebrafish during sex-reversal. *Comparative Biochemistry and Physiology 137A*, 11–20.