BS. VET. MED. J. JANUARY 2009 VOL. 19, NO.1, P. 23-26

Beni-Suef Veterinary Medical Journal

Detection of testosterone residues in farm fish tissue

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A total of one hundred samples of marketed fish farm; 25each of tilapia (Oreochromis niloticus), Mugil cephalus, Carp (Silver) and fry; were collected from different localities at Al-Behera and Kafr El-Shakh Provinces. The samples were examined for detection of testosterone hormone by Thin Layer chromatography (TLC) as well as it was quantitatively assayed by Radio-Immuno-Assay (RIA). The obtained result revealed that the testosterone residues were detected in flesh of farm fish of Tilapia (Oreochromis nilotocus) and Carp (Silver), each constituting 24% and 4 %; respectively but it could be failed to detect in each of Mugil cephalus and fry. The hormonal residues of testosterone was ranged from 3.25 to 34.9 ng/g with mean value 4.22±1.1 ng/g in positive samples of Tilapia, while only one sample of Carp showed 22.0 ng/g, Mugil cephalus and Fry showed no detectable level of hormonal residues. The public health significance of detectable levels was discussed as well as the recommendations to avoid health hazards from such fish were mentioned.

The consumption of fish flesh containing methyl testosterone residues may be potentially hazardous to consumer's health. The dietary administration of methyl testosterone to improve body weight, it increased residual testosterone and decrease lipids the required for human consumption, which might be hazardous for human (Schardein, 1980).

Protocols to produce 90-100% sex- reserved fish have been described for European Sea bass. George and Pandian (1998) achieved maximum production of 59% males of black molly, even at the highest dose of 400 mg/kg diet- containing natural (androstenedione) or synthetic (methyl testosterone) steroid. Hormonal control of sex of fishes is accomplished by administering a specific hormone to fry before sexual differentiation occurs. Thus, a sex changed individual has the genotype of one sex, but has the phenotypic characteristic of other (Nakamura and Takahashi, 1973; Rizakalla *et al.,* 2004).

There are many androgens that can used for musclinization of tilapias which lead to maximum growth. Methyl testosterone is a synthetic steroid used in aqua culture as a growth promoters and for production of males of mono sex type which commercially important because males generally grow faster and larger than females (El-nemr et al., 1999; Rizkalla et al., 2004 and Hegazy, 2007). The incorporation, distribution, and depletion of total 17α methyl testosterone radioactivity are consistent with several authors (El- nemr et al. 1999).

Literature regarding are steroid residues in tissues of fish treated with androgenic steroids have been reviewed by (Donaldson *et al.,* 1979; Higgs *et al.,* 1982; Keshk, 2004; Rizkall *et al.,* 2004; Hegazy 2007). Based on the previous literature, it is apparent that 17α methyl testosterone has an important economic potential for use in fish culture. However, this synthetic steroid used on large scale in many fish forms in Egypt.

Therefore, the present study was carried out to detect the level of hormonal residues in marketed fish farms which collected from different localities at Al-Behera and Kafr El-sheikh Provinces; as well as the recommendations to avoid public health hazards were discussed

Material and Methods

Collection of samples. A total of one hundred samples of marketed fish farms; 25 each of tilpia (*Oreochromis niloticus*), *Mugil cephalus*, Carp (Silver) and fry; were collected from different localities at Al-Behera and Kafr El-sheikh Provinces. The samples were collected in polyethelene bags; then identified. The collected samples were rapidly transferred to laboratory in ice box for detection of hormonal residues.

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Preparation of samples. Five grams of muscle tissues from each fish sample was separately prepared, extracted, extracted and cleaned up according to methods described by (Verdeke, 1979).

Hormonal analysis. Testosterone hormone in extract was detected by Thin Layer Chromotography (TLC) according to technique recommended by Coffin and Pllon, (1973).

Testosterone hormone was quantitatively assayed by Radio-Inmmuno-Assay (RIA) technique by using CIS Biointeruational Kit, France according to (Jaffe and Behrman, 1974).

The obtained results were statistically analyzed according to (Smedecor,1969).

Results and Discussion

From the present data in table (1), it is evident that the testosterone residues were detected in flesh of farm fish of Tilapia (*Oreochromis niloticus*) and Carp (Silver), each constituting 24% and 4%; respectively but it could be failed to detect in each of *Mugil cephalus* and Fry (0%).

The hormonal residues of testosterone was detected in Tilapia (*Oreochromis niloticus*) with range of 3.25 to 34.9 ng/g. with mean value 4.22 ± 1.1 ng/gram in positive samples as well as only one samples of Carp (Silver) showed residual level, constituting 22.0 ng/gram. However, each *Mugil cephalus* ad Fry showed no detectable level of hormonal residues (Table 2).

Concerning the frequency distribution of testosterone residues in flesh of farm fish (ng/gram) based on health hazards; 8% of examined Tilapia (*Orechromis nitoticus*) showed level exceeded 5 ng/gram flesh as well as only one sample (4%) of Carp (Silver) exceeded such level (Table 3).

The detection of hormonal residues in some local fish (Tilapia and Carp) may be attributed to widely use of synthetic androgen as methyl testosterone in fish production in Egypt for its anabolic and androgenic action in fish. This agrees with that stated by (Mansour and Satyanareyana,1989; Hegazy 2007).

In this respect, Goudie *et al.* (1986, a&b) stated that Tilapia had shown a detectable amount of radioactive methyl testosterone in the fish flesh within one hour initial feeding and reached the highest level six hours and a rapid depletion of 90% from muscles were eliminated within 24 hours as well as less than 1% representing 5 ng/g tissues could be detected 21 days after steroid withdrawal.

On country, Rothbard *et al.* (1996) found a detectable amount of steroid only on the first day after the termination of feeding with 17 α ethyl testosterone in the muscle samples of tilapia fishes. However, 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 fry are generally given feed containing 30-120 mg/kg diet of synthetic androgen for 28 hours.

On the other hand, Johnstone *et al.* (1983) stated that, under normal circumstances, it would be unreasonable to suggest that hazardous level of 17 α methyl testosterone might be ingested by the consumption of adult fish treated as juveniles with this steroid.

The presence of testosterone residues in Tilapia muscles may be attributed to long half-life of methyl testosterone as compared to that of testosterone due to the strong binding of methyl testosterone to plasma protein, reaching to maximum levels during 6 to 10 hours after starting of feeding and then declined and reached a radir by 22 hours after cessation of feeding. This in-accordance with that mentioned with (Westphal and Ashley,1962; Rinchard *et al.,* 1991).

In this regard, Green and Teichert-Coddington, (2000) concluded that the use of methyl testosterone feed will not cause significant adverse effects. Standardized calculations were made to estimate methyl testosterone concentration in effluents based on stocking rate and water exchange. Guide lines are provided for management of farms to ensure that effluent methyl testosterone concentration remain <1 µg/liter.

The levels of residues of the synthetic anabolic steroids found in the present and previous works should be rationally considered, taking into account the amount of endogenous steroids naturally occurring in fish tissues.

From the present data, it could be concluded that the hormonal residues (testosterone) was detected in Tilapia (*Oreochramis nitoticus*) with incidence 24% with mean 4.22 ± 1.1 ng/g flesh that did not exceed the level which cause health hazards to consumers.

Table (1): Incidence of testosterone residues in flesh of farm fish.

Type of fish	Number of examined samples	Number of positive samples	%
Tilapia (Oreochromis niloticus)	25	6	24
Mugil cephalus	25	0	0
Carp (Silver)	25	1	4
Fry	25	0	0

Table (2): Statistical analysis of testosterone residues in flesh of farm fish (ng/gram).

Type of fish	Minimum	Maximum	Mean±SE
Tilapia (Oreochromis niloticus)	3.25	34.9	4.22±1.1
Mugil cephalus	ND	ND	0±0
Carp (Silver)	ND	22.0	-
Fry	ND	ND	0±0

ND: Non detectable

Table (3): Frequency distribution of testosterone residues in flesh of farm fish (ng/gram).

Frequency	Tilapia (*Orechromis niloticus*)		Mugil cephalus		Carp (Silver)		Fry	
	No.	%	No.	%	No.	%	No.	%
ND	19	76	25	100	24	96	25	100
<5	3	12	25	100	24	96	25	100
>5	2	8	0	0	1	4	0	0
>10	1	4	0	0	1	4	0	0

ND: Non detectable

The safety of this technology (dietary administration of anabolic steroids) is of some concern since chronic consumption of the steroid 17 α methyl testosterone, the drug most frequently used to produce all- male fish populations, may produce adverse human effects including liver dysfunction and hepatic adencarcinoma (Muard and Haynes, 1985).

Sex reversal is assumed to be most effective if hormone treatment coincides with the period of gonadal differentiation. Moreover, a very long interval between the treatment and harvest to produce male fish with minimum level of testosterone residues in fish flesh to avoid health hazards was recommended.

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الكشف عن بقايا التيســـتســـتيرون فى أنسجة أسماك المزارع

أدى استخدام هرمون التيستستيرون فى مزارع الاسماك لتوحيد الجنس بغرض التسمين للعمل على الكشف عن بقايا هذا الهرمون فى اسماك المزارع المتداولة فى الاسواق المحلية حفاظا على صحة المستهلك. أجريت الدراسة على مائة عينة من اسماك المزارع المسوقة حيث تم جمع 25 عينه من كلا من البلطى و البورى و المبروك و الزريعة من أماكن مختلفة من محافظتى البحيرة و كفر الشيخ. تم الكشف عن تواجد بقايا هرمون التستستيرون فى أسنجة الاسماك باستخدام جهاز Thin Layer Chromatography كما تم تحديد كمية تلك البقايا فى العينات الموجبة بواسطة Radio Immuno Assay (RIA) أظهرت النتائج عن تواجد بقايا هرمون التستستيرون فى لحوم اسماك بلطى المزارع والمبروك بنسب 24% و4% على التوالى ولم يلاحظ فى كلا من البورى و الزريعة. بينت النتائج أن مستوى هرمون التستستيرون فى اسماك البلطى يتراوح بين 3.25 الى 34.9 بمتوسط 4.22 ± 1.1 نانو جرام / جرام فى العينات الموجبة بينما عينة واحدة من سمك المبروك أظهرت مستوى 22.0 نانوجرام/ جرام ولم يلاحظ أى مستوى فى أسماك كلا من البورى و الزريعة. تم مناقشه الأهمية الصحية لمستوى الهرمون المتواجد فى أنسجه الأسماك كما ذكرت التوصيات لتجنب المخاطر الصحية من تلك الأسماك.