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# Current situation of some residues in common meat products

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**ABSTRACT:**

This study was designed to evaluate some synthetic hormones and total aflatoxin residues in meat and poultry products using Enzyme-linked ImmunoSorbent Assay (ELISA) method. A total 90 of random samples represented by beef and chicken burger, chicken pane ,minced meat, beef and chicken Kofta (15 of each), were collected from different supermarkets in Cairo governorate. Results revealed that the mean values of Trenbelone acetate hormone 0.4±0.01, 0.59±0.02, 0.58±0.01, 0.94±0.02, 1.9±0.07 and 2.03±0.1 ppb .While, the mean values of Zeranol residues 1.09±0.05, 1.68±0.09, 2 ±0.15, 0.72 ±0.04, 0.98±0.03 and 1.19±0.04 ppb. Moreover the mean values of total aflatoxin residues 4.36±0.44, 5.5 ± 0.63, 5.1±0.52, 2.3±0.28, 1.7±0.25 and 1.71±0.24 ppb in chicken pane, chicken burger, chicken kofta, beef burger, beef kofta and minced meat; respectively. So public health should place a high priority on raising awareness of hormone and aflatoxin residues in food and how to control them.

## INTRODUCTION:

The global production and consumption of processed meat products have been steadily rising in recent times, driven by their convenient nature and abundant nutritional benefits **(Rajic´et al. 2007.)**

Meat products have gained popularity as convenient and delectable food options, making them the preferred choice for individuals worldwide. Their availability, quick preparation, and savory taste make them highly sought after **(Heinz and Hautzinger, 2007)**.

The Food and Agriculture Organization of the United Nations (FAO) states that meat is an animal product that offers valuable elements to the diet, including important amino acids, lipids, proteins, vitamins, and minerals **(FAO, 2014)**.

Additionally, meat products are highly well -liked by kids since they have a distinct flavor and aroma from red meat **(Elhelaly et al. 2022 and Morshedy et al. 2022).**

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According to FAO data, at least some mycotoxin is present in 25% of crops **(OMS, 2018)**. Poor handling techniques can cause contamination of spices at any stage of the production chain, including pre-harvest, harvest, processing, storage, drying, and transportation **(Ardic et al. 2008, Khayoon et al. 2012 and Tosun and Arslan 2013)** , particularly in situations where the growth of fungi is encouraged by environmental factors like temperature and humidity **(Ardic et al. 2008 and Hammami et al. 2014).**

However, in an effort to produce large yields faster, hormones and hormone-like compounds have recently been employed in animal husbandry. These anabolic substances are used to increase body mass, enhance nutrient uptake, accumulate protein, and decrease adiposity. However, the potential for anabolic steroid residues to be present in meat and meat products could be dangerous to human health, depending on the use of anabolic agents in animal feed **(Asiya and Akzira, 2016).**

In animal production, the use of toxic compounds like hormones and growth boosters is a common issue. These substances are frequently used to lower feeding expenses and boost output **(Toffolatti et al. 2006).**

As previously mentioned, the other two hormones replicate the biological activity of the natural hormones: zeranol mimics the action of estradiol 17-ß, and trenbolone mimics the action of testosterone **(Ali, 2009)**

*Aspergillus Fusarium and Penicillium* are two examples of molds that produce mycotoxins, which are extremely poisonous compounds that negatively impact food goods' market quality and cleanliness. Food-producing molds that produce mycotoxin pose a serious risk to public health as well as a big financial issue (**Dalie et al. 2010**).

The most well-known mycotoxin is aflatoxins (AFS), a class of heterocyclic metabolites generated by Aspergillus fungi, especially *Aspergillus flavus and Aspergillus parasiticus*. AFS frequently contaminates human and animal food, leading to illness and even death in those who consume it **(Giambrone et al. 1985 and Magnussen and parsi, 2013)**. Aflatoxin B1, B2, G1, and G2 are the four naturally occurring AFs. They are all harmful, mutagenic, and carcinogenic substances **(CAST, 2003)**, having been categorized as group 1 (substances that are harmful for humans) by the International Agency for Research on Cancer **(IRAC. 1993)**. There have also been reports of possible immunosuppressive and nutritional influence **(Williams et al. 2004)**, as well as be teratogenic, mutagenic, and hepatotoxic **(Kensler et al. 2011).**

When aflatoxins consumed by people or animals, extremely poisonous fungal metabolites that can have a wide range of negative effects. Certain Aspergillus species, especially *Aspergillus aflatoxin* and *Aspergillus parasiticus*, are the main producers of aflatoxins, which are carcinogenic substances. On a range of diets and feeds, this fungus can thrive when the temperature and humidity are correct. Contamination with aflatoxin can happen anywhere in the food chain **(Giray et al. 2007)**.

The main way that humans become exposed to AFTs is through eating directly contaminated food, including as fruits, cereals, seeds, and other foods. Or indirectly through consuming food items and by products that came from animals that ate tainted feed **(Galvano et al. 2005).**

They have deleterious effects on the liver in particular, as well as mutagenic, teratogenic, and immunosuppressive properties. A number of physical, chemical, and biological techniques have been developed to manage and remove aflatoxins from contaminated feeds and commodities (**Morteza et al. 2013**).

The current survey sought to identify and measure certain residues of hormones and aflatoxins in beef and chicken products, as well as to evaluate the dangers associated with them, both now and in the future, for customers who are adults and children.

## MATERIAL AND METHODS:-

### 2.1. Collection of samples:

Ninety randomly selected samples of meat and poultry items, including of fifteen each of

beef and chicken Kofta, chicken pane, minced meat, and beef and chicken burgers, were gathered from various supermarkets within the governorate of Cairo. Every sample, which weighed roughly 100 g, was aseptically transported to the lab right away in an insulated icebox and examined for the presence of hormone and total aflatoxins (B1+B2+G1 and G2) residues.

**2.2. Apparatus**:

Microtiter plate spectrophotometer (450nm), centrifuge, RIDA C18 column, mixer and shaker were used for the analysis.

**2.3. Detection of Hormonal residues:-**

**2.2.1. Estimation of Trenbolone acetate hormone in meat and poultry products (Mor et al. 2011):**

**Extraction of samples:-**

The sample was grounded after the fat was removed.
Ten gm of ground sample homogenized with 10 ml of 67 mM PBS buffer and shaked for 5 min.
A centrifugal screw cap vial containing two grams of homogenized sample and five milliliters of tertiary butyl methyl ether (TBME) was tightly shaken for thirty to sixty minutes using a vortex.
The contents were centrifuged for 10 minutes at 3000 rpm.
The TBME extraction was carried out again using the retained supernatant.
After combining and evaporating the supernatants, the dry extract was mixed in one milliliter of 80% methanol.
The methanolic solution was put on a RIDA C18 column (a solid phase extraction column with a C18 end-capped sorbent with an average particle size of 50 µm) after being diluted with 2 mL of 20 mM PBS-buffer.
To conduct the test, an aliquot of the elute was diluted with water and the resulting solution was used in 20µL per well.

**Test procedures:**

The testing was conducted in accordance with the chart that was included in the kits of Europroxima Manufacture: R-Biopharm Nederland B.V, doc .No.5081TRENBO- CA V02.

**2.3.1. Estimation of Zeranole hormone in meat and poultry products (Mor et al. 2011):**

**Extraction of samples :-**

- Ten gm of the sample was homogenized.
- One gm was taken in test tube then 4 ml of acetonitrile was added
- Homogeneous emulsion made by vortex
- The sample mixed for 15 min head over head
- The contents were centrifuged at 2500 x g for 10min.
- One ml of the supernatant was kept in glass tube.
- The supernatant was evaporated under steam of nitrogen at 50 °c
- The residue was reconstituted with 100 µl of 100% methanol.
- An aliquot of 50µl used as sample in ELISA.

**Test procedures:-**

The testing was conducted in accordance with the chart that was included in the kits of Europroxima Manufacture: R-Biopharm Nederland B.V, doc .No.5081ZERAN (3) - CA V02.

**Estimation of total aflatoxins residues in meat and poultry products (Barragan et al. 2021)**

Using a competitive direct enzyme linked immune sorbent assay (CD-ELISA), the total amount of aflatoxins was quantitatively analyzed. The approach predicated on precise mycotoxin monitoring. The veratox test kits (Neogen Crop., Lansing, and MI.USK.) approved by USDA-GIPSA (2008-011) and the AOAC Research Institute (certificate No 950702) were utilized. The analysis was completed in compliance with the manufacturer's guidelines. Aflatoxin concentration was computed using the log/log it software from Awareness Technology Inc. **(Stoloff et al.1999 and Anonymous, 2000).**

**CALCULATION**

In order to get quantitative data, the absorbance values for the standards and the samples were multiplied by 100 (the percentage

maximum absorbance) and divided by the absorbance value of the first standard, or zero standard. As a result, 100% is set as the zero-standard, and absorbance values are expressed as percentages. Plotting the standard curve on a semilogarithmic graph paper with the corresponding absorbance value on the Y-axis and the standard value on the x-axis. The TAF concentration. The standard curve relating optical density versus TAF standards was used to determine the levels in the tested samples.

## 3. RESULTS

Table 1. Acceptability and Outcomes of Trenbolone acetate residues (ppb) in the examined chicken and beef product samples:

| Examined products | N0. of sample analyzed | Min. | Max. | Mean ±SE | Means of products ± SE | Accepted samples According to CAC 2017* | |
|---|---|---|---|---|---|---|---|
| | | | | | | No. | % |
| Chicken pane | 15 | 0.34 | 0.45 | 0.4±0.01[a] | | 15 | 100 |
| Chicken burger | 15 | 0.52 | 0.72 | 0.59±0.02[b] | 0.52±0.02[a] | 15 | 100 |
| Chicken kofta | 15 | 0.49 | 0.63 | 0.58±0.01[b] | | 15 | 100 |
| Beef burger | 15 | 0.86 | 1.06 | 0.94±0.02[a] | | 15 | 100 |
| Beef kofta | 15 | 1.5 | 2.16 | 1.9±0.07[b] | 1.6 ± 0.1[b] | 11 | 73 |
| Minced meat | 15 | 1.17 | 2.91 | 2.03±0.1[b] | | 10 | 66.6 |

**CAC*: Codex Alimentarius commission (2017)** stated the **(MRL)** Maximum residue limit in Muscle (2ppb) in liver (10ppb).
There are significance P ≤0.05 between different letters (a and b) in the same column**.**

Table 2. Acceptability and Outcomes of Zeranol residues (ppb) in the examined chicken and beef product samples:

| Examined products | N0. of samples analyzed | Min. | Max. | Mean ±SE | Means of products ± SE | Accepted samples According to CAC 2017* | |
|---|---|---|---|---|---|---|---|
| | | | | | | No. | % |
| Chicken pane | 15 | 0.87 | 1.32 | 1.09±0.05[a] | | 15 | 100 |
| Chicken burger | 15 | 1.25 | 2.05 | 1.68±0.09[b] | 1.6±0.09[a] | 13 | 86.6 |
| Chicken kofta | 15 | 1.4 | 2.83 | 2.0 ±0.15[c] | | 12 | 80 |
| Beef burger | 15 | 0.54 | 0.91 | 0.72 ±0.04[a] | | 15 | 100 |
| Beef kofta | 15 | 0.87 | 1.12 | 0.98±0.03[b] | 0.96±0.04[b] | 15 | 100 |
| Minced meat | 15 | 0.96 | 1.34 | 1.19±0.04[c] | | 15 | 100 |

**CAC*: Codex Alimentarius commission (2017)** stated the **(MRL)** Maximum residue limit in Muscle (2ppb) in liver (10ppb).
There are significance P ≤0.05 between different letters (a, b and c) in the same column**.**

Table 3. Acceptability and Outcomes of total Aflatoxine residues (ppb) in the examined chicken and beef product samples:

| Examined products | N0. of samples analyzed | Min. | Max. | Mean ±SE | Means of products ± SE | Accepted samples according to FDA (2011)* | |
|---|---|---|---|---|---|---|---|
| | | | | | | No. | % |
| Chicken pane | 15 | 2.6 | 6.3 | 4.36±0.44[a] | | 15 | 100 |
| Chicken burger | 15 | 2.3 | 7.8 | 5.5 ± 0.63[a] | | 15 | 100 |
| Chicken kofta | 15 | 2.5 | 7.04 | 5.1±0.52 [a] | 4.9±o.3[a] | 15 | 100 |
| Beef burger | 15 | 0.5 | 3.3 | 2.3±0.28 [a] | | 15 | 100 |
| Beef kofta | 15 | 0.6 | 2.8 | 1.7±0.25 [a] | | 15 | 100 |
| Minced meat | 15 | 0.3 | 2.6 | 1.71±0.24 [a] | 1.9 ± 0.15[b] | 15 | 100 |

Pl. according to FDA (2011) (20 ppb)
There are significance ≤0.05 between different letters (a and b) in the same column.

## DISCUSSION

A comprehensive investigation into the presence of banned chemicals and residues of chemical, biological, and veterinary medicine products in animals, biological material, and food derived from animals is carried out in order to guarantee consumer health and food safety.

The mean values of trenbelone acetate residues are shown in Table (1) based on the ELISA test findings 0.4±0.01, 0.59±0.02 and 0.58±0.01 ppb with minimum values of 0.34,0.52 and 0.49 ppb, while maximum values of 0.45, 0.72 and 0.63 ppb in chicken pane, chicken burger and chicken kofta ;respectively. All samples were accepted because of trenbelone hormone synthetic for testosterone and rarely to use for chicken as growth promoter. There were significant differences between chicken pane and chicken burger, chicken kofta but there weren't significance between chicken burger and chicken kofta.

Moreover in Table(1) the mean values of trenbelone acetate residues 0.94±0.02, 1.9±0.07 and 2.03±0.1 ppb with minimum values of 0.86, 1.5 and 1.17 ppb, while maximum values of 1.06, 2.16 and 2.91 ppb in beef burger, beef kofta and minced meat ;respectively. There were 4 samples not accepted in beef kofta and 5 samples not accepted in minced meat that exceed permissible limit. There were significant differences between beef burger, beef kofta and minced meat but there weren't significance between beef kofta and minced meat.

The conclusion reached was that there is significant difference between poultry products and meat products obtained by T- test.

Lower results were reported by **Nazli et al. (2005)** 4 (40%) of the 10 ready-made minced beef samples that were gathered from Istanbul, Turkey marketplaces showed 0.10-0.5 ppb TBA.

However, **Jannat et al. (2007)** found higher results, estimating the average amount of trenbolone in cattle meat to be 3.76±5.26 ppb and **(Mor et al. 2011)** examined the presence of trenbolone in samples of meat, liver, and kidney from cattle raised in the Burdur district. Trenbolone was found in 50–100 ppb of the 30 meat samples that were tested, 100–150 ppb of 21 samples, and 151-200 ppb of 6 samples.

This was validated by the trenbolone allowed limit levels, which are 2 ppb in muscle. **(Codex Alimentarius, 2017& (EU) 2017/625)** pertains to food regulation and establishes new national surveillance initiatives to track down leftovers of illegal drugs. For instance, 2 ppb of trenbolone in muscle and 10 ppb in liver are acceptable limits.

The Joint Expert Committee on Food Additives **(JECFA, 2000)** has established 0.02µg/kg body weight (BW) as the maximum recommended daily intake (ADI) for trenbolone. As a result, it appears that the current availability of this anabolic hormone in the market carries potential risks. These findings indicate a significant rise in human exposure to trenbolone, especially among children, which could have detrimental effects on health. Consequently, as part of food quality control procedures, it is essential to routinely test this chemical.

The data reported in Table (2) found that the average zeranol residue levels were 1.09±0.05, 1.68±0.09, and 2.0±0.15 ppb, with the lowest values 0.87, 1.25 and 1.4 ppb, while maximum values 1.32, 2.05 and 2.83ppb in chicken pane, chicken burger and chicken kofta ; respectively. There were 2 samples in chicken burger and 3 samples in chicken kofta not accepted as they exceed the permissible limit that due to use of zeranol in chicken farms because it synthetic hormone for estradiol used as growth promoter.

There were significance difference between chicken pane, chicken burger and chicken kofta with each other's

Additionally, Table (2) shows the average zeranol residue values 0.72 ±0.04, 0.98±0.03 and 1.19±0.04ppb with minimum values 0.54, 0.87and 0.96 ppb, while maximum values of 0.91, 1.12 and 1.34 ppb in beef burger, beef kofta and minced meat; respectively. There weren't any sample exceed the permissible limit also there were significance difference between beef burger, beef kofta and minced

meat with each other's.

And also, there were significant difference between poultry products and meat products obtained by T- test.

Lower results reported by **(Sadek et al. 1998)**, who not identifying any zeranol residues in chicken muscle. However, **Xiamong et al. (2002)** discovered zeranol residues (2.5 ppb) in chicken liver samples, yielding more conclusive results. Also, **Mor et al. (2011)** found zeranol residues at 100–150 ppb in 4 samples, 151-200 ppb in 5 samples, and 201–500 ppb in 2 samples from the cattle's liver, kidney, and flesh.

Table (2) revealed that five samples exceeded the maximum residue limit (MRL) of 2 ppb in muscle and 10 ppb in liver, as per the **Codex Alimentarius Commission (2017)**. Although the anabolic hormones on the market do not pose a high risk, their cumulative effect on consumer health may warrant consideration.

According to the Food and Drug Administration **(FDA, 1999)**, aflatoxins, particularly B1, B2, and G1, are the most often occurring toxins in food items consumed by humans. Acute toxicity and delayed mental development are two of the health effects of aflatoxins.

Table (3) demonstrated that the amounts of AFT residuals in all meat product samples under examination fell under the 20 ppb maximum allowable threshold of total aflatoxin residues set by the FDA (2000) and FAO (2004). The mean values of total aflatoxins residues 4.36±0.44, 5.5± 0.63, 5.1±0.52, 2.3±0.28, 1.7±0.25 and 1.71±0.24ppb with minimum values2.6, 2.3 , 2.5, 0.5, 0.6 and 0.3 ppb while with maximum values 6.3, 7.8, 7.04, 3.3, 2.8 and 2.6 ppb in chicken pane, chicken burger, chicken kofta, beef burger, beef kofta and minced meat; respectively. There weren't significant difference between any products with each other. But there were significant differences between poultry products and meat products obtained by T- test.

**In chicken products:** Lower result obtained by **Alaa Eldin et al. (2015)** who recognized AFT residues in, chicken burger 1.78 ppb and chicken fillet 0.31 ppb. The higher values were found by **Mohamed, (2004)**, **Wadee, (2010)** and **Hasanen et al. (2016)** where total AFT was 8.9±1.5 ppb in chicken tissue.

**In beef products:**

In beef burger: **El-Mossalami (2010)** found a decreased aflatoxin B1 residual level in burgers (0.41 ppb). **El-Shafei , (2007)** reported higher results, with 40% of the burger samples contaminated with 14.89 ppb. **Abd-Elghany and Sallam, (2015)** found very identical results, with 100% of the 25 burger samples analyzed and collected from Mansoura city contaminated with 3.22 ppb total aflatoxin (AFT).

In minced meat: lower AFT residual levels was 0.65±0.14, where 18 (72%) were positive (**Ibrahim et al. 2018)**, conversely, larger AFT residual values were reported by **El-Shafei , (2007)**, who found that 20% of the samples under examination had AFT residues of 8.52 ppb.

In Kofta: higher level reported by **Shabana et al. (2008)**, where AFB1 was 6.70 ±0.89 in Kofta; additionally, AFB1 was 0. 15 to 6.36 in beef products, according to **Herzallah , (2009)**.

## CONCLUSSION

Based on the collected data, it can be concluded that the levels of hormonal residues in the tested chicken and meat products were higher than allowed. This could be related to laws that forbid the use of anabolic agents as growth promoters because of the negative health effects they have on consumers, or it could be because of change in chemical composition of meat as increase of moisture content instead of protein that become un fit for human consumption. Also from obtained results, we could be concluded that the aflatoxin residues were detected in considerable levels in poultry and meat products. The goal of mycotoxin control in Egypt is to enhance public health. As a result, several approaches to the decrease and management of

mycotoxins have been explored in various parts of the world, including African nations. The management of mycotoxins entails:
Stopping the growth of fungi in crops and other feedstuffs.
Cleaning up feeds and foods contaminated with mycotoxin as a backup plan.
Constantly monitoring mycotoxin levels in human food, animal feedstuffs, and agricultural crops **(Tola and Kebede, 2016)**.

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