

Existence of some synthetic hormonal residues in some chicken meat cuts

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Abstract

In order to boost meat production in a shorter amount of time, anabolic hormone growth promoters have been utilized extensively in chicken husbandries. However, if administered improperly or for an extended period of time, they may possess a serious risk to the consumers' health. The goal of the present survey was to ascertain whether the ninety randomly selected samples of chicken breast, thigh, and wing (each comprising thirty samples) contained any synthetic hormone growth promoters, specifically 17-estradiol and zeranol residues using ELISA assay. The samples were collected from different selling points of Shebin elkom markets, Monoufiya governorate. Thigh samples had greater residual amounts of 17-estradiol than wing and breast samples, with mean values of 0.58, 0.42, and 0.27 ppb for each, and a total incidence of 8.9%. In the meantime, zeranol was found in 7.8% of the samples that were tested, with mean values of 0.21, 0.09, and 0.07 ppb, respectively. Based on the indicated MRL in the Codex Alimentarius Commission, all of the analyzed samples were deemed fit for human consumption, provided that acceptable animal husbandry methods were followed. Since the use of these hormones in chicken farms seems to be harmful to the general public's health, it is important to routinely monitor food quality by looking for chemical residues.

Keywords: Chicken meat products, anabolic hormones, Growth promoters, Hormonal residues.

1. Introduction

Poultry meat and meat products are widely accessible, reasonably affordable, and can be very helpful in compensating for important dietary deficiencies, particularly for developing populations. Rich in essential nutrients, poultry products lower the risk of several metabolic illnesses, which are often caused by dietary shortages in important minerals, vitamins, and amino acids [1].

Veterinary drugs are routinely administered to animals raised for food in order to promote growth, boost feed effectiveness, and cure and prevent sickness. It is said that these compounds increase the deposition of proteins, lower the amount of fat on the carcass, and make the meat more tender. Their application has made it possible to produce chicken on a big scale and profitably, giving consumers access to premium meat at competitive prices [2].

Some anabolic substances have estrogenic qualities, while others are androgens that disrupt the normal physiological processes of humans. Estradiol-17 β is a prominent example of a steroid hormone that has been employed to improve bird performance. Steroid hormones are still used indiscriminately to treat bacterial infections or as growth promoters, despite the fact that the majority of countries have outlawed a wide variety of growth promoters [3].

Estrogenetic medications administered during the rearing period result in a heavier carcass with increased protein, moisture content, and decreased fat content [4]. The use of anabolic steroids is commonplace despite the fact that doing so poses a serious risk to the public's health due to their resemblance to sex hormones like androgens, progesterone, and estrogens; especially in developing countries such as Egypt [5].

Although estradiol significantly lowers luteinizing hormone (LH) levels, it also increases the release of growth hormone, which has an impact on body weight. Estradiol withdrawal is largely influenced by the amount of fat present in various organs [6].

On the other side, The Fusarium fungus produces the mycotoxin zeralenone, which is used to make the synthetic hormone zeranol. It has an estrogenic effect and is used to increase growth in animals that are being fattened. It functions similarly to 17-estradiol. Its enduring presence in milk and meat may have an impact on kids' early sexual development [7].

The tissue residues left by anabolic steroids and their metabolites are the most dangerous possible side effects. These residues are more harmful to humans since they can lead to cancer, liver tumors, early puberty in both sexes, and increased embryo death [8, 9].

Therefore, the aim of this work is to detect the hormonal residues (17 β estradiol and zeranole) in some local market chicken's products in Shebin elkom city.

2. Material and Methods

This research was approved by Institutional Animals Care and Use Committee of faculty of veterinary medicine, Benha University (approved number BUFVTM (37-11-23)

2.1. Collection of samples

From Shebin Elkom city, Monoufiya governorate, thirty randomly selected samples of chicken meat products, comprising 30 pieces of each of the breast, thigh, and wings, were collected from various supermarkets.

The hormonal residues were determined according to **Asiya and Akzira [10]** using manual kits ELISA R-Biopharma AG, Darmstadt, Germany

2.2. Determination of 17 β - Estradiol residues

Following the removal of the skin and fat, 10 g of each sample were mixed for five minutes with 10 mL of 67 mM PBS buffer. Then, in a centrifugal screw cap vial, 2 g of the homogenized material and 5 mL of tertiary butyl methyl ether (TBME) were combined. The mixture was shaken vigorously for 30 to 60 minutes using a shaker, and then centrifugation was performed for 10 minutes at 3000 RPM. After combining and evaporating the supernatant, the dry extract was dissolved in one milliliter of 40% methanol. A RIDA C18 column (a solid phase extraction column with C18 end-capped sorbent with an average particle size of 50 μ m) (Art. No. R2002) was used to receive the diluted methanolic solution, which was applied at a flow rate of one drop per second.

After loading 3 mL of material onto the column and rinsing it with 3 mL of 100% methanol, 2 mL of PBS-Buffer (20 mM) were injected to equilibrate it. One milliliter (80%) of methanol was gently injected to elute the material. After diluting an aliquot of the eluate with water, the test employed 20 μ L of the resultant solution per well.

2.3. Determination of Zeranol residues

The sample was crushed after the fat was removed. One gram of ground sample was homogenized in a centrifugal screw cap vial with one milliliter of 20 mM PBS buffer, then thoroughly mixed. Ten milliliters of tertiary butyl methyl ether (TBME) were then added, and the mixture was gently shaken for thirty minutes before being centrifuged for ten minutes at 4000 RPM. After being moved into a second centrifugal screw cap vial, the supernatant (ether layer) was evaporated at 60°C until it was completely dried. Following a strong 30-second vortex, the dried residue was dissolved in 1 milliliter of chloroform and 3 milliliters of 1M NaOH. It was then centrifuged for 10 minutes at 4000 RPM. After the NaOH extract (upper aqueous layer) was transferred, it was

centrifuged for 10 minutes at 4000 RPM and mixed for 30 seconds. It was then frozen at -25 °C for approximately 60 minutes (the lower phase needs to be frozen). The centrifugal screw cap vial held 250 μ l acetic acid (96%) and 5 ml tertiary butyl methyl ether (TBME). A second centrifugal screw cap vial was filled with the supernatant (ether layer), which was then evaporated at 60°C until it was completely dried. Sample dilution buffer (2 ml) was used to dissolve the dried residue.

The test procedures were done according to the chart enclosed in the kits of RIDAR and RIDS screen of R-Biopharm AG. Manufacture: R-Biopharm AG, Darmstadt, Germany. R-Biopharm AG is ISO certified.

The results were calculated by this equation:

% absorbance = (OD sample/ OD standard) x 100, results were calculated as ppb.

2.4. Statistical Analysis

Data collected were analyzed using one-way analysis of variance (ANOVA) with Duncan by SPSS® version 16.0. Statistical probability (p value) less than 0.05 was considered statistically significant.

3. Results

Out of the examined samples, thigh samples showed higher estradiol residual concentration than wing and breast samples; with mean values of 0.58, 0.42 and 0.27 ppb, respectively. Regarding with their fitness for human consumption, in reference to the noted MRL in CAC standards, all of the examined samples were fit for human consumption on condition of good animal husbandry practice (**Table (1) and Fig. (1)**).

Moreover, **Table (2) and Fig. (2)** showed that, out of the examined samples, thigh samples had higher zeranol residual concentration than wing and breast samples; with mean values of 0.21, 0.09 and 0.07 ppb, respectively. Regarding with their fitness for human consumption, in reference to the noted MRL in CAC standards, all of the examined samples were fit for human consumption on condition of good animal husbandry practice

Table (1) Incidence and hormonal residues of 17 β - estradiol (ppb) in the examined samples of chicken cuts (n=30).

Chicken samples	+ve samples		Min	Max	Mean \pm S.E	Fitness ratio		
	No.	%				MRL* (ppb)	NO.	%
Breast	2	6.7	0.05	0.49	0.27 \pm 0.01 ^B	2	30	100
Thigh	4	13.3	0.12	0.91	0.58 \pm 0.02 ^A		30	100
Wing	2	6.7	0.09	0.74	0.42 \pm 0.01 ^{AB}		30	100
Total	8	8.9					90	100

ABC. Means with different superscript letters in the same column are significantly different (P<0.05).

* Codex Alimentarius Commission (2018)

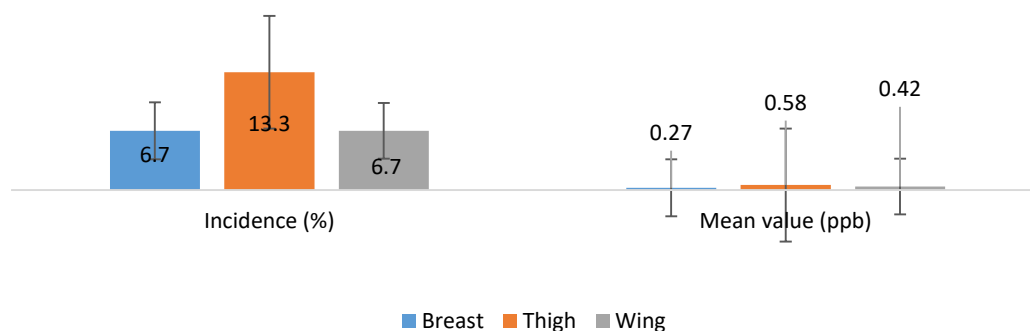


Fig. (1) Incidence and Mean values of 17β- estradiol (ppb) residue in the examined chicken samples.

Table (2) Incidence and hormonal residues of zeranol(ppb) in the examined samples of chicken carcasses (n=30).

Chicken samples	+ve samples		Min	Max	Mean ± S.E	Fitness ratio		
	NO.	%				MPL*(ppb)	NO.	%
Breast	2	6.7	0.13	0.49	0.07 ± 0.01 ^B	2	30	100
Thigh	3	10	0.30	0.91	0.21 ± 0.02 ^A		30	100
Wing	2	6.7	0.18	0.74	0.09 ± 0.01 ^{AB}		30	100
Total	8	7.8					90	100

Means with different superscript letters in the same column are significantly different (P<0.05).

* Codex Alimentarius Commission (2018)

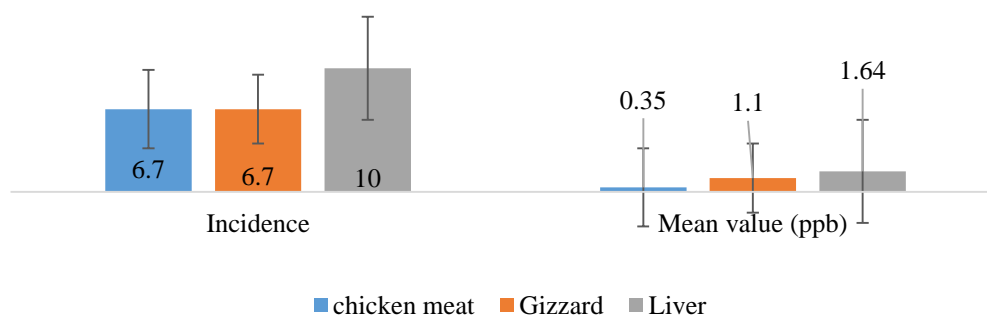


Fig. (2) Incidence and Mean values of zeranol residue in the examined chicken samples.

4. Discussion

The public's health has been seriously at risk from hormone residues found in food-animal carcasses, as evidenced by toxicological research that suggested these residues may be connected to cancer. The primary driver of increasing animal protein production rates at low cost and minimal delay is thought to be the scarcity of sources of animal protein. Using growth enhancer to boost meat production is one of these initiatives. Feed ingredients are treated with chemicals known as growth promoters in order to improve daily weight gain. Consequently, a variety of illicit techniques were used to accelerate the rate of animal production [11].

Recently, high yielding food animals have been produced faster by using hormones and hormone-like compounds. These substances are intended to decrease fatness, preserve protein, enhance meal nutritional value, and encourage weight growth. It is possible that certain medications or hormones will be used as growth accelerators during the illicit trials. Anabolic agents aid in the nitrogen retention and protein deposition of farm animals and share physiological traits with human sex hormones [12].

The general public's knowledge of the potential concerns to human health linked with residue building in meat, the widespread use of drug therapy, and these issues has increased recently. Numerous growth promoters on the market today

are classified as dangerous, allergenic, and carcinogenic. Some of which could interfere with a person's regular bodily functions. It is critical to identify these residues in grill meat meant for human consumption for the safety of customers [13].

According to the results that were recorded in **Table (1) and Fig. (1)**, thigh samples showed higher estradiol residual concentration than wing and breast samples; with mean values of 0.58, 0.42 and 0.27 ppb, respectively. Regarding with their fitness for human consumption, in reference to the noted MRL in CAC [14], all of the examined samples were fit for human consumption on condition of good animal husbandry practice. Lower findings were obtained by **Doyle [15]** who found that 17 β - estradiol in chicken meat was ranged from < 0.03- 0.02 ppb; and **Akhmet et al. [16]** who collected a chicken breast and wing samples from USA, Russia and Ukraine, where estradiol was detected in the mean value of 0.004, 0.001 and 0.002 ppb in wing samples, while was 0.004, 0.001 and 0.001 ppb in breast samples, respectively. While, higher findings were obtained by **Sadek et al. [17]** who found 17 β - estradiol in chicken meat and Liver were 0.865, 4.216 ppb, respectively; **Kadim et al. [18]** (0.704 ppb in chicken meat samples), and **Ibrahim et al. [19]** who recorded detection of 17 β - estradiol in all of the examined chicken meat samples with mean values of 0.782 and 1.53 ppb, respectively.

While, **EL-Neklawy [20] and Doyle [15]** discovered that out of their examined samples, 44 and 55%, respectively, exceeded the MPL, the acceptability of the examined samples according to **CAC [14]** revealed that there were no samples exceeded the permissible limit, making all of the examined samples fit for human consumption. The fact that gonads and adrenals secrete natural steroid hormones may explain why there are natural steroid hormones in chicken meat [21].

Table (2) and Fig (2) revealed that liver samples had higher estradiol residual concentration than gizzard and muscle samples; with mean values of 1.64, 1.1 and 0.35 ppb, respectively. Results did not agree with those obtained by **Sadek et al. [17]** who did not find any residues of zeranol in chicken muscle. While, higher findings were obtained by **Xiaoming et al. [22]** who found zeranol residues (2.5 ppb) in liver samples of chickens. Moreover, lower findings were recorded by **Ibrahim et al. [19]** (0.106 and 0.123 ppb for meat and liver samples, respectively).

On the other hand, **Elshikh et al. [23]** did not found 17 β - estradiol or zeranol in any of their examined chicken meat samples.

Variation between different author's records may be referred to difference in location of collection, rearing protocols and difference in authority standards.

Accordingly, it appears that the current status of these anabolic hormones on the Shebin elkom City

market is not in danger. On the other hand, these results do not rule out the possibility that these anabolic hormones will be misused in the future, significantly increasing human exposure to gonadal synthetic steroids, particularly among children, which may have a negative impact on their health. As a result, regular monitoring of these chemical residues is required as a food quality control strategy.

5. Conclusion

The anabolic hormonal residue in chicken breast, thigh, and wing samples that was currently studied revealed that the thigh samples had the highest level of hormonal residues, followed by the breast and wing samples, respectively. Additionally, the fact that none of the studied samples exceeded the allowable levels indicated that they were all safe for human food on the assumption of proper child rearing, routine monitoring procedures, and care, together with the advised withdrawal period before slaughter.

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