Hematological and biochemical characters of monosex tilapia (*Oreochromis niloticus*, Linnaeus, 1758) cultivated using methyltestosterone

Alaa El-Din H. Sayed *, Rehab H. Moneeb

Zoology Department, Faculty of Science, Assiut University, 71516 Assiut, Egypt

Received 16 October 2014; revised 15 February 2015; accepted 30 March 2015

**KEYWORDS**
Nile tilapia; Hematology; Biochemistry; Methyltestosterone; Aquaculture

**Abstract** The use of steroid-treated feeds as 17 α-methyltestosterone for the production of all-male populations is widespread in tilapia aquaculture. The aim of the present study was to investigate the misuse effects of methyltestosterone on monosex farmed Nile tilapia, *Oreochromis niloticus* by evaluation of hematological and biochemical values. The fishes were obtained from four localities (Assiut as a control, Beheira, Alexandria and Kafr el-Sheikh: three farms from each governorate as farmed monosex produced using methyltestosterone). The total erythrocyte count, hemoglobin rate, hematocrit percentage, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), white blood cell count, blood platelets, lymphocyte, monocyte, neutrophils and basophils were determined. The results showed that there were changes in the erythrocytic series and in the defense white blood cells. Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), glucose, cholesterol, total protein, uric acid and creatinine were determined for biochemical study. These alterations are considered an indication for performance and health of fish in the monosex culture medium indicating the side effects of overdose induction of MT.

Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

**Introduction**

Tilapia is likely to be the most important of all aquaculture fish in the 21st century (Fitzsimmons, 2000). Tilapia has certain favorable characteristics, which make its cultivation most tolerant to adverse environmental conditions. Also it can survive at low dissolved oxygen, euryhaline, has relatively fast growth and efficient food conversion (Asad et al., 2010).

Tilapia is farmed in at least 85 countries, making it the most widely farmed finfish worldwide and second in volume only to carps and salmonides. Tilapia production has expanded dramatically in recent years, from about 1 million metric tons in 1998, to nearly 2.4 million tons in 2006, of which almost 2 million tons were contributed by a single species, the Nile tilapia (FAO, 2006a).

Because of their high protein content, large size, rapid growth (6–7 months to grow to harvest size) and palatability, tilapia is in the focus of major aquaculture efforts.

* Corresponding author. Tel.: +20 882411444; fax: +20 882342708. E-mail address: alaa_h254@yahoo.com (Alaa El-Din H. Sayed).

Peer review under responsibility of The Egyptian German Society for Zoology.

http://dx.doi.org/10.1016/j.jobaz.2015.03.002

2090-9896 Published by Elsevier B.V.

This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Nile tilapia is a native fish species of Egypt that has become popular worldwide mainly as a valuable fish, easy to breed and grows in a variety of aquaculture systems (El-Sayed, 2006). The production of all-male populations of tilapia (Oreochromis sp.) is an important tool in aquaculture to avoid energy consumption in reproduction and to produce the sex with the larger growth potential (Green et al., 1997; Macintosh and Little, 1995). One of the most common techniques for producing mono-sex populations is steroid-induced sex inversion such as 17 α-methyltestosterone. The use of steroid-treated feeds for the production of all-male populations is widespread in tilapia aquaculture (Green et al., 1997).

Hematological parameters are an indicator of water balance, nutritional status and overall health condition of fish (Chang and Hur, 1999; Denson et al., 2003). For this reason hematological variables have been used as indicators of fish health status in a number of fish species to detect physiological changes as a result of stress condition such as exposure to pollutants, hypoxia, transportation, anesthetic and acclimation (Akinrotimi et al., 2009).

Measurement of serum biochemical parameters can be especially useful to help identify target organs of toxicity as well as the general health status of animals and has been advocated to provide early warning of potentially damaging changes in stressed organisms (Folmar, 1993; Jacobson-Kram and Keller, 2001).

The dietary administration of MT to improve body weight, increased residual testosterone whereas decreased the lipids required for human consumption, which might be hazardous for humans (Schardein, 1980).

Due to the misuse of hormonal treatments in sex reversal of tilapia especially in the Egyptian private sector hatcheries, the main objective of this study is to evaluate the hematological and biochemical values for mono-sex farmed Nile tilapia, Oreochromis niloticus and also estimation of the methyltestosterone in the serum and muscle of monosex tilapia.

**Materials and methods**

**Sample collection**

Six fishes of the Nile tilapia, O. niloticus were caught from Assiut farms (only males were taken) as base line data and three farms of Beheira, Alexandria and Kafr el-Sheikh as monosex farms in Egypt (Fig. 1). The data of those farms and fishes are reported in Table 1.

**Water quality assessment**

Water-quality criteria [pH, dissolved oxygen, water temperature, conductivity, salinity, turbidity, phenols, chloride, fluoride, sulfate, nitrate, cyanide and ammonia] of the chosen sites were monitored during the fish collection. Total Fe, Cd, Pb, Zn, Cr, and Hg were measured using graphite furnace AA (GFAA) spectroscopy. Sampling and assessment of water quality were done according to the traditional manual methods (APHA, 2005). Data of the selected farms are shown in Table 2.

**Blood analysis**

Blood samples (6 fishes/farm) were collected from the caudal vein of the fish in a small plastic tube containing heparin solution (0.2 ml/ml blood) as anticoagulant. The concentration of Hb and blood cells count were immediately estimated. RBCs, WBCs, blood platelets, hematocrit (HCT), hemoglobin (Hb) were determined by using automated technical analyzer (Mindray Bc-2800). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated using the formulae mentioned by (Dacie and Lewis, 1991).

*Figure 1*  Map showing the sampling sites of the selected fish farms under investigation.
MCHC (g/dl) = Hb/Ht * 100
MCH (pg) = Hb/RBC’s * 10
MCV (μm³) = Ht/RBC’s * 10

Total white blood cells (WBC) were counted using an improved Neubauer hemocytometer (Mgbenka and Oluah, 2003; Shah and Altindg, 2005). Blood were diluted 1:20 with a diluting fluid and placed in hemocytometer. 4 large (1 mm²) corner squares of the hemocytometer were counted under the microscope (Olympus) at 640·. the total number of WBCs was calculated in mm³·10³ (Wintrobe, 1967) and differential count (lymphocytes, monocytes, neutrophils, eosinophils) was displayed by staining blood films with Giemsa stain.

Some other blood samples were collected and left to coagulate for 15–20 min at 4°C prior to centrifugation for 20 min at 3000 rpm to separate serum. The fresh serum was subjected to biochemical analysis. Serum glucose, cholesterol total protein, uric acid and creatinine were determined colorimetrically using assay kit supplied by Diamond Diagnostics, Egypt.

Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were determined colorimetrically using assay kits (Spectrum Diagnostics, Egypt) according to (Reitman and Frankel, 1957) method. The samples were measured by spectrophotometer (Ultrospec 3100 pro, Biochrom Ltd).

**Estimation of the MT concentration**

Estimation of the MT concentration in fish muscles and serum was done according to the protocol of Risto et al. (2013) using a kit purchased from R-Biopharm AG, Darmstadt, Germany.
**Statistical analysis**

Data statistical analysis will be done using the statistical program (Statistical Package for the Social Science; Inc., Chicago, IL, USA) (SPSS, 1998), version 16.

**Results**

**Physicochemical water parameters**

Table 2 shows mean ± SD of the measured physico-chemical parameters of the water samples collected from Assiut farms as control and three farms of Beheira, Alexandria and Kafr el-Sheikh as monosex farms. Most of these parameters showed the highest values in the water of the monosex farms in comparison to Assiut farms.

**Hematological studies**

Table 3 reveals hematological parameters of the sampled fishes from the monosex farms in comparison to Assiut farms. RBCs number (million/mm$^3$) and blood platelets (Thousands/mm$^3$) decreased significantly ($p \leq 0.05$) while hematocrit percentage (%) decreased insignificantly. Also hemoglobin content (g/dl) showed significant changes ($p \leq 0.05$). Mean corpuscular volume; MCV ($\mu m^3$) and mean corpuscular hemoglobin; MCH (Pg) were increased significantly ($p \leq 0.05$) while mean corpuscular hemoglobin concentration; MCHC (%) and white blood cells (Thousands/mm$^3$) increased insignificantly.

Differential count of white blood cells showed significant changes in the sampled fishes from the monosex farms as compared to control fish. As Table 3 shows, the lymphocytes (%) decreased significantly while neutrophils (%) increased significantly ($p \leq 0.05$). Monocytes (%) and eosinophils (%) changed significantly ($p \leq 0.05$) in the different farms.

**Biochemical studies**

Table 4 reveals biochemical parameters of the sampled fishes from the monosex farms in comparison to Assiut farms. ALT and ALP (µl) decreased significantly ($p \leq 0.05$) and also AST (µl) except Beheira farms were increased, while glucose (mg/dl) was decreased insignificantly. Cholesterol (mg/dl), Uric acid (mg/dl) and Creatinine (mg/dl) were increased significantly ($p \leq 0.05$) while total protein increased insignificantly.

---

**Table 3** Hematological changes (mean value ± SE) of *Oreochromis niloticus* from Assiut and monosex farms, ($N = 6$) for each group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Assiut</th>
<th>Alexandria</th>
<th>Kafr el-Sheikh</th>
<th>Beheira</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (million/mm$^3$)</td>
<td>1.76 ± 0.09a</td>
<td>1.47 ± 0.03c</td>
<td>1.65 ± 0.03ab</td>
<td>1.51 ± 0.03bc</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>8.24 ± 0.15a</td>
<td>7.6 ± 0.25b</td>
<td>8.42 ± 0.17a</td>
<td>8.13 ± 0.14a</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>25.36 ± 0.99a</td>
<td>23.13 ± 0.95a</td>
<td>25.13 ± 0.58a</td>
<td>24.91 ± 0.23a</td>
</tr>
<tr>
<td>MCV (µm$^3$)</td>
<td>145.91 ± 6.73b</td>
<td>157.11 ± 4.97ab</td>
<td>152.8 ± 2.2ab</td>
<td>165.14 ± 3.9a</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>47.49 ± 1.89b</td>
<td>51.64 ± 1.15ab</td>
<td>51.28 ± 1.05ab</td>
<td>53.89 ± 1.36a</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>32.8 ± 1.17a</td>
<td>33.02 ± 0.8a</td>
<td>33.57 ± 0.6a</td>
<td>32.64 ± 0.4a</td>
</tr>
<tr>
<td>Platelets (Thousands/mm$^3$)</td>
<td>319.11 ± 3.26a</td>
<td>297.56 ± 6.58a</td>
<td>318.89 ± 13.24a</td>
<td>310.33 ± 2.33a</td>
</tr>
<tr>
<td>WBC (Thousands/mm$^3$)</td>
<td>829.33 ± 27.64a</td>
<td>806 ± 19.52a</td>
<td>836.33 ± 8.77a</td>
<td>834.67 ± 3.53a</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>90.89 ± 0.35a</td>
<td>90.56 ± 0.47a</td>
<td>87.56 ± 0.29b</td>
<td>90.56 ± 0.47a</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>2 ± 0.17b</td>
<td>1.56 ± 0.24b</td>
<td>3 ± 0.24a</td>
<td>1.78 ± 0.32b</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>5.67 ± 0.37b</td>
<td>7.11 ± 0.35a</td>
<td>7.44 ± 0.18a</td>
<td>6.56 ± 0.34ab</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>1.44 ± 0.18b</td>
<td>0.78 ± 0.15c</td>
<td>2 ± 0a</td>
<td>1.11 ± 0.11bc</td>
</tr>
</tbody>
</table>

Different letters indicate there is a significant difference at $p \leq 0.05$.

RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells.

**Table 4** Biochemical results (mean value ± SE) of *Oreochromis niloticus* from Assiut and monosex farms, ($N = 6$) for each group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Assiut</th>
<th>Alexandria</th>
<th>Kafr el-Sheikh</th>
<th>Beheira</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (µl)</td>
<td>58.98 ± 1.3b</td>
<td>56.49 ± 1b</td>
<td>54.69 ± 0.71b</td>
<td>63.39 ± 1.4a</td>
</tr>
<tr>
<td>ALT (µl)</td>
<td>29.92 ± 0.57a</td>
<td>26.58 ± 1.02b</td>
<td>28.7 ± 0.61ab</td>
<td>29.48 ± 0.95ab</td>
</tr>
<tr>
<td>ALP (µl)</td>
<td>31.21 ± 0.4a</td>
<td>29.49 ± 0.87a</td>
<td>30.46 ± 0.4a</td>
<td>26.5 ± 0.48b</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>105.78 ± 4.04a</td>
<td>98.27 ± 4.08a</td>
<td>102.29 ± 2.23a</td>
<td>105.76 ± 2.69a</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>183.11 ± 7.32b</td>
<td>209.89 ± 9.04a</td>
<td>203.06 ± 2.6ab</td>
<td>201.57 ± 4.14ab</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>5.12 ± 0.95a</td>
<td>6.07 ± 0.32a</td>
<td>6.14 ± 0.14a</td>
<td>5.5 ± 0.13a</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>14.78 ± 0.48a</td>
<td>16.42 ± 0.57ab</td>
<td>16.43 ± 0.26ab</td>
<td>17.09 ± 0.51a</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.29 ± 0.05b</td>
<td>0.72 ± 0.03a</td>
<td>0.6 ± 0.02a</td>
<td>0.6 ± 0.05a</td>
</tr>
</tbody>
</table>

Different letters indicate there is a significant difference at $p \leq 0.05$. 

RBC, red blood cells; Hb, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood cells.
Estimation of the MT concentration

Table 5 reveals the methyltestosterone concentration in the serum and muscle of sampled fishes from the monosex farms in comparison to Assiut farms. MT concentration in the serum (ng/ml) and muscle (ng/g) of Assiut farms showed no detectable levels of hormonal residues while the monosex farms showed high levels of MT concentration in the serum and muscle of the sampled fishes.

### Discussion

The synthetic hormone, 17-α-methyltestosterone (MT), is used in fish hatcheries to induce male monosex. By feeding small amounts of male hormone to tilapia fry before and during sexual differentiation, virtually all the treated fish develop as males morphologically and the potential of the stock to reproduce is thereby eliminated.

This form of sex control has the added benefit that male tilapias generally grow faster than females, with a result that all-male fish are larger and more uniform in size than mixed sex tilapias (Smith and Phelps, 1997; Hussain et al., 2005).

These desirable growth characteristics are shown particularly by MT treated Nile tilapia (*O. niloticus*), which is the major tilapia species farmed commercially worldwide (FAO, 2006b). To our knowledge this is the first investigation which deals with the study of the effects of use of methyltestosterone in tilapia sex reversal at Egypt in the field not experimentally.

Water quality which investigated along the studying areas showed similar mean values of nearly all the detected physico-chemical parameters in water collected from sampling sites. Those results indicated that the water quality of the ones collected from the fish farms of the selected areas is high compared with those collected from the river Nile in a previous study (Osman et al., 2012). The results of (Osman et al., 2012) prove the presence of large quantities of organic and inorganic pollutants in the water along the river Nile and this is supported by (Osman et al., 2012) who prove the presence of large amounts of male hormone to tilapia fry before and during sex reversal at Egypt in the field not experimentally.

Kidney function has been reported using creatinine and uric acid levels (Abdel-Tawwab and Wafeek, 2008). Cholesterol is a chemical compound that is naturally produced by the body, and is a combination of lipid (fat) and steroid. Cholesterol is a building block for cell membranes and for sex hormones like estrogen and testosterone. About 80% of the cholesterol is produced by the liver (Hasheesh et al., 2007). These enzymes are also considered to be important in assessing the state of the liver and some other organs (Verma et al., 1981).

In this study, methyltestosterone in *O. niloticus* from the monosex farms resulted in a significant decrease in the activities of AST (except Beheira farms), ALT and ALP as compared with the control. Decreasing ALT activity in *O. niloticus* were recorded by Onier et al., (2009) after exposure to metals.

Liver function using AST and ALT as biomarkers was studied. Those enzymes belong to the plasma nonfunctional enzymes which are normally localized within the cells of liver, heart, gills, kidneys, muscle and other organs (Hadi et al., 2009). These enzymes are also considered to be important in assessing the state of the liver and some other organs (Verma et al., 1981).

In the present study, it has been determined that methyltestosterone in *O. niloticus* from the monosex farms has significantly increased serum cholesterol, uric acid and creatinine. This agrees with (Kefi et al., 2013) who recorded high levels of cholesterol in *Oreochromis andersonii* feed 40 mg/kg methyltestosterone. This is likely to be a sign of stress associated with the increase in the cortisol level (Borges et al., 2007). Also Tasgin et al. (2010) recorded high levels of cholesterol with the effects of testosterone implication in rats.
Blood glucose levels have long been used as indicators of stress in fish (Abdel-Baky, 2001). Glucose level decreased insignificantly in the studied fishes from the monosex farms while total protein was increased insignificantly.

Hormonal disruption in the present study was recorded. The monosex farms showed high levels of MT concentration in the serum and muscle in comparison to Assiut farms. These results may go hand in hand with those recorded by (El-Neklawey et al., 2009; El-Khaky et al., 2012) who found high values of testosterone in tilapia farm fish. On the other hand, higher results of testosterone in tissues of tilapia were reported by Tag El-Din et al. (2009a,b) who recorded 7.52 ± 0.67 (ng/g) of testosterone in fresh water prawn tissues. The detection of hormonal residues in some local fish (tilapia and carp) may be attributed to the wide use of synthetic androgen as methyl testosterone on fish production in Egypt for its anabolic and androgenic action in fish. This agrees with that stated by Mansour and Satyanarayana (1989) and Hegazy (2007).

5. Conclusion

In conclusion, based on the results obtained from the present study, it was found that MT-induced monosex production has severe side effects depending on the high doses estimated in the serum and muscles of the fishes. Alterations in the erythrocytic series, white blood cells, liver functions, kidney functions, glucose, cholesterol, total protein, and hormones were reported. These alterations are considered an indication for performance and health of fish in the monosex culture medium indicating the side effects of overdose induction of MT.

Acknowledgment

This study was financially supported by Science and Technology Development Fund (STDF), Egypt (Project ID: 5585).

References

FAO, 2006b. Fisheries and Aquaculture Development, Rome, Italy.


