J. Bio-Sci. 29(1): 131-141, 2021 (June) **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index
DOI: https://doi.org/10.3329/jbs.v30i1.63108

A MALE HORMONE-INDUCED CHANGES IN GROWTH PERFORMANCE AND HISTOPATHOLOGY IN THE STINGING CATFISH *HETEROPNEUSTES FOSSILIS* (SILURIFORMES: HETEROPNEUSTIDAE) UNDER LABORATORY CONDITIONS

Sabina Sultana* and Fahmida Farha

Department of Zoology, University of Rajshahi, Rajshahi 6205, Bangladesh

Abstract

The present study was conducted to assess the features of the stinging catfish *Heteropneustes fossilis* (Bloch) in terms of total length (TL), total weight (TW) and growth performance and survival rate after treatment of fish feed with a synthetic male hormone, 17 α-methyltestosterone (17 α-MT) under laboratory conditions. Results showed that TL in the untreated (C) and T1 group of fishes differed significantly ($p<0.05$) even though the overall effect of the hormone did not influence TL of the fishes under study ($p = 0.085$). A similar result was observed for TW, but the overall impact of 17-αMT on TW of *H. fossilis* was highly significant one ($p<0.001$), indicating that the TW was increased much more than the TL due to the hormone treatment in the experimental fishes. Growth performances of the fishes in terms of their gains in TL and TW due to the hormone treatment and over a rearing period of four months from March to June showed that compared to the control groups, all three treatment groups increased in both the morphometric parameters in *H. fossilis*. The increases in TW were more drastic than the corresponding increases in TL. Notably, the hormone treatments did not affect the survival of the experiment fishes under study. Histopathological changes in the intestine of T2 fishes were characterized by hemorrhage and degeneration of villi, whereas hemorrhage, degeneration of serosa and rapture of villi were remarkable in T3 group. Enlarged glomeruli, lots of vacuoles, disorganization of renal tubules and necrosis were observed in T2 whereas the T3 group was characterized by vacuoles, disorganization of tubules, enlarging glomeruli and wider tubular lumen. Since the present results clearly demonstrated that 17 α-MT treatments enhanced the total length and weight as well as growth performance of *H. fossilis*, carefully designed experiments with appropriate doses of the hormone in fish feed would be beneficial for the commercial production and conservation of this popular catfish in the country.

***Key words*:** Growth performance, *Heteropneustes fossilis*, Histopathology, Survival rate, Total length, Total weight, 17α-methyl testosterone

Introduction

The stinging catfish, *Heteropneustes fossilis* (Bloch 1974) is a commercially important fish species in Asia including Bangladesh. This is an important food fish as it is enriched with high amounts of protein, iron and calcium (Hasan et al. 2022). However, the monoculture technology for *H. fossilis* and other species could be one is the best technologies in aquaculture to meet up the protein deficiency and socio-economic status of

*Author for correspondence: seager18@yahoo.com

the general people of our country. Such a technology would be extremely helpful for the protection of *H. fossilis* from extinction as well as for its production at commercial levels (Chakraborty and Mirza 2008). Roy et al. (2019), on the other hand, demonstrated the potential of *H. fossilis* production through new technique in tank culture system.

17 α-methyl testosterone (17 α-MT) is a synthetic steroid androgen hormone which is fed to fish fingerlings with the purpose of inducing sex reversal and/or monosex culture to produce predominately male populations of Nile tilapia (*Oreochromis* spp.) and climbing perch (*Anabas* spp.) in commercial fish farms at home (Ferdous and Ali 2011, Rima et al. 2017, Hossain et al. 2018, Hasan et al. 2022); and abroad (Barry et al. 2007, Jensi et al. 2016, Singh et al. 2018, Rivero-Wendt et al. 2020). In recent years, therefore, it is best known for accelerating growth parameters and retrench the feed cost in animal husbandry, that have enchanted the fish farmers' attraction. As a consequence, the hormone is rampantly used in aquaculture as a gender transformer into monosex and as a growth promoter. But the use of 17 α-MT in fish farms has been concerned by both consumers and aquaculturists because of its potential risks to human health and environmental contamination by its residues (Karaket et al. 2021).

Studies on such morphometric features as total length (TL), standard length (SL), body weight (BW) and gonado-somatic indices (GSI) of individual *H. fossilis* have been reported by a number of authors. Chakraborty and Mirza (2008) assessed the production potential of *H. fossilis* monoculture for a period of 8 months at a private fish seed farm in Mymensingh, while Roy et al. (2019) investigated weight, length and survival rate *H. fossilis* and assessed the potential of its production in tank culture system in Dhaka University. Nushy et al. (2020) compared the effects of prepared feed with commercial feed on growth performance of this fish for a period of 5 months at a farmer's pond in Gazipur. Working in a wetland ecosystem at Gajner Beel in the northwestern Bangladesh, Hasan et al. (2022) emphasized that effective but environmentally safe concentrations of 17 α-MT could be applied with an aim of sustainable production and conservation of *H. fossilis* through induced breeding and good aquacultural practices.

Histopathology of liver and kidney in experimentally infected *H. fossilis* was reported earlier by Islam et al. (2008). Nasiruddin et al. (2012**)** evaluated histopathological changes in the gill, liver and intestine of the fish exposed to aqueous and ethanolic seed extracts of three plant species. Khanam and Latifa (2013) assessed hematological parameters in peripheral blood of *H. fossilis* with the change in fish physiology under different environments, time, seasons, maturity, nutritional state, activity levels, and physical and chemical changes in the rearing water. Effects of a common pollutant pesticide Diazinon in the aquatic environment on the histopathology of gills, liver, heart, intestine and kidneys of *H. fossilis* have been analyzed by Islam et al. (2019). Uddin et al. (2019) reported clinical and histopathological changes in the gills and liver of this fish species at various fish farms in Mymensingh region during summer and winter months.

Keeping the aforesaid literature and results in mind, the present study was designed to assess the impacts of 17 α-MT on the growth performance, survival and histopathology of such two vital organs as liver and kidney of *H. fossilis* under laboratory conditions. The present findings might help in boosting *H. fossilis* monoculture technology, which in turn, might be extremely helpful for the mass production, protection and conservation of this popular and commercially important catfish species in the country.

Materials and Methods

Culture of the experimental catfishes

Culture of the experimental *H. fossilis* was maintained in 30L glass aquaria (45 × 30 × 30 cm) in the Fisheries Laboratory, Department of Zoology, Rajshahi University, during a period of four months from March to June 2021. A highly nutritious and protein-enriched commercial fish feed (*Quality Gold*®) was used that contained 10% moisture, 33% crude proteins, 4% crude fats and <4% fibre. The feed also contained fish meal, corn gluten, fish oil, soya bean, wheat flour, yellow corn, some vital minerals such as copper, zinc, iodine, iron and manganese, and vitamins A, C, D3, E and K3 as ingredients. The feed was free from antibiotics, hormones and preservatives. An aerator was used to ensure proper oxygen supply to the fishes. A total of 28 fish fries, seven for each treatment group, were reared throughout the experiment.

Source of fingerlings experimental design

The fingerlings of *H. fossilis* used in this experiment were collected Katakhali fish market, Rajshahi. Total lengths and weights of the fingerlings were 12.675±0.27cm and 8.65 ±0.43g respectively. The experiment was split four treatment groups designated as C (control), T1, T2 and T-3. The fried were reared for 120 days with hormone containing fish feed.

17-αMT treatments

17-αMT is an anabolic steroid type androgen (male) hormone. Discovered and introduced in 1935 for medical applications, it is odourless, white or slightly yellowish white in colour, slightly hygroscopic, solid crystals or powder like. It is infusible in water, entirely soluble in methyl alcohol and chloroform but sparsely soluble in vegetable oils. It is sold under several trade names *viz*., Agoviron, Testovis, Virilion, Android, Metandren, Oraviron, Oreton and others. The usual routes of administration include mouth, buccal or sublingual. Its chemical formula is $C_{20}H_{30}O_2$, that has a molar mass of 302.458 g.mol^{-1}, melting point of 163.0°C and half life of 150 min (~2.5–3 hrs). Its solubility is 33.9 mg/l and its stability is affected by light (Kicman, 2008).

Four treatment groups namely,0.5 mg (T1),0.7 mg (T2),0.9 mg (T3), and 0.00 g (C) of the hormone per g of the fish feed were maintained for estimating growth performance and histopathological studies. The hormone was administered through oral route, mixed with the feed. Since 17-αMT was insoluble in water, the aforesaid doses were first dissolved in 5 ml absolute alcohol, then they were mixed with the fish feed. All the treatments were air-dried for 12-16 hrs before dropping them into the aquaria. Food was supplied to the experimental fishes twice a day while the treated feed (~2g pellets) was supplied once a day. The growth performance in terms of length (cm) and weight (g) per month, and survival rate of the fishes were calculated as follows (Roy et al. 2019; Nushy et al. 2020):

$$\text{Growth percentage} = \frac{\text{Final data} - \text{Initial data}}{\text{Initial data}} \times 100$$

$$\text{Survival rate} = \frac{\text{Number of Fish Alive}}{\text{Number of fish used}} \times 100$$

Histopathological procedures

Histopathological studies included transverse sections of the tissues from the intestine and kidney of the untreated and 17α-MT-treated fishes, which were processed according to the standard procedure for routine microtome techniques as described by Islam et al. (2008), Nasiruddin et al. (2012) and Uddin et al. (2019).

Statistical analysis

Morphometric data on total lengths and weights were analyzed using SPSS for Windows (version 19.0) and presented in Mean±SD. All experimental data between the control and treatment groups were subjected to one-way analysis of variance (ANOVA) followed by the post hoc least significant difference (LSD) tests to separate the means, where the levels of significance were set at $p<0.05$ (Steel and Torrie 1984).

Results

Changes in TL and TW

17-αMT-induced alterations in total length (TL) and total weight (TW) of *H. fossilis* at control (C), T1, T2 and T3 groups under laboratory conditions have been presented in Table 1. Data revealed that even though TL in C and T1 group of fishes differed significantly ($p<0.05$) from that in T2 and T3 groups, the overall effect of the hormone did not influence TL of the fishes under study ($p = 0.085$). A similar result was observed for TW, but the overall impact of 17-αMT on TW of *H. fossilis* was highly significant one ($p<0.001$), indicating that the TW was increased much more than the TL due to the hormone treatment in the experimental fishes.

Table 1: 17-αMT-induced change in total length and total weight of *H. fossilis* under laboratory conditions.

Doses of 17- αMT	N	Total length (TL) in cm ± SD (Range)	Total weight (TW) in g ± SD (Range)
0.0 (Control)	25	13.58 ± 0.94^{a} (12.0-15.8)	9.77 ± 1.57^{a} (7.17-12.86)
0.5 mg/g (T1)	27	13.40 ± 2.92^{a} (12.25-16.0)	10.37 ± 2.68^{a} (9.30-13.64)
0.7 mg/g (T2)	28	14.56 ± 1.75^{b} (12.0-17.8)	12.19 ± 2.89^{b} (11.07-16.93)
0.9 mg/g (T3)	27	14.24 ± 1.29^{b} (12.5-16.5)	11.56 ± 2.27^{b} (10.51-14.86)
F-values at 3, 103 df		2.269ns (P = 0.085)	5.515 (p<0.001)

Dissimilar superscript letters in the same column differ significantly by least significant difference (LSD) tests at $p<0.05$; df = degrees of freedom; ns = not significant.

Month-wise changes in TL and TW of *H. fossilis* are shown in Fig. 1, which suggest that both length and weight of the fishes increased significantly ($p<0.001$) owing to the hormone treatment. Here notably, both length and weight of the experimental fishes increased gradually over the rearing period of four months from March to June 2021. Similar to the TW data shown in Table 1, TW data in Fig. 1 also indicated that weight of the experimental catfishes was much influenced by 17-αMT treatment as compared to their length.

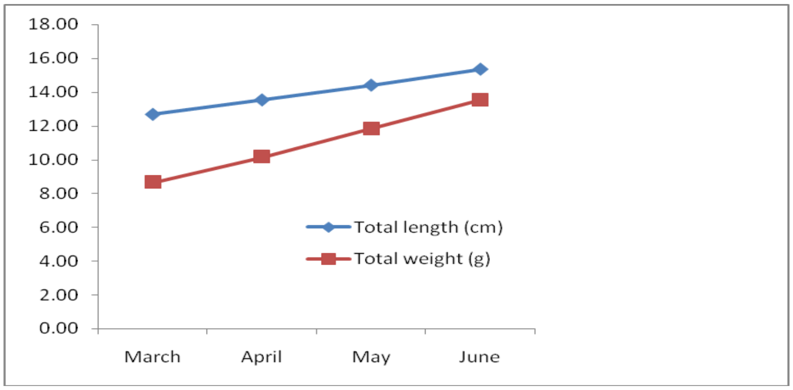

Fig. 1: 17α-MT-induced changes in the length and weight of *H. fossilis* during rearing in the laboratory from March to June 2021.

Growth performance

Growth performances of the fishes in terms of their gains in TL and TW due to the hormone treatment and over a rearing period of four months from March to June have been shown in Fig 2. Compared to the control groups, as expected, all three treatment groups increased in both the morphometric parameters in *H. fossilis*. The increases in TW were more drastic than the corresponding increases in TL.

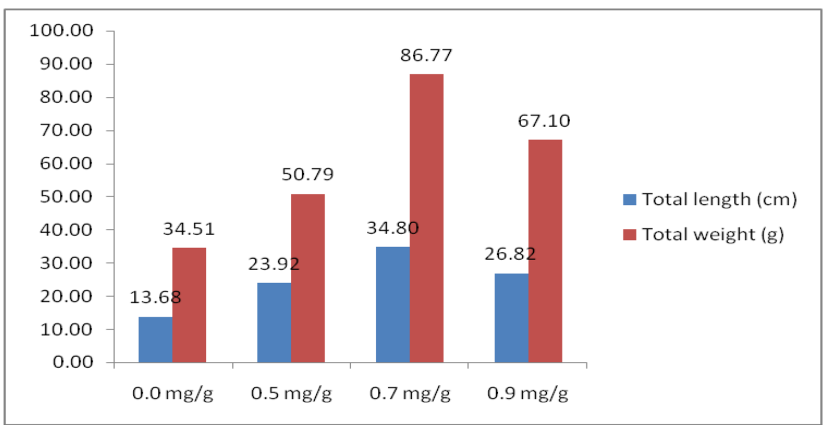

Fig. 2: 17α-MT-induced changes in the growth performance of *H. fossilis* under laboratory conditions.

Survival rate

The survival rate of the experimental catfishes following 17α-MT treatment (Table 2) showed that all the fishes in C and T2 groups' survival during the rearing period of four months from March to June 2021. Of three fishes that died during the observation period, two were from T1 and one was from T3. Thus, the hormone treatments did not affect the survival of the experiment fishes under study.

Table 2. Survival rate of *H. fossilis* following 17-αMT treatments under laboratory conditions during March through June 2021.

Doses of 17α-MT	March		April		May		June	
	No. alive fishes	Survival rate (%)	No. alive fishes	Survival rate (%)	No. alive fishes	Survival rate (%)	No. alive fishes	Survival rate (%)
C (0.0 mg/g)	7	100	7	100	7	100	7	100%
T1 (0.5 mg/g)	7	100	7	100	6	85.71	6	85.71%
T2 (0.7 mg/g)	7	100	7	100	7	100	7	100%
T3 (0.9 mg/g)	7	100	7	100	7	100	6	85.71%

Histopathology

17 α-MT induced changes in the intestine

The wall of intestine in fishes is made of four distinct coats: (a) the outer most layer is serosa, which is made of visceral peritoneum; (b) muscular layer lies just beneath the serosa; (c) sub-mucosa layer, made of connective tissue containing elastic fibres, blood and lymphatic vessels, nerve and glands; and (d) the inner most layer of the intestine is mucosa. TS of the intestine in the untreated (C) fish showed regular structure (Fig. 3a); also no prominent changes were noticed in T1 group of treated fish, except for a tiny degeneration of epithelium (Fig. 3b). However, histopathological changes in T2 group were characterized by hemorrhage and degeneration of villi (Fig. 3c), whereas hemorrhage, degeneration of serosa and rapture of villi were remarkable in T3 (Fig. 3d) group of *H. fossilis* under study.

17 α-MT induced changes in the kidney

Kidney, being an osmoregulatory organ, plays an important role in the excretion of all vertebrates including fishes. The present results showed well-developed glomeruli and numerous renal corpuscles with a system of renal tubules in the control group of fishes (Fig. 3e) and no significant changes like damage of blood vessels and vacuolization were detected in T1 (Fig. 3f). But enlarged glomeruli, coupled with lots of vacuoles, disorganization of renal tubules and necrosis were observed in T2 (Fig. 3g). Finally, TS of kidney in T3 group was characterized by vacuoles, disorganization of tubules, enlarging glomeruli and wider tubular lumen (Fig. 3h).

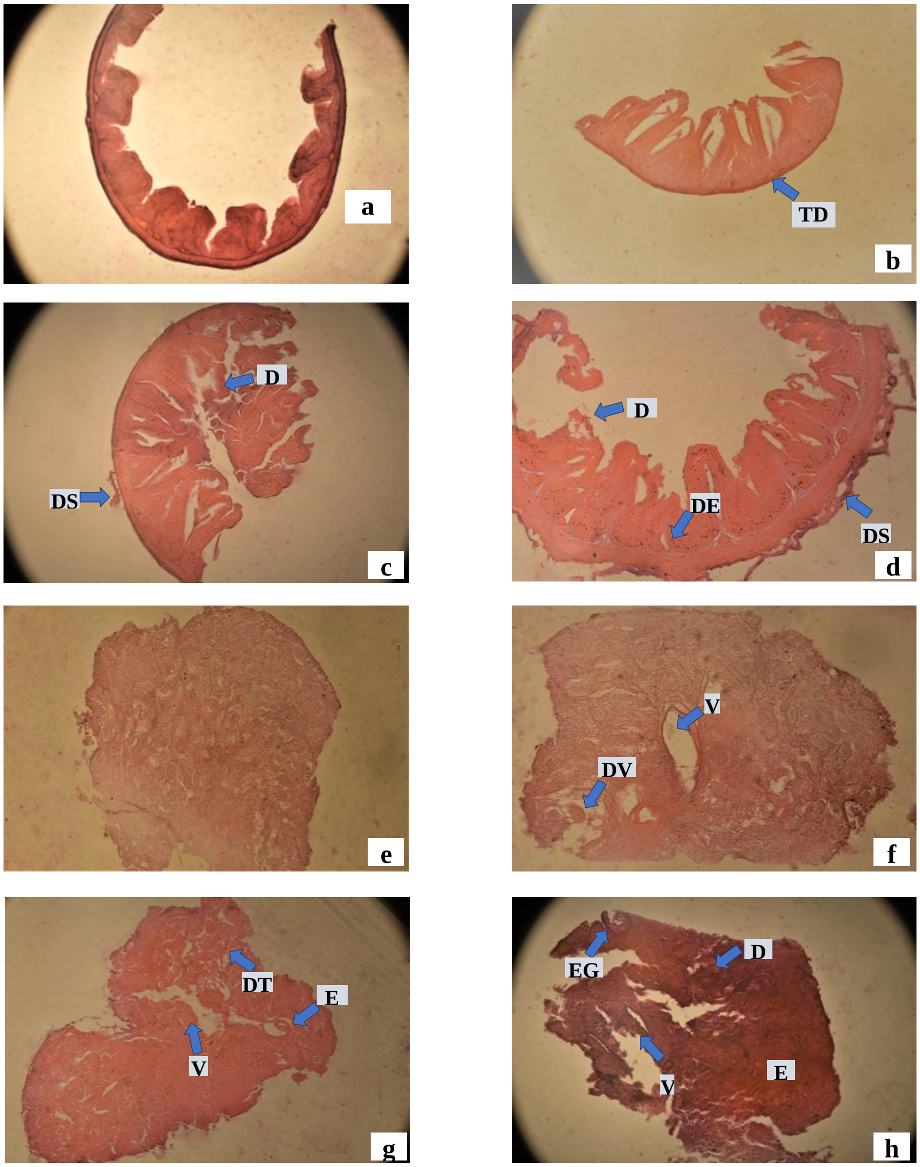

Fig. 3: Transverse sections at 10× magnifications of intestine (a-d) and kidney (e-h) collected from the control (C, 0.0 mg/g), T1 (0.5 mg/g), T2 (0.7 mg/g) and T3 (0.9 mg/g) groups of 17 α-MT-treated experimental *H. fossilis*, respectively. Various abnormalities of the organs have abbreviated as DBV = damaged blood vessel, DE = degeneration of epithelium, DS = degeneration of serosa, DT = disorganization of renal tubules, DT = disorganization of tubules, DV = degeneration of villi, EG = enlargement of glomeruli, H = hemorrhage, TDE = tiny degeneration of epithelium, and V = vacuolization.

Discussion

Results of the current experiment clearly indicated that dietary administration of 17 α-MT at 0.7 $mg.g^{-1}$ artificial feed acted as a better anabolic steroid in *H. fossilis* in comparison with 0.0, 0.5 and 0.9 $mg.g^{-1}$ doses. Although the hormone treatment enhanced the growth performance in the experimental fishes, it also affected the internal organs, including changes and degenerate of function and structures of the internal organs of the kidney and intestine, which are in good agreement with the previous findings by a number of researchers at home and abroad.

Thus, the hormone promoted growth in *Coho salmon* where the optimum dose of 10 $mg.kg^{-1}$ was recorded by McBride and Fagerlund (1973). Kefi et al. (2012) raised *O. andersonni* on 60 $mg.kg^{-1}$ and observed the highest final average weight compared to other treatments. In Tamil Nadu, India, Jensi et al. (2016) used 17-αMT as a feed additive to produce predominately male populations of tilapia where the most effective dose was 60 $mg.kg^{-1}$ and the maximum body length was 80±3.87 mm and the maximum body weight was 11.56±4.027g. Singh et al. (2018) administered five different doses of the hormone for 30 days in Rajasthan, India, where the highest sex reversal rate was observed at 50 $mg.kg^{-1}$ feed and the growth of the fish was also higher in treatments than control. The hormone treated feed was offered to the tilapia fries for 21-28 consecutive days and the efficacy and cost of the treatment regime have been accepted by most aquaculturists in India (Karaket et al. 2021).

In Bangladesh Agricultural University, Mymensingh, Ferdous and Ali (2011) reported that the optimum dose of the hormone was 60 $mg.kg^{-1}$ with a feeding period of 28 days after hatching. Moreover, growth performance data in the tilapia showed significant variation in weight and percent weight gain with the increase of hormone dose. In Noakhali Science and Technology University, on the other hand, Rima et al. (2017) reported the optimum dose of 50 $mg.kg^{-1}$ feed which was better both for growth and male optimization in tilapia, and Hossain et al. (2018) administrated 100 $mg.kg^{-1}$ of 17-αMT with feed for 6-12 days to produce over 85% of monosex tilapia.

Chakraborty and Mirza (2008) assessed production potential of *H. fossilis* monoculture for a period of 8 months at a private fish seed farm in Mymensingh, while Roy et al. (2019) investigated weight, length and survival rate *H. fossilis* and assessed the potential of its production in tank culture system in Dhaka University. Muniasamy et al. (2019) showed in India that 60 $mg.kg^{-1}$ concentrations yielded better results in *C. mrigala* and 100 $mg.kg^{-1}$ in *C. punctatus*. Nushy et al. (2020) compared the effects of prepared feed with commercial feed on growth performance of this fish for a period of 5 months at a farmer's pond in Gazipur. Working in a wetland ecosystem at Gajner Beel in the northwestern Bangladesh, Hasan et al. (2022) emphasized that effective but environmentally safe concentrations of 17 α-MT could be applied with an aim of sustainable conservation of *H. fossilis* through induced breeding and good aquacultural practices. The growth performance of the present experimental *H. fossilis* was higher in treated groups than the control where the percentages of length and weight were 34.80% and 86.77%, respectively at 0.7 $mg.g^{-1}$ in comparison with 13.68% and 34.51%, respectively in the control. The survival rate at this dose was also higher compared to other doses.

Histopathology of *C. starius* and *H. fossilis* inhabiting in the polluted waters by Kumari and Kumar (1997) revealed necrosis of internal organs and degenerative alternations in serosa, mucosa and sub-mucosa layers of the intestine. Histopathology of experimentally infected *H. fossilis* showed massive atrophy and focal necrosis in the kidneys, liver and intestine (Islam et al. 2008). Hemorrhage, vacuolation and atrophy of hepatic sinusoids and degeneration of hepatic tissues were noted. Atrophy, hemorrhage, missing of epithelium and villi were found in intestine whereas tissue abscess characterized by focal necrosis, hemorrhage and vacuolation were found in the kidneys. These changes are almost similar to that caused by heavy metal and pesticides as reported by Mohamed (2009) and Mekkawy et al. (2013).

Nasiruddin et al. (2012) evaluated histopathological changes in gill, liver and intestine of *H. fossilis* exposed to aqueous and ethanolic seed extracts of three plant species that caused subtle cellular damages like disintegration of gill filaments and lamellae, degeneration of hepatocytes and blood vessels and necrosis of the intestinal epithelia. Khanam and Latifa (2013) assessed hematological parameters in peripheral blood of *H. fossilis* with the change in fish physiology under different environment, time, season, maturity, nutritional state, activity levels, and physical and chemical changes in the rearing water. Similar to our present study where degeneration of villi and serosa and lysis of intestinal vacuoles, however, Maharajan et al. (2016) also reported swelling of intestine, fused microvilli, lysis of intestinal vacuoles and erosion of intestinal villi caused by toxic substances.

Effects of Diazinon in the aquatic environment on the histopathology of gills, liver, heart, intestine and kidneys of *H. fossilis* were analyzed by Islam et al. (2019), where lamellar fusion, sinusoid vacuoles, fused villi and hemorrhagic renal tubules were observed. From January to April 2018, health status of *H. fossilis* was investigated through clinical and histopathological observations at various fish farms in Mymensingh, where pathological changes in the gill were lamellar missing, split gill lamellae, hemorrhage, hypertrophy, vacuums and presence of parasites. Moreover, hemorrhage, vacuums, necrosis and fat bodies were noticed in the liver (Uddin et al. 2019). The aforesaid changes corroborate to the present 17 α-MT treatments in *H. fossilis*, where degeneration of renal tubules, vacuolization and enlargement of glomeruli were obvious.

Conclusion

Present results clearly indicated that 17 α-MT treatments could enhance the total length, total weight and growth performance of *H. fossilis*. Carefully designed experiments with appropriate doses of the hormone in fish feed, therefore, would be beneficial for the commercial production and conservation of this popular catfish in Bangladesh.

Acknowledgements

We are grateful to the Chairman, Department of Zoology, RU, for providing laboratory facilities to conduct the experiments. Prof. Dr. M. Saiful Islam of the same department deserves special thanks for his critical reviews of the manuscript and updating the literature.

References

Barry TP, Marwah A and Marwah P (2007). Stability of 17α-methyltestosterone in fish feed. Aquaculture. 271: 523-529.

Chakraborty BK and Mirza JA (2008). Growth and Yield Performance of Threatened Singi, *Heteropneustes fossilis* (Bloch) under semi intensive aquaculture. J. Fish. Soc. Taiwan, 35(2): 117-125.

Ferdous Z and Ali MM 2011. Optimization of hormonal dose during masculinization of tilapia (*Oreochromis niloticus*) fry. J. Bangladesh Agril. Univ., 9(2): 359-364.

Hasan MR, Hossain MY, Mawa Z and Hossain MAR 2022. Reproductive biology of *Heteropneustes fossilis* in a wetland ecosystem (Gajner *Beel*, Bangladesh) in relation to eco-climatic factors: Suggesting a sustainable policy for aquaculture, management and conservation. Saudi J. Biol. Sci., 29(2): 1160-1174.

Hossain MS, Hossain MS, Hossain MM, Mustafa MG, Siddique MA (2018). Standardization of the dose of 17α methyltestosterone to produce monosex population of tilapia. Poster presented at the 1st International Conference on Biological and Environmental Research: Recent Development, Challenges and Future Prospects.

Islam MS, Haque MM, Uddin MN and Hasanuzzaman M (2019). Histopathology in the fish *Channa punctatus*, *Heteropneustes fossilis* and *Anabas testudineus* exposed to diazinon. Int. J. Fish. Aquatic Stud., **7**(6): 47-54.

Islam MT, Mostafa K and Rashid MM 2008. Histopathological studies of experimentally infected Shing, *Heteropneustes fossilis* with *Aeromonas hydrophila* bacteria. Progress. Agric., 19(1): 89-96.

Jensi A, Marx KK, Rajkumar M, Shakila RJ and Chidambaram P. 2016. Effect of 17 α-methyl testosterone on sex reversal and growth of Nile tilapia (*Oreochromis niloticus* L., 1758). Eco. Env. Cons., 22(3): 1493-1498.

Karaket T, Reungkhajorn A and Ponza P (2021). The optimum dose and period of 17α-methyltestosterone immersion on masculinization. https://doi.org/10.1016/j.aaf.2021.09.001

Kefi AS, Kang'ombe J, Kassam D and Katongo C (2012). Effect of 17alpha methyltestosterone on haematology and histology of liver and heart of *Oreochromisandersonii* (Castelnau, 1861). J Marine Sci. Res Dev., 3: 130.

Khanam T and Latifa GA 2013. Hematological parameter of *Heteropneustes fossilis* (Shing fish). J. Sci. Foundation,11(1): 3-10.

Kumari AS and Kumar NSR (1997) Effects of water pollution on histology of intestine of two fresh water fishes from Hussainsagar lake (A. P.), Indian J. Environ. Toxicol., 7: 68-70.

Maharajan A, Kitto MR, Paruruckumani PS and Ganapiriya V (2016). Histopathology biomarker responses in Asian sea bass *Lates calcarifer* Bloch exposed to copper. J. Basic Applied Zool., 77: 21-30.

McBride JR and Fagerlund UHM (1973). The use of 17 a-methyl testosterone for promoting weight increases in juvenile Pacific salmon. J. Fish. Res. Board Canada, 30: 1099-1104.

Mekkawy IAA, Mahmoud UM, Wassif ET and Naguib M (2013). Effect of cadmium on some histopathological and histochemical characteristics of the kidney and gills tissues of *Oeochromis niloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E. J. Fish. Aquat. Sci., 8: 553-580.

Mohamed FAS (2009). Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. World J. Fish Mar. Sci., 1: 29-39.

Muniasamya PS, Benzigera AY, Kumarb MA, Haniffa , Paraycb BA, Albeshr MF and Al-Umri S (2019). Effect of 17a-methyl testosterone incorporated diets on growth of spotted snakehead, *Channa punctatus* and white carp, *Cirrhinus mrigala.* Saudi Journal of Biological Sciences, 26: 541-546.

Nasiruddin M, Azadi MA and Jahan A (2012). Histopathological changes in gill, liver and intestine of *Heteropneustes fossilis* (Bloch) treated with three dry seed extracts. J. Asiat. Soc. Bangladesh, Sci., 38(2): 217-226.

Nushy NH, Zafar A, Khatun M, et al. (2020). Comparative growth performance assessment of Shing (Heteropneustes fossilis) feeding with prepared and commercial diet. J. Aquac. Mar. Biol., 9(1):10-13. doi: 10.15406/jamb.2020.09.00270.

Rima NN, Rahman MM and Sarker MJ (2017). Optimization of 17-alpha methyltestosterone (MT) hormone dose during masculinization of Nile tilapia (*Oreochromis niloticus*) fry. J. Noakhali Sci. Technol. Univ.,1(2): 35-41.

Rivero-Wendt CLG, Miranda-Vilela AL, Domingues I, Oliveira R, Monteiro MS, Moura-Mello MAM, Matias R, Maia-Soares AMV and Grisolia CK (2020). Steroid androgen 17 alpha methyltestosterone used in fish farming induces biochemical alterations in zebrafish adults. J. Environ. Sci. Health (Part A) 55(11): 1321-1332. doi: 10.1080/10934529.2020.1790954.

Roy D, Al Masud A, Saha PK, Kutubuddin MM and Islam MM (2019). Water quality, growth and production performance of stinging catfish, *Heteropneustes fossilis* (Bloch) in cemented tanks with two different stocking densities. Bangladesh J. Zool., 47(1): 107-119.

Singh E, Saini VP and Sharma OP (2018). Orally administered 17 α methyl testosterone at different doses on the sex reversal of the red tilapia (*Oreochromis niloticus*). Int. J. Fish. Aquat. Stud.,6(3): 301-305.

Steel RGD and Torrie JH (1984). Principles and Procedures of Statistics: A biometrical approach. McGraw Hill, Tokyo, Japan.

Uddin MA, Islam MA, Islam R, Yesmin R, Halim KMA and Hasanuzzaman KM 2019. Study on health status of farmed Shing (*Heteropneustes fossilis*) in Mymensingh region. Res. Agric. Livest. Fish. 6(3): 445-455.

(Manuscript received on 14 June 2022; Revised on 21 June 2022)