



Detection of methyltestosterone and trenbolone acetate hormones residues in Nile tilapia (*oreochromisniloticus*)

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ABSTRACT

The study was planned out to estimate the Methyltestosterone (MT) and Trenbolone acetate (TB) residues in 100 samples of Nile Tilapia. The samples were randomly collected from different localities in Cairo and Giza markets and were analyzed using Enzyme-linked immune sorbent Assay (ELISA) method. The mean values of MT&TB in the fish muscle were 2.057 ± 0.200 ppb and 0.227 ± 0.007 ppb, respectively. The obtained results showed no evidence for the illegal use of hormones, but these results do not exclude the possibility of misuse of these potentially harmful hormones. There is, therefore, need to routinely monitor these hormones as a food quality and health control measure.

Keywords: Methyltestosterone, Trenbolone acetate, Tilapia masculinization

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1. INTRODUCTION

Tilapia species constitute a major and important item in the Egyptian fish farming. Tilapias are among the important fishes for aquaculture because of many positive characteristics and have been cultured in more than 100 countries (Altun *et al.*, 2006). Dietary treatment with 17α -Methyltestosterone (MT) and Trenbolone acetate (TPA) as an effective mean of producing all-male tilapia populations; however, the treatment requires a minimum of several weeks exposure. Administration of steroids to the water containing sexually undifferentiated fish has also been effective in altering sex ratios and may provide aquaculturists with a safe and cost-effective alternative to treating fry with food that contains MT or TPA (Piferrer and Donaldson, 1989). The most serious potential hazards arising from using of anabolic steroids are the tissue residues of these substances and their metabolites. The effect of these residues is greater on human as it can cause early puberty for girls and boys, liver tumors, carcinoma and increase embryo mortality (Ibrahim, 2009). Thus,

this work was done to determine the level of hormonal residues in tissue of tilapia.

2. MATERIALS AND METHODS

2.1. Collection of samples:

A total of 100 samples of Nile tilapia (about 165-207g in weight) were collected from different localities in Cairo and Giza markets. The raw samples were collected in polyethylene bags and rapidly transferred to laboratory for detection of their hormonal residues according to manual kits ELISA R-BiopharmAG, Darmstadt, Germany.

2.2. Preparation of samples:

- skin and scales were removed from the muscle of fish
- Ten grams of the ground muscle was homogenized with 10mL of 67mM PBS buffer by mixer for 5min.
- Two grams of homogenized sample were mixed with 5mL of tertiary butyl methyl ether (TBME) in a centrifugal

screw cap vial and shaken vigorously by shaker for 30-60 min.

- d. The contents were centrifuged at 3000rpm for 10min.
- e. The supernatant was kept and the extraction with TBME was repeated.
- f. The supernatants were combined and evaporated by N₂ evaporator then the dried extract was dissolved in 1mL of 80% methanol.
- g. The methanolic solution was diluted with 2mL of 20mM PBS-buffer and applied to a RIDA C18 column (solid phase extraction column with C18 end-capped sorbent of an average particle size of 50µm) for filtration of the samples then the filtrate was used in ELISA kit.

2.3. Test procedures:

The test procedures were done according to the chart enclosed in the kits of RIDA^R and RIDS screen. R is register trademarks of R-Biopharm AG. Manufacture: R-Biopharm

AG, Darmstadt, Germany. R-Biopharm AG is ISO certified.

3. RESULTS

In order to obtain the MT and TB concentration in (ng/1) ppt actually contained in the samples. The concentration was read from the calibration standard curve for MT (Fig.1) and TB (Fig. 2). The result was calculated by this equation: % absorbance = (OD sample/ OD standard) x 100, results were calculated as (ng/1). 3.1. mean, minimum and maximum values of Methyltestosterone in fish muscle samples were 2.057±0.200 ,1.4 and 3.1µg/ kg, respectively.

3.2. Mean, minimum and maximum values of Trenbolone acetate in fish muscle samples were 0.227±0.007, 0.1 and 0.4 µg/ kg, respectively.

Table (1): the mean, minimum, maximum values of Methyltestosterone and Trenbolone acetate hormones residues in fish samples (ppb).

Hormones	Min	Max	Mean ±SE
Methyltestosterone	1.4	3.1	2.057±0.200
Trenbolone acetate	0.1	0.4	0.227±0.007

Fig. 1: standard curve of methyltestosterone.

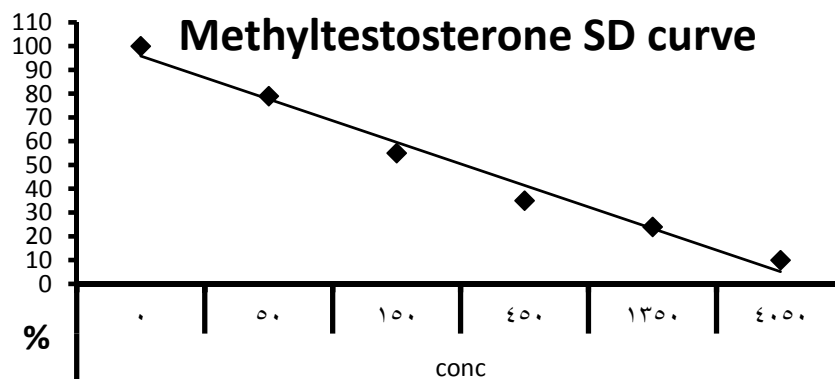
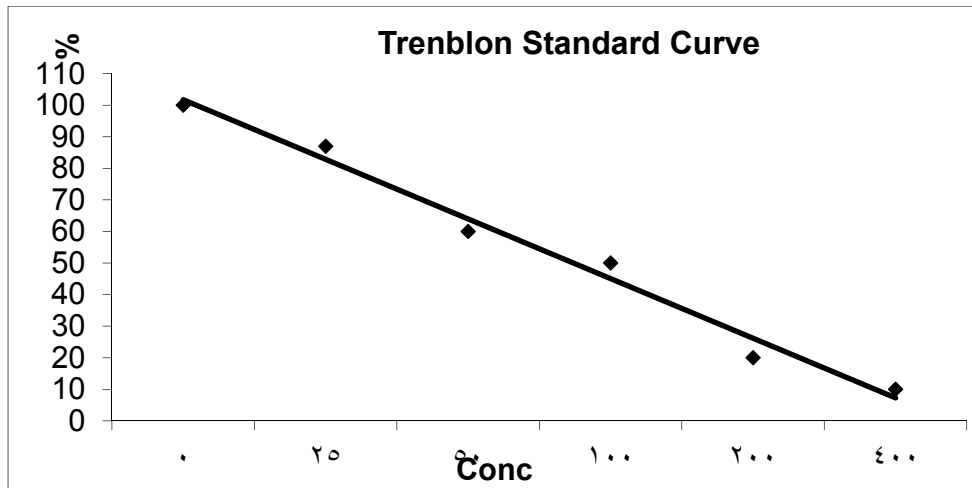


Fig. 2: standard curve of Trenbolone acetate



4. DISCUSSION

In the present study, the ELISA method was used to achieve the unambiguous identification of methyltestosterone and trenbolone acetate in fish muscle. The method of choice should be accurate, sensitive, specific and precise, so that both false negative and false positive results should be obtained. For this purpose routine methods are needed. Recently, Enzyme-Linked ImmunoSorbent Assays (ELISA) have been established as screening methods (Dursch and Meyer, 1992). Raw fish and fish products, which play an important role in human nutrition, should be safe and should not contain any factors or substances harmful for human health. However, the anabolic agent used for various purposes in animal husbandry tend to leave residues and this causes some problems in consumer health. (Hoffman, 1996) and (Nazli et al; 2005). Moreover, after administration high dose of methyltestosterone in humans causes negative mood as irritability, mood swings, violent feelings, and hostility, then cognitive impairment as distractibility, forgetfulness, and confusion (Su et al; 1993). Because of negative effects, the European Economic Community (EEC) prohibited the use of anabolic compounds as growth accelerators in food animals and fishes (European Commission Decision, 2002). The detection of hormonal residues in some local fish (Tilapia and Carp) may

be attributed to widely use of synthetic androgen as methyltestosterone in fish production in Egypt for its anabolic and androgenic action in fish. This agrees with the results stated by Mansour and Satyanareyana (1989) ; Hegazy (2007). According to the obtained results in table (1) the mean value of MT as 2.057 ± 0.200 ppb with minimum and maximum values of 1.4 and 3.1 ppb while lower findings were obtained by Hegazy (2007) who estimated testosterone hormone residues in flesh of some local fish farms which used methyltestosterone and the study revealed that the hormonal residues in the treated groups decreased gradually till reached 0.753 ppb in the 12th week, while in the control groups 0.635 ppb, respectively. While, higher finding was obtained by El-Asaly (2004) who determined the testosterone residues in muscle samples of fish aged 120 and 180 days that previously feeding with 60mg/kg feed for 30 days were 6.865 ppb and 6.946 ppb for treated group respectively. While, they were 5.847 ppb and 6.610 ppb for control group at 120 and 180 days, respectively. Pandian and Kirankumar (2003) stated that the estimated residue steroids of less than 5 ng/g fish is too low to cause any concern or hazards to human. Moreover, Rizkalla et al. (2004) concluded that no potential hazards exists for people who eat fish that have been fed 17 α methyl testosterone as fries; for this purpose, are generally given feed

containing 30-120 mg/kg diet of synthetic androgen for 28 hours.

Moreover, table (1) shows that the mean value of TB was 0.227 ± 0.007 ppb with minimum and maximum values of 0.1 and 0.4 ppb. Lower results were reported by Mahgoub et al. (2006) who found that the mean values of trenbolone in Somali sheep 0.034 ppb and in Omani goat 0.004 ppb. While higher results were obtained by Jannat et al. (2007) who determined the average value of trenbolone in cattle meat was 3.76 ± 5.26 ppb and this confirmed with the permitted limit values for trenbolone which are 2 ppb in muscle (Codex Alimentarius, 1997; European Commission, 1999). Acceptable daily intake (ADI) maxima of trenbolone were established by JECFA at $0.02 \mu\text{g}/\text{kg}$ body weight (BW). Therefore, it seems that the present status of this anabolic hormones in market is not at risk but on the other hand, these results do not exclude the possibility of misuse of this anabolic hormone in future and significantly increase exposure of humans, particularly children, to trenbolone which may adversely affect health. There is, therefore, need to routinely monitor this chemical as a food quality control measure.

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