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

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KEYWORDS

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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.

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.

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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, chloridate, fluridate, 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).

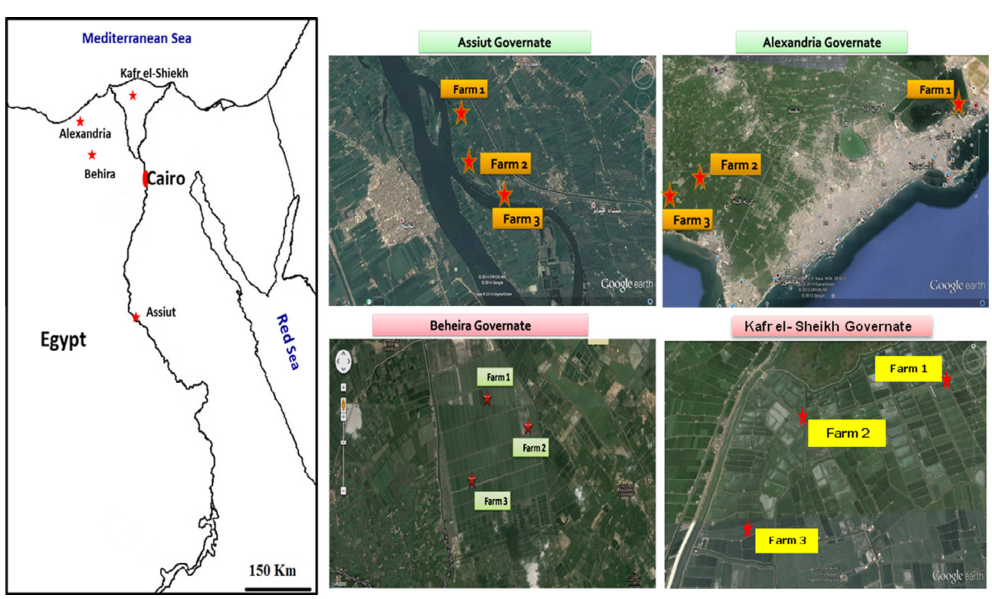

Figure 1 Map showing the sampling sites of the selected fish farms under investigation.

Table 1 Age, weight and length of the collected fishes from different farms and also the farm's area.

Governorates	Farm No.	Farm area (Acre)	Age of fish (Month)	Weight (Grams)	Length (cm)
Beheira	Farm 1	250	7	61–235	15–20
	Farm 2	85	10		
	Farm 3	30	12		
Alexandria	Farm 1	38	6	45–583	14–32
	Farm 2	37	5		
	Farm 3	22	23		
Assiut	Farm 1	1.5	22	43–248	15–22
	Farm 2	2.2	18		
	Farm 3	5	12		
Kafr el-Sheikh	Farm 1	6	12	33–129	12–19
	Farm 2	22	10		
	Farm 3	8	7		

Table 2 Physicochemical parameters of the water samples collected from Assiut farms as control and three farms of Beheira, Alexandria and Kafr el-Sheikh as monosex farms.

Parameters (Unit)	Governorates				
	Assiut Mean ± SD	Alexandria Mean ± SD	Beheira Mean ± SD	Kafr el-Sheikh Mean ± SD	Permissible limit**
pH (ppm)	7.33 ± 0.15	7.33 ± 0.23	7.6 ± 0.2	7.3 ± 0.3	7–8.5
Dissolved oxygen (ppm)	8.63 ± 0.25	6.57 ± 0.72	5.8 ± 0.44	7.03 ± 0.208	not less than 5
Temperature (°C)	27.4 ± 0.26	27.03 ± 0.15	27.1 ± 0.1	27.27 ± 0.058	usually up 5
Conductivity (ms/cm)	0.67 ± 0.06	0.7 ± 0	0.6 ± 0.06	0.7 ± 0	..
Salinity (%)	0.01 ± 0	0.08 ± 0.001	0.07 ± 0.004	0.08 ± 0.01	..
Turbidity (NUT)	0.13 ± 0.056	0.43 ± 0.076	0.51 ± 0.1	0.34 ± 0.069	not more than 0.25
Phenols (ppm)	0.01 ± 0.006	0.03 ± 0.005	0.04 ± 0.01	0.03 ± 0.006	0.1
Chloridate (ppm)	7.1 ± 0.1	6.5 ± 0.36	5.97 ± 0.15	6.6 ± 0.2	..
Fluridate (ppm)	0.29 ± 0.04	0.26 ± 0.02	0.35 ± 0.01	0.23 ± 0.044	0.5
Sulfate (ppm)	68 ± 3.61	85.33 ± 8.14	94 ± 2	80.67 ± 4.16	200
Nitrate (ppm)	0.87 ± 0.16	1 ± 0.21	1.07 ± 0.16	2.07 ± 0.085	45
Cyanide (ppm)	0.006 ± 0.0014	0.0063 ± 0.00057	0.007 ± 0.004	0.0078 ± 0.00057	..
Ammonia (ppm)	ND*	0.133 ± 0.0058	0.367 ± 0.058	0.2 ± 0.1	0.5
Fe (ppm)	0.073 ± 0.0058	0.09 ± 0.01	0.143 ± 0.04	0.097 ± 0.0057	1
Cd (ppm)	ND	0.007 ± 0	0.009 ± 0.0006	0.0067 ± 0.00057	0.01
Pb (ppm)	ND	0.0367 ± 0.0153	0.043 ± 0.0058	0.03 ± 0.01	0.05
Zn (ppm)	0.217 ± 0.0153	0.483 ± 0.058	0.813 ± 0.0929	0.34 ± 0.01	1
Cr (ppm)	ND	0.00016 ± 0.027	0.0002 ± 0.0002	ND	0.05
Hg (ppm)	ND	ND	0.008 ± 0.0014	ND	0.001

* ND, not detected.
** According to APHA (2005).

MCHC (g/dl) = Hb/Ht * 100
MCH (pg) = Hb/RBC's * 10
MCV (μm^3) = 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 mm^2) corner squares of the hemocytometer were counted under the microscope (Olympus) at 640×. the total number of WBCs was calculated in $mm^3 \times 10^3$ (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 ($p \leqslant 0.05$) while hematocrit percentage (%) decreased insignificantly. Also hemoglobin content (g/dl) showed significant changes ($p \leqslant 0.05$). Mean corpuscular volume; MCV (μm^3) and mean corpuscular hemoglobin; MCH (Pg) were increased significantly ($p \leqslant 0.05$) 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 ($p \leqslant 0.05$). Monocytes (%) and eosinophils (%) changed significantly ($p \leqslant 0.05$) 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 (μ/l) decreased significantly ($p \leqslant 0.05$) and also AST (μ/l) 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 ($p \leqslant 0.05$) 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.

Parameters	Governorates			
	Assiut	Alexandria	Kafr el-Sheikh	Beheira
RBC (million/mm^3)	1.76 ± 0.09a	1.47 ± 0.03c	1.65 ± 0.03ab	1.51 ± 0.03bc
Hb (g/dl)	8.24 ± 0.15a	7.6 ± 0.25b	8.42 ± 0.17a	8.13 ± 0.14a
HCT (%)	25.36 ± 0.99a	23.13 ± 0.95a	25.13 ± 0.58a	24.91 ± 0.23a
MCV (μm^3)	145.91 ± 6.73b	157.11 ± 4.97ab	152.8 ± 2.2ab	165.14 ± 3.9a
MCH (Pg)	47.49 ± 1.89b	51.64 ± 1.15ab	51.28 ± 1.05ab	53.89 ± 1.36a
MCHC (%)	32.8 ± 1.17a	33.02 ± 0.8a	33.57 ± 0.6a	32.64 ± 0.4a
Platelets (Thousands/mm^3)	319.11 ± 3.26a	297.56 ± 6.58a	318.89 ± 13.24a	310.33 ± 2.33a
WBC (Thousands/mm^3)	829.33 ± 27.64a	806 ± 19.52a	836.33 ± 8.77a	834.67 ± 3.35a
Lymphocytes (%)	90.89 ± 0.35a	90.56 ± 0.47a	87.56 ± 0.29b	90.56 ± 0.47a
Monocytes (%)	2 ± 0.17b	1.56 ± 0.24b	3 ± 0.24a	1.78 ± 0.32b
Neutrophils (%)	5.67 ± 0.37b	7.11 ± 0.35a	7.44 ± 0.18a	6.56 ± 0.34ab
Eosinophils (%)	1.44 ± 0.18b	0.78 ± 0.15c	2 ± 0a	1.11 ± 0.11bc

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

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

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

Table 5 Methyltestosterone concentration in blood serum and muscle (mean value ± SE) of *Oreochromis niloticus* from Assiut and monosex farms, ($N = 3$) for each group.

Governorates	Serum (ng/ml)	Muscle (ng/g)
Assiut	ND	ND
Alexandria	2.32 ± 0.31	1.54 ± 0.15
Kafr el-Sheikh	2.03 ± 0.16	0.93 ± 0.07
Beheira	3.47 ± 0.19	2.01 ± 0.19

ND, non detectable.
Note: The detection limit of the apparatus 0.1 ng/g or ml.

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 physicochemical 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 finding was expected due to the fact that the level of contamination in the river Nile is greater than fish farms.

Hematological parameters have been employed in assessing the condition of the fish. However, hematological parameters have been described to be reliable (Katalay and Pariak, 2004; Akinrotini et al., 2012), as they indicate the nutritional status and overall health indication of the fish (Akinrotini et al., 2012). The decrease in Ht and Hb of the fish given MT may signify condition deterioration as a result of androgen administration (Kefi et al., 2013). The same results of erythrocytes, hematocrit and hemoglobin decrease were reported in fish blood after exposure to UV radiation (Sayed et al., 2007), diazinon (Adedeji et al., 2009), cadmium (Mekkawy et al., 2010) and atrazine (Mekkawy et al., 2013).

Lower hemoglobin levels may decrease the ability of fish to enhance its activity in order to meet occasional demands according to Joshi et al. (2002). Also toxic components induced anemia and interfered with platelet production in the animals which reduce their values (Sudakov, 1992) and this appeared in the present study in the sampled fishes from the monosex farms.

Significant increase in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) and insignificant increase in mean corpuscular hemoglobin concentration (MCHC) were recorded in the blood of fishes from the monosex farms. These results are in agreement with Diethyl phthalate treated fishes (Sepperumal and Saminathan, 2013). The increase in MCV may result from the presence of a large amount of older or larger red blood cells as described by Hardig and Hoglund (1983); and could be due to an increase in immature RBC (Carvalho and Fernandes, 2006).

The major functions of WBCs are to fight infection, defend the body against foreign organisms and in immune response. In the present study WBCs increased insignificantly while differential WBC count showed significant increase in neutrophils with significant decrease in lymphocytes numbers and these results are in agreement with results of Davis et al. (2008), while monocytes and eosinophils showed significant changes between monosex farms. These changes can be used as indicator for immunity decrease after treatment with methyltestosterone in the present study and also after exposure to toxic substances (Adedeji et al., 2009).

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 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 Öner et al., 2009) after exposure to metals.

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., 2011).

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 urea concentration 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 was 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 to 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 cray fish 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.

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