

Detection of Testosterone hormone and 17 α -Methyltestosterone, residues in some fish products at retailed markets.

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Abstract

This study was done to estimate the testosterone and 17 α -methyl- testosterone residues in 45 samples of fish burger, fish kofta and fish fingers. Samples were collected randomly from different localities at Cairo retailed markets and were analyzed using Emzyme – Linked Immuno Sorbent Assay (ELISA) method. The mean values of testosterone residues in fish burger, fish kofta and fish fingers were $0.41 \pm 0.21 \mu\text{g/kg}$, $0.11 \pm 0.04 \mu\text{g/kg}$ and $0.62 \pm 0.24 \mu\text{g/kg}$ respectively. While, the mean value of 17 α -methyltestosterone were $0.81 \pm 0.03 \mu\text{g/kg}$, $0.94 \pm 0.19 \mu\text{g/kg}$ and $1.01 \pm 0.16 \mu\text{g/kg}$, respectively. The obtained values were found to be within the acceptable daily intake. So, it seems that the present status of this hormones in marketed fish products does not cause any public health hazard but required a routinely monitor of such chemicals as a food quality control measurement.

Introduction

The shortage of animal protein sources is considered the main reason behind the different trials for raising the production rates of animal protein with low cost and minimum delay. One of such trials is the use of growth promoters for increasing meat production (**Galli *et al.*, 1989**).

Growth promoters are substances, which are added to feed components to improve the daily body gain. Therefore, many illegal methods were applied for abnormal increase of animal production rates. The illegal trials may include the use of some chemicals or hormonal substance as growth promoters.

The synthetic steroid 17 α -methyltestosterone is a male specific hormone commonly used to induce sex reversal in fish. It is a synthetically produced anabolic and androgenic steroid hormone, otherwise it promotes both muscle growth and development of male sexual characters.

Administration of steroids to the water containing sexually undifferentiated fish has also been effective in altering sex ratios and may provide aqua culturists with a safe and cost-effective to treating fry with food that contains 17 α -methyltestosterone (**Piferrer and Donaldson, 1989**). The most serious potential hazards arising from using of anabolic steroids are the tissue residues of these substance and their metabolites. The effect of these residues is greater on human as it can cause puberty for girls and boys, liver tumors, carcinoma and increase embryo mortality (**Ibrahim, 2009**).

In animals, testosterone or testosterone propionate, alone or in combination with other hormonally active substances, is used primarily to improve the rate of weight gain and feed efficiency. This effect is most likely a consequence of the anabolic action of androgens. On the other hand, **Grace (1986)** stated that the withdrawal time of hormone is 60-90 days and the hormonal residues in tissue depends on the dosage, withdrawal time and site of subcutaneous implants.

Sex steroid hormones are capable of increasing the risk of cancer in certain target organs (mammary gland, cervix, uterus and prostate gland) and therefore classified as carcinogens (**Hoffmann and Evers, 1986 and FAO/WHO, 1988**). Testosterone can cause cervical-uterine tumors in female rats and prostate cancer in males (**Henderson and Feigelson, 2000**).

Therefore, this study was carried out to estimate testosterone and 17 α -methyltestosterone hormonal residual levels in some fish products (fish burger, fish kofta and fish finger) using Enzyme-linked immune sorbent assay (ELISA) technique.

Material and Methods

Collection of samples

A total of 45 samples of marketed fish burger, fish kofta and fish finger (15 from each) were collected from different localities at Cairo markets. The samples were collected in polyethylene bags, and then identified. The collected samples were rapidly transferred to laboratory in an ice box for determination of hormonal residues according to, manual kits ELISA R-Biopharm AG, Darmstadt, Germany.

Preparation of samples

- 1- Samples were grinded and to (10 g) of each homogenized ground sample, 10 ml of 67 mM phosphate buffer pH 7.2 was added ,then shaken strongly for 5 min.
- 2- Mix 2 g of homogenized with 5 ml of tert- butyl-methyl ether in screw cap vial and shake vigorously for 30-60 min.
- 3- Centrifuged for 10 min./3000 g/10-15°C.
- 4- The supernatant was transferred to another centrifugal screw cap vial.
- 5- The extraction was repeated with 5 ml tert-butyl-methyl ether.
- 6- Evaporate the combined ether layers to dryness and dissolve with 1ml of methanol (80%).
- 7- dilute the methanolic solution with 2ml of 20mMPBS buffer and further clean with RIDA C18 column flow rate: 1 drop /sec.
 - Rinse column with 3ml methanol.
 - Equilibrate column with 2 ml of 20 mMPBS buffer.
 - Apply sample (3ml).
 - Rinse column with 2ml of methanol (40%).

- Press out residues of rinse- solution.
- Dry column for 3 min by pressing air or N₂ through it.
- Elute sample slowly (15drop/min) with 1 ml methanol (80%).
- Evaporate an aliquot of the elute to dryness and redissolve in 1ml of 10% methanol.

8- Use 20 µl per well of the resulting solution in the assay.

Test principle

The basis of the test is the antigen-antibody reaction. The micro titer wells are coated with capture antibodies directed against anti-testosterone antibodies. Standards or sample solution, testosterone enzyme conjugate and anti-testosterone antibodies are added. Free and enzyme conjugated testosterone compete for the antibody binding sites (competitive enzyme immunoassay). At the same time, the anti-testosterone antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in a washing step. Enzyme substrate and chromogen are added to the wells and incubated. Bound enzyme conjugate converts the colorless chromogen into a blue product, the addition of the stop solution leads to a color change from blue to yellow. The measurement is made photometrically at 450nm. The absorption is inversely proportional to the testosterone concentration in the sample.

Test procedure:

The test procedure were done according to the chart enclosed in the kits of RID A^R and RIDS screen R is register trademarks of R-Biophram AG .Manufacture: R-Biophram AG, Darmstadt, Germany R- Biopharm AG is ISO certified.

Results and Discussion

According to the results in table (1) the mean value of testosterone residue in fish burger samples was $0.41 \pm 0.21 \mu\text{g/kg}$ with minimum and maximum values 0.10 $\mu\text{g/kg}$ and 1.32 $\mu\text{g/kg}$, respectively. While, the mean value of testosterone residue in fish kofta samples was $0.11 \pm 0.04 \mu\text{g/kg}$ with a minimum and maximum values of 0.01 $\mu\text{g/kg}$ and 0.20 $\mu\text{g/kg}$, respectively. Moreover the mean value of testosterone residue in fish finger samples was $0.62 \pm 0.24 \mu\text{g/kg}$ with a minimum and maximum values of 0.13 $\mu\text{g/kg}$ and 1.28 $\mu\text{g/kg}$, respectively. The obtained results in Table (2).showed that the mean value of 17 α -methyl testosterone (17 α MT) residue in fish burger samples was $0.81 \pm 0.03 \mu\text{g/kg}$ with minimum and maximum values were 0.7 $\mu\text{g/kg}$ and 0.9 $\mu\text{g/kg}$, respectively. While, the mean value of 17 α MT residue in fish kofta samples was $0.94 \pm 0.19 \mu\text{g/kg}$ with a minimum and maximum values of 0.7 $\mu\text{g/kg}$ and 1.7 $\mu\text{g/kg}$, respectively. Moreover, the mean value of 17 α MT residue in fish finger samples was $1.01 \pm 0.16 \mu\text{g/kg}$ with minimum and maximum value of 0.7 $\mu\text{g/kg}$ and 1.6 $\mu\text{g/kg}$, respectively.

The ELISA method is applicable in official control laboratories as a rapid screening method for determination of testosterone and methyltestosterone in fish **Risto et al. (2013)**. While, **Pnadian and KiranKumar (2003)** stated that the estimated residue steroids of less than 5ppb is too low to cause any concern or hazard to human. Moreover, **Rizkalla et al.(2004)** concluded that no potential hazards exists for people who eat fish that have feed 17α -methyltestosterone as fries.

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 human 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**)

According to the results recorded in table (1) and table (2), there were 100% accepted samples according to the permissible limit stipulated by (**Codex Alimentarius, 2007**) which determined by (2 ppb in muscle).

Conclusion and recommendation

From the present data it could be concluded that the examined samples does not constitute any risk for human. This may be attributed to legislation and laws, which prohibit the use of anabolic agents as growth promoters registered to their harmful effect on health of consumers. To avoid the presence of hormonal residues in fish products. It was recommended that :-

1. The illegal use of hormones as growth promoters should be banned for food producing fish.
2. Care should be taken when steriois are employed.
3. When hormone used to induce sex reversal in fish a suitable withdrawal time should be elapsed.
4. Laboratory quality assurance program monitoring of analysis and validation of analytical methods.
5. Inspection should be regularly performed to the form fish before marketing to ensure that farms follow the rules of pre-marketing withdrawal period.
6. Periodical examination of fish and fish products in local market for presence of hormonal residues.

Table (1) : Mean values of testosterone hormones residues in Fish Burger, Fish, Kofta and fish Finger (ppb). (n=15 each sample type)

Type of Samples	Minimum	Maximum	Mean \pm SE
Burger	0.10	1.32	0.41 \pm 0.21
Kofta	0.01	0.2	0.11 \pm 0.04
Finger	0.13	1.28	0.62 \pm 0.24

Data are expressed as mean \pm SE

Maximum permissible limit according to codex 2007 is 2 ppb

Table (2) : Mean values of 17 α methyl testosterone hormones residues in Fish Burger, Fish Kofta and fish Finger (ppb). (n=15 each sample type)

Type of Samples	Minimum	Maximum	Mean \pm SE
Burger	0.7	0.9	0.81 \pm 0.03
Kofta	0.7	1.7	0.94 \pm 0.19
Finger	0.7	1.6	1.01 \pm 0.16

Data are expressed as mean \pm SE

Maximum permissible limit according to codex 2007 is 2 ppb

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