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Some Hematological Studies on Induced Monosex Nile Tilapia (Oreochromis niloticus) by using 17 α Methyltestosterone

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Abstract

The present study aimed at investigates the hematological characteristics of monosex male Nile tilapia, Oreochromis niloticus, induced by feeding on 17 α methyltestosterone (17-α-MT). A total number of 2000 fry were divided into two groups based on the exposure time (15 and 30 days). Each group was subdivided into three groups based on the concentration of 17-α-MT in the feed. First (control) group fed basal diet 0 mg 17-α-MT /kg food, group 2 fed 40 mg 17-α-MT /kg food and group 3 fed 60 mg 17-α-MT /kg food. Fish in all groups were cultured under similar feeding regime and stocking density for six months. At 5^{th}, 5.5^{th} and 6^{th} month of age, the sex ratio, total red and white blood cells count, hemoglobin (Hb) concentration, HCT and the total and differential leukocytic count were determined. The erythrocytic indices were also calculated. The obtained results showed that there was a significant ($p < 0.05$) increase in male ratio in treated groups. There was a significant decrease in total RBCs count Hb concentration of treated males compared with the control males at the different experimental ages. However, treatment with 17-α- MT caused a significant increase in MCV and MCH in 40 and 60 mg treated males when compared with the control males at the different experimental ages. HCT and MCHC not significantly changed. While, treatment with 17-α-MT caused no significant variation in the total and differential WBCs count. It could be concluded that induction of all male monosex Nile tilapia by using 17-α-MT caused changes.

Keywords: Hematological parameters, Nile tilapia, Sex reversal, 17 α methyltestosterone.

1. Introduction

Induction of sex reversal by administration of 17-α methyltestosterone (17-α-MT) in the feed during the period of gonadal differentiation of the fish has become a common practice and widespread in tilapia aquaculture in many parts of the world to produce all-male tilapia stocks, which consistently grow to a larger/more uniform size than mixed sex or all-female stocks [44,35]. The total global production of tilapias and other cichlids reached 2.6 million ton in 2010 [16,13]. For 2011, [14] reported production rates for Nile tilapia above 3 million tons per year. For 2014, world tilapia production reached 4.850.000 mt **[15]**; hence the use of MT for production of all- male monosex Nile tilapia is expected to be increased in outspreading for its economical advantages.

In the recent years, more attention was given to studying the hematological characteristics of fish as an integral part of evaluating their health status, performance, productivity and physiological state [10,25,26,35]. Also, the assessment of hematological parameters might be used as a quick tool for diagnosing stress and malnutrition in a number of fish species [2,20]. However, the diet composition and variation in fish activity and feeding of sex steroids are the main causes of the changes in hematological parameters of fish [32,23]. Moreover, sex steroids are the main regulators for the erythropoiesis via the erythropoietin hormone in fish [41,31,37]. Therefore, the present study was undertaken to evaluate the impact of feeding 17-α-methyltestosterone (17-α-MT) on the hematological characteristics of the monosex male Nile tilapia (O. niloticus).

2. Materials and methods

2.1 Experimental design

A total number of 2000 yolk sac larvae were obtained from a private hatchery, Kafr El-Sheikh Governorate at the end of August, 2014 and were placed in well prepared glass aquaria at 28 ◦C for about 24h for acclimation and then flushed with potassium permanganates 5 mg/l [38], as a prophylactic measure. The yolk sac larvae were maintained in these aquaria until absorption of yolk-sac. At the start of exogenous feeding, about 1000 fry were distributed randomly into ten experimental glass aquaria representing different 6 groups as illustrated in Table (1) Then the fry transported into a ten plastic aquaria at the beginning of the 2^{nd} month of age. Each plastic aquarium was stocked with 100 fingerlings of (O. niloticus). Fish in all groups were cultured under similar feeding regime and stocking density for sex months. The basal ration obtained from (Joe Trade Company) with about 45% crude protein was mixed with the 17-α-MT hormone to prepare hormone treated diet by using the alcohol

evaporation technique according to [30]. After yolk sac absorption, the fry received the treated (40 and 60 mg 17-α-MT /kg food) and control (0 mg 17-α-MT /kg food or basal diet) diet as a paste moisten with water at a rate of 20% of the body weight (BW)/day as initial feeding till the end of the first month from the beginning of the exogenous feeding. Then the feeding rates was decreased to 12% of body weight/day till the half of the 3rd month (Shelton et al., 1978) then to 6% till the end of 3rd month. Then 4% till the end of the experiment (6 months) [44]. At the end of the experiment (at the 5th, 5.5 th and 6th month of age), the following parameters were determined.

Table (1) Grouping of fry according to exposure time and concentration of 17 α methyl testosterone in food

Group number	Exposure time(days)	Feeding
1		Control (basal diet 0 mg 17-α-MT /kg food) *
2	15	40 mg 17-α-MT /kg food**
3		60 mg 17-α-MT /kg food**
4		Control (basal diet 0 mg 17-α-MT /kg food) *
5	30	40 mg 17-α-MT /kg food**
6		60 mg 17-α-MT /kg food**

2.1 Determination of sex ratio

The sex ratios were determined by gross inspection of the gonads of about 80 fish (n=80) to confirm the efficacy of 17 α methyltestosterone in production of male monosex populations. With respect to that females were excluded from hematological and immunological analysis.

2.1 Determination of hematological parameters

Blood samples were collected at each age from a set of ten male tilapia (n=10) from each aquarium (randomly chosen). Blood samples were collected using one ml sterile insulin syringes containing EDTA 0.1% by direct puncture of the heart to obtain large blood volume as much as possible. Whole blood samples were transferred into dry and clean epindroff tubes and taken to the laboratory for determination of hematological parameters. During the blood collection, no anesthetic agent was used to avoid the increases in PCV in teleost fish [5]. Total erythrocytic (RBCs) and leukocytic (WBCs) count were determined optically by using an A_o Bright – Line Hemocytometer model with Natt and Herrick's solution as a diluting fluid according to the method described by [8,9]. Hb concentration was determined by using Drabkin's reagent according to the method described by [39,6].

Hematocrit was determined by the microhematocrit centrifugation technique [39]. differential leukocytic count, the Giemsa staining method was used [39,42]. Erythrocytic indices were calculated using the total red blood cell count (RBCs), hemoglobin concentration (Hb) and hematocrit (Hct) according to [19,9].

2.2 Statistical analysis

All collected numerical data were tested statistically by using One- way ANOVA at 5% level of significance followed by Fishers Least Significant Difference test (LSD). Duncan multiple tests at ($p < 0.05$) [11] were applied to evaluate the differences among means. The statistically homogenous means were denoted by similar alphabets. Sex ratios were compared using the chi-square test. All analyses were performed using SPSS 16.0 version for Windows.

3. Results

3.1 Sex ratio

Table (2) revealed significant increases in male ratio in all treated groups as compared with the control groups. The results showed significant differences concerning the doses and the periods; the highest male percentage (100%) was obtained in groups treated with 17 α Mt at 60 mg for 30 days followed by 40 mg MT for 30 days (97.5%). While, the lowest male percentage was observed in groups treated with 40 mg MT for 15 days (87.5%).

Results showed in Table (3,4,5) revealed that, treatment of O. niloticus fry with 17-α-MT for 30 and 15 days significantly ($p< 0.05$) decreased the RBCs, and Hb concentrations and significantly ($p< 0.05$) increased the MCV and MCH at the different experimental ages in treated groups compared with control groups. PCV and MCHC showed no significant differences among the different experimental groups at all periods of treatment.

Concerning the leukogram, treatment with 17α MT for 15 and 30 days resulted in no significant differences in the total white blood cells count (WBCs), lymphocytes, monocytes, neutrophils, eosinophils and basophils percentages among the different experimental groups at all experimental periods

Table (6,7,8) Regard to the dose and exposure time, there were no significant differences in all hematological parameters between 40 and 60 mg

treated groups and between 15 and 30 days at the different experimental periods.

4. Discussion

Concerning to sex ratio, the obtained results showed significant differences concerning the doses and the periods; the highest male percentage (100%) was obtained in groups treated with 17 α Mt at 60 mg for 30 days. These results agree with those obtained by [28,29,24,12,3,44,22]. On the other hand, lower percentages were obtained by [18,33] who induced 62.1 and 92% O. niloticus males, respectively at a dosage of 60 mg/kg food for 28 days.

Regarding to hematological parameters, the obtained results revealed that, treatment of O. niloticus fry with 17-α-MT significantly ($p< 0.05$) decreased the red blood cells counts (RBCs), and Hb concentrations and significantly ($p< 0.05$) increased the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) at the different experimental ages in treated groups compared with control groups. These results are nearly similar with the results of [21,35] who found that RBCs number and hemoglobin content decreased significantly while hematocrit percentage (%) decreased insignificantly in treated Oreochromis fishes as compared with normal males. Moreover they recorded that the mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were significantly increased in monosex Oreochromis fishes as compared with normal males.

On the contrary, [43] found that there was a statistically significant decrease in the haematocrit of tench (Tinca tinca) males treated with 17-α-MT compared with the control (untreated males). The difference in results may be attributed to different fish species.

The lower red blood cells count (RBCs) and hemoglobin content (Hb) of the MT treated monosex tilapia compared to the normal male may be attributed to the lower plasma testosterone level in treated male compared with normal male [1,44] because the exogenous testosterone treatment directly suppresses the release of gonadotropin hormone from the anterior pituitary gland, so lowers the production of natural testosterone hormone from the gonads of hormone treated males (negative feedback mechanism) [27,36,1]. Moreover, [44] observed the lower plasma testosterone level in induced males was attributed to the degeneration of some germ cells in seminiferous tubules of testis of treated male compared with the normal seminiferous tubules of testis of the normal male.

This lower plasma testosterone level in treated (induced) males decreased erythropoiesis because androgens stimulate the rate of erythropoiesis either directly, i.e., acting on bone marrow (in mammals) or indirectly by way of the kidney in various vertebrate species including fish [37] via increase the proportion of erythroblasts (functional Hematopoietic Stem Cells) in haematopoietic organs (kidney) [17] [in fish] and [7] [in mammals].

The increase in MCV in treated males compared with the normal males may be due to an increase in immature RBC after methyltestosterone administration [35]. Also, may due to significant decrease in total RBCs and non-significant decrease in PCV. Also, the increase in MCH in treated males compared with the normal males, may could be due to significant decrease in total RBCs and Hb concentration.

Concerning to the leukocytes, treatment with 17α MT for 15 and 30 days caused non significant increases in the total white blood cells count (WBCs) and the percentage of lymphocytes in treated males as compared with the normal males at all ages.

Table (2) Effect of 17 α- methyltestosterone treatment on sex ratio of O. niloticus fry at 6 months age:

Period	Treatment	Fish No.	Male		Female	
			No.	%	No.	%
15 days	Control	80	48	60	32	40
	MT 40 mg	80	69	87.5	11	12.5
	MT 60 mg	80	72	90	8	10
30 days	Control	80	56	70	24	30
	MT 40 mg	80	78	97.5	2	2.5
	MT 60 mg	80	80	100	0	0

Male percentages differed among treatments by chi square.

Table (3) Effect of 17 α methyltestosterone treatment on hematological parameters of O. niloticus at 5 months age (mean ± SE)

Period	Paramete Treatment	RBCs (10^6/mm^3)	PCV (HCT) (%)	Hb (g/dl)	MCH (pg)	MCHC (g/dl)	MCV (fl)
15 days	Control	2.5± 0.12^a	23.54±0.33^a	7.56±0.26^a	31.59±0.71^b	30.24±1.72^a	94.16±3.50^b
	40mg	1.8±0.10^b	21.79±0.57^a	6.29±0.21^b	35.04±1.59^a	28.87±0.67^a	121.09±3.31^a
	60mg	1.7±0.10^b	23.00±0.77^a	6.40±0.17^b	37.57±0.99^a	27.83±1.29^a	135.39±2.11^a
30 days	Control	2.5± 0.12^a	23.66±0.33^a	7.76±0.26^a	30.94±0.71^b	30.59±1.72^a	94.65±3.50^b
	40mg	1.6±0.12^b	20.66±0.66^a	5.93±0.22^b	37.06±0.75^a	28.70±0.54^a	129.12±3.20^a
	60mg	1.8±0.12^b	22.71±0.91^a	6.33±0.37^b	35.17±1.47^a	27.97±1.06^a	126.17±2.26^a

Means with different letters in the same column are significantly different in the same period of the treatment ($p < 0.05$).

Table (4) Effect of 17 α methyltestosterone treatment on hematological parameters of O. niloticus at 5.5 months age (mean ± SE)

Period	Parameter Treatment	RBCs (10^6/mm^3)	PCV (HCT) (%)	Hb (g/dl)	MCH (pg)	MCHC (g/dl)	MCV (fl)
15 days	Control	2.3±0.21^a	24.51±0.37^a	7.91±0.22^a	34.39±0.51^b	30.26±2.52^a	105.95±3.09^b
	40mg	1.7±0.10^b	23.75±0.75^a	6.35±0.32^b	37.27±0.93^a	26.74±0.80^a	139.07±1.83^a
	60mg	1.7±0.12^b	24.33±0.33^a	6.50±0.23^b	38.13±0.64^a	26.72±0.86^a	143.12±2.14^a
30 days	Control	2.3±0.21^a	24.60±0.37^a	7.91±0.22^a	34.39±0.51^b	30.16±2.52^a	106.82±3.09^b
	40mg	1.7±0.12^b	24.33±0.33^a	6.50±0.23^b	38.13±0.64^a	26.72±0.86^a	143.12±2.14^a
	60mg	1.6±0.10^b	23.40±0.25^a	5.93±0.54^b	37.06±0.71^a	25.54±2.35^a	145.15±4.53^a

Means with different letters in the same column are significantly different in the same period of the treatment ($p < 0.05$).

Table (5) Effect of 17 α methyltestosterone treatment on hematological parameters of O. niloticus at 6 months age (mean ± SE)

Period	Parameter Treatment	RBCs (10^6/mm^3)	PCV (HCT) (%)	Hb (g/dl)	MCH (pg)	MCHC (g/dl)	MCV (fl)
15 days	Control	2.4±0.10^a	23.97±0.78^a	7.26±0.13^a	30.25±0.89^b	29.20±1.91^a	99.87±2.13^b
	40mg	1.6±0.10^b	22.33±0.69^a	5.73±0.51^b	35.81±0.72^a	25.66±1.99^a	139.56±1.97^a
	60mg	1.6±0.10^b	22.07±0.84^a	5.87±0.21^b	36.48±0.99^a	26.59±0.74^a	137.95±1.73^a
30 days	Control	2.4±0.10^a	23.97±0.78^a	7.26±0.13^a	30.25±0.89^b	29.20±1.91^a	99.87±2.13^b
	40mg	1.7±0.12^b	23.33±0.33^a	5.86±0.23^b	34.57±0.91^a	25.12±1.89^a	137.25±1.51^a
	60mg	1.7±0.10^b	22.80±0.96^a	6.16±0.29^b	36.23±0.55^a	27.02±0.52^a	135.22±2.47^a

Means with different letters in the same column are significantly different in the same period of the treatment ($p < 0.05$).

Table (6) Effect of 17 α methyltestosterone treatment on total and differential leukocytic count of O. niloticus at 6 months age (mean ± SE)

Period	Parameter Treatment	WBCs (10^3/mm^3)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
15 days	Control	19.10±0.87[a]	55.33±0.67[a]	4.33±0.33[a]	36.33±0.33[a]	4.00±0.57[a]	0.17±0.10[a]
	40mg	21.97±0.86[a]	56.67±0.67[a]	4.00±0.28[a]	36.16±0.60[a]	3.50±0.57[a]	0.17±0.19[a]
	60mg	20.58±0.85[a]	56.83±0.70[a]	3.70±0.26[a]	36.17±0.47[a]	3.33±0.33[a]	0.17±0.15[a]
30 days	Control	19.10±0.87[a]	55.33±0.67[a]	4.33±0.33[a]	36.33±0.33[a]	4.00±0.57[a]	0.17±0.10[a]
	40mg	21.73±0.77[a]	57.33±0.61[a]	3.75±0.38[a]	36.00±0.51[a]	3.37±0.49[a]	0.17±0.13[a]
	60mg	21.37±0.84[a]	56.33±0.50[a]	4.25±0.28[a]	36.00±0.36[a]	3.37±0.56[a]	0.25±0.12[a]

Means with different letters in the same column are significantly different in the same period of the treatment ($p < 0.05$).

Table (7) Effect of 17 α methyltestosterone treatment on total and differential leukocytic count of O. niloticus at 5.5 months age (mean ± SE)

Period	Parameter Treatment	WBCs (10^3/mm^3)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
15 days	Control	19.67±0.73[a]	54.56±0.35[a]	2.90±0.21[a]	39.6±0.36[a]	2.93±0.26[a]	0.00±0.00[a]
	40mg	21.80±0.67[a]	55.37±0.33[a]	2.73±0.33[a]	39.27±0.12[a]	2.63±0.10[a]	0.00±0.00[a]
	60mg	21.90±0.95[a]	55.13±0.40[a]	2.97±0.29[a]	39.35±0.28[a]	2.55±0.10[a]	0.00±0.00[a]
30 days	Control	19.67±0.73[a]	54.56±0.35[a]	2.90±0.21[a]	39.6±0.36[a]	2.93±0.26[a]	0.00±0.00[a]
	40mg	20.73±0.88[a]	55.47±0.30[a]	3.33±0.15[a]	38.87±0.32[a]	2.46±0.10[a]	0.00±0.00[a]
	60mg	22.65±0.95[a]	55.40±0.37[a]	2.72±0.17[a]	39.08±0.24[a]	2.80±0.11[a]	0.00±0.00[a]

Means with different letters in the same column are significantly different in the same period of the treatment ($p < 0.05$).

Table (8) Effect of 17 α methyltestosterone treatment on total and differential leukocyte count of O. niloticus at 6 months age (mean ± SE)

Period	Parameter Treatment	WBCs (10^3/mm^3)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
15 days	Control	20.63±0.80[a]	63.83±0.54[a]	2.63±0.27[a]	30.37±0.19[a]	2.23±0.23[a]	0.00±0.00[a]
	40mg	21.27±0.77[a]	64.47±0.47[a]	2.60±0.20[a]	30.30±0.25[a]	2.63±0.31[a]	0.00±0.00[a]
	60mg	23.20±0.70[a]	65.60±0.45[a]	2.40±0.36[a]	29.83±0.20[a]	2.17±0.20[a]	0.00±0.00[a]
30 days	Control	20.63±0.80[a]	63.83±0.54[a]	2.63±0.27[a]	30.37±0.19[a]	2.23±0.23[a]	0.00±0.00[a]
	40mg	21.80±0.83[a]	64.57±0.61[a]	2.83±0.28[a]	30.03±0.18[a]	2.57±0.30[a]	0.00±0.00[a]
	60mg	22.93±0.80[a]	65.63±0.35[a]	2.43±0.32[a]	29.83±0.12[a]	2.17±0.23[a]	0.00±0.00[a]

Means with different letters in the same column are significantly different in the same period of the treatment ($p < 0.05$).

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