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Study on Hormonal and Heavy metals residues in fresh beef meat

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Abstract

This study was carried out to investigate hormonal and heavy metal residues in beef meat in different seasons. Meat is a very complex and continuously changing system of molecular substances that can be used for satisfying needs of the human for metabolic energy, building material and fulfilling the other vital functions whereas today most people ask for safe and healthy food with high quality. Therefore, a total of 120 samples of fresh beef meat were collected randomly from different markets in Dakhlia Governorate (30 samples for each season). Collected samples were subjected to estimate hormonal and heavy metal residues concentrations using Gas chromatography-mass spectrometry (GC-MS) for hormonal residues and Flame Atomic Absorption Spectrometer (AAS) for heavy metals

Data analysis revealed that estradiol concenteration was highest in summer (0.18 ± 0.03) then in autumn season 0.15 ±0.02 .progestrone level was higher in autumn (0.26 ± 0.03) than in summer season (0.24 ± 0.04) .Summer season was higher than winter and spring(0.21 ± 0.02 – 0.19 ± 0.02) respectively. Testesteron was highest in autumn season(0.19 ± 0.02). Concerning heavy metals, they showed the highest concentration in summer season . Lead concetration in summer season was (0.082 ± 0.006) ppm. Cadmium in summer season was (0.069 ± 0.004) ppm and copper in summer season was(0.11± 0.008) ppm . It can be concluded that meat could be exposed to chemical pollutants, in addition to presence of some pollutants residues in meat, these residues will be accumulated on the long run and may be carcenogenic. The use of natural growth promotor is advisable not hormonal growth promotors and to reduce the negative impacts of stressors especially heat stress, to the lowest possible degree. Proper nutrition, and optimum climatic conditions are indispensable to avoid heavy metal residues entry to food chain through contaminated feed and water. Immediate measures must be carried out for the treatment and detoxification of industrial and domestic effluents intended to be used for agricultural purposes.

Key words: hormonal and heavy metal residues - GC-MS -AAS - Meat - different seasons.

Introduction

Meat is a relevant dietary source of proteins, essential amino acids, chemical elements (as iron, zinc) and vitamins (including B12 and D) **(Tonu, 2013)**. A united approach with consistent standards based on sound science and robust controls is necessary to ensure consumers' health and to maintain consumers' confidence.Yet, the healthy image of meat is tarnished by its negative association with saturated fat and cholesterol, and with non-nutritional issues like the presence of various toxic contaminants, including the most commonly found persistent pollutants **(Tonu 2013)**

Hormonal growth promoters have been extensively used for many decades in animal husbandry to improve the rate of growth and efficiency of feed conversion. In European Union theiruse in food producing animals has been banned by **Council Directive 19 96/22/EC**, **(Commission of the European Communities, Council1996)**.

Because of possible harmful effects on human health through carcinogenic mechanisms or endocrine disrupting, the control of the absence of these forbidden substances in animal foods and feeds is regulated inthe **European Union by Directive 96/23/EC**. There are three natural hormones (estradiol or estrogen, progesterone, and testosterone) and their synthetic alternatives including zeranol, melengestrol acetate, and trenbolone acetate **(Maia *et al*., 2008).**

Monitoring for illicit use of hormones is carried outunder the terms of EU Directive 96/23/EC which is implemented through surveillance according to the Nationals Plans ofthe individual Member States. For controls at retail level, as wellas for products imported into the EU, efficient methods applicable to meat samples are necessary. To establish the presence of anabolic agents and their metabolites in tissue samples, analytical techniques for detecting trace amounts of these compounds are required **(Hartwing *et al.*, 1997 and Smets, *et al*., 1993).**

Contents of naturally occurring steroid hormones in beef and veal have been frequently reported because of their misuse as anabolic performance enhancers in cattle production **(Scippo *et al.*, 1993).** It is known that steroid hormones also occur in tissues of non treated cattle **(CEC, 1989 and Hartwig *et al.*, 1997).**

The estrogens, which are fat anabolics and regarded as typical female hormones, are formed from the androgen testosterone or the weakly androgenic intermediate androstenedione. Another female hormone is progesterone**. (Erb *et al.*, 1977 and Scippo *et al.*, 1993).** Not only the sex steroids have physiological effects; theintermediate dehydroepiandrosterone (DHEA) is alsosaid to have biological significance **(Regelson and Kalimi, 1994).** It has an antagonistic effect to corticosteroids**.** It may also act as estrogen or androgen depending on the hormonal state of the individual,.like pregnenolone, it is considered as a neurosteroid due to its gradual decrease with advancing age **(Birkenhager-Gillesse *et al.*,1994),** DHEA is also of interest as a possible 'anti-aging pill'.Analysis of steroids was based on the official methodin the Federal Republic of Germany for the determination of hormonally active agents in meat, liver, kidney and fatty tissue **(Bundesgesundheitsamt, 1989).**

Therefore a few selected foodstuffs could be investigated, to give an over view of the occurrence of steroid hormones in human nutrition. If available, data from the literature were brought into support estrogenic agents with anabolic properties are stilbenes, which the main representative isdiethylstilbestrol (DES), compound known from carcinogenic properties. It was the first synthetic estrogen that has been widely used as feed additive or as an implant into the ear to promote growth in cattle and sheep **(EU Directive 1996)**.

The hormonal content of the latter could not be quantified, however, because of interferences in the chromatogram. Progesterone is administered to cattle in combination with estradiol benzoate at a ten to one ratio (progesterone 100~200 mg with estradiol 10~20 mg) as an ear implant, to increase rates of weight gain and feed efficiency. Progesterone is also used to synchronize estrus in lactating and non-lactating dairy cows and goats via an intravaginal sponge. Exogenously administered progesterone is structurally identical to the progesterone produced in animals and humans. Progesterone is poorly absorbed by oral ingestion and inactivated in the gastrointestinal tract and/or liver, which makes its bioavailability less than 10% after oral administration **(Simon *et al.*, 1993).**

Testosterone is synthesized in testicular Leydig cells, ovarian thecal cells, and the adrenal cortex, and it exerts activity via binding with androgen receptors. Testosterone is a precursor of other steroid hormones. The active metabolite of testosterone is dihydrotestosterone (DHT), which is metabolized to androsterone, androstanedione, and 3α- and 3β-androstanediol. The physiological concentration of circulating testosterone is 3~10 ng/ml in men **(Miyamoto *et al.*, 1998)** .

The major functions of testosterone are pubertal development for spermatogenesis. The most comprehensive data concerning androgens, progestogens and their precursors and metabolites in meat of bulls, steers and heifers have been published recently by **Hartwig *et al.*, (1997).**

Continued exposure to heat stress has several known physiological effects such as an increase in plasma progesterone in open and cycling cows, which results in problems with breeding **(Abilay*et al.*, 1975).**

EC regulations **(EC, 2008)** set the same maximum residue levels (MRLs) for Pb in meat of bovine, sheep, pig, and poultry (0.1 mg/kg) and for edible offals of these animals (0.5 mg/kg). Heavy metals pollution is considered as one of the most important environmental problems in Egypt. It results from industrial and agricultural wastes that spread in air, water and soil. These pollutants have tendency to accumulate in tissues of animals. Cumulative toxic effect of heavy metals are recognized due to low elimination rates from body and cause serious health hazard to human depending on their level of contamination **(Lars , 2003).**

The toxic elements of cadmium, lead and copper are widely distributed in environment.Cadmium is the mining and electroplating industries and found in fertilizers and fungicides, all its chemical form are toxic **(Underwood 1977).** Lead is used in many industrial processes, lead paint and gasoline. It is common material for spraying fruit trees. Absorbed lead accumulates in tissues of the animals. Lead inhibits the activity of enzymes dependent upon the presence of free sulphydryl groups for their activity **(Willoughby *et al.*, 1976).**

Material and Methods

Thirty samples of fresh beef meat for each season were randomly collected from different location at Dakhlia Governorate during Autumn, Winter, Spring and Summer season in the year 2014-2015. Immediately after collection, meat samples were cooled to 4°C and quickly transferred in an ice box to the Lab, where they kept frozen at -20°C until analysis. Before analysis, the samples were homogenized. Each meat sample was divided into two parts; the first was analyzed for hormonal residues while the second for heavy metal residues.

Analysis

Twenty five gm of meat samples were submitted to analysis. Sample preparation depended on the food stuffs' matrix and the occurrence of conjugated steroids. To obtain the sum of all steroids present in food, hydrolytic liberation of steroids was carried out if the presence of conjugated steroids was expected.

Samples were treated with mineral acid to liberate any conjugates. Samples with more than 30% fat had to be dissolved first in lipophilic solvents to allow the extracting agent of methanol to penetrate through the sample.

Sample pretreatment

After homogenizing the samples testosterone (2.0 ng µ methanol) and D3-17B-estradiol (1 .0 ngµ methanol) were added as internal standards to eliminate preparation losses.

Meat samples were incubated overnight at 37°C in 25 ml acetate buffer (0.04 mol litre) at pH 5.1-5.3 **(Bundesgesundheitsamt, 1989).** Homogenized material was suspended with 50 ml water and heated under reflux. After addition of 5ml concentrated HCl the mixture was boiled for 15min. The suspension was then neutralized with NaOH and centrifuged.

Fat and fatty tissues were first dissolved in 30 ml hexane at 40°C .The different sample preparations were checked by analysing the recovery of spiked samples. Isolation and purifcation of the steroids **(Bundesgesundheitsamt, 1989).**

To extract the liberated steroids, the samples were homogenized with 90 ml methanol and 25 ml water (depending on the original water content of the food stuff and the sample pretreatment). The supernatant was extracted with 2 x 40 ml hexane to remove fat. The methanol/waterlayer was then extracted three times with dichloromethane (70, 40 and 30ml). The crude extract was purified on an Amberlite XAD-2 column followed by afractionation in phenolic and neutral steroids through Celite/KOH column coupled to an A1203 column as described by **Bundesgesundheitsamt (1989).**

The chosen sample preparation was proved to be suitable for most foodstuffs. Interferences were effectively removed and so chromatograms which were very suitable for interpretation were obtained. The method was recommended for analysis of offal and meat products by **Bundesgesundheitsamt (1989).**

Gas chromatography-mass spectrometry

Both fractions were analysed separately by GC-MS. Mass Spectrometer (Applied Biosystems|MDS, SCIEX, Toronto Canada. The |steroids were derivatized with 50 µg N-methyl Ntrimethylsilyltrifluoroacetamide/ trimethyliodosilane/ dithioerythritol (1000:2:2) at 60°C for 15 min **(Smets, *et al.*, 1993).** GC-MS conditions: GC: Varian3400 (column: DB-5 MS, 30 mx0.25 mm, 0.25 µm film), MS: Finnigan INCOS 50 B (EI, electron energy: 70eV, ion source temperature: 180°C). Masses for selected ionmonitoring: androsterone, dihydrotestosterone, testosterone: 432, 417; androstenedione: 430, 415.

Heavy metals analysis: Digestion of samples were done according to the recommended method **(Al-Ghais 1995)** using Perkin Elmer model (Spectra- AA 10, USA) Flame Atomic Absorption Spectrometer (AAS) with computer system .The examined samples were calculated as ppm (mg\kg) on wet weight **(Seady 2001).**

Results and Discussion

Table (1) showed that the estradiol concenteration was highest in summer (0.18±0.03µg|kg) then in autumn (0.15±0.02µg|kg). Progesteron showed higher concentration in autumn and summer (0.26±0.03and 0.24±0.04 µg|kg) respectively, than in winter and spring (0.21± 0.02 and 0.19 ± 0.02 µg|kg) respectively. Testosterone showed the higher concentration in autumn and summer (0.19 ±0.02 and 0.17 ±0.03 µg|kg) respectively, than in winter and spring (0.16 ± 0.03 and 0.12 ±0.02 µg|kg) respectively The results in this study were in agree with **Sonja *et al.,* (1998) and Mader *et al.,* (2006).**

Estrogen, progesterone, and testosterone are naturally present in beef cattle regardless of whether or not they have been treated with hormones. Despite the fact that these hormones are also present in human, the contribution of estrogen, progesterone and testosterone from beef is miniscule compared to the quantities produced naturally in the body. Further information is available on the content of testosterone,progesterone and estrogens in veal and beef in view published by the European Communities **(CEC,1989)** and on androstenedione and testosterone in tissues of calves, bulls and heifers **(Gaiani and Chiesa,1986),** on testosterone and estrogens in veal **(Scippo *et al.,* 1993)** and on estrogens and progesterone in tissues of steers and cows **(Tsujoka *et al.,*1992).**

Tissues from adult cattle can reach higher testosterone and progesterone concentrations than calves. However calves show comparatively high amounts of estrogens. These values are only exceeded by pregnant cows **(CEC, 1989).** The hormone patterns of male and female cattle obviously differ with heifers showing high levels of progesterone but lower levels of testosterone than male animals. Whereas fatty tissues accumulate lipophilitic hormones

There are climate stressors such as cold, heat, humidity, rain, ice, and wind that can affect the endocrine system and influence the reproductive system and normal estrous cycle performance of a cow. However, current knowledge available to scientists and producers is mostly based on heat stress. Heat stress is defined as a point on a temperature-humidity index (THI) above that is considered the thermo-neutral zone **(Mader *et al.,* 2006).** Continued exposure to heat stress has several known physiological effects such as an increase in plasma progesterone in cows, which results in problems with breeding **(Abilay *et al.*, 1975 and White *et al.*, 2002).**

Cows were in estrus longer in summer than winter or mounted more times per estrus during winter than in spring or summer and that the intervals between each mount was longer in summer, which infests the obvious, that there were fewer mounts throughout the day. Stressors, especially heat stress, can also affect an animals **(White et al., 2002).** EC regulations **(EC, 2008)** Thus, the quantities of hormones found in a serving of meat are far below the level considered to be a risk to the development of cancer **(Commission of the European Communities, Council1996).** Accumulations of these residues on the long run will be carcinogenic.

On other hand, beef may be exposed to toxic metals by air, water and ingestion of Polluted food, feeding animals with forage produced in contaminated areas resulting in increasing the concentration of heavy metals in meat and milk **(Jarup 2003).**

Table (2) showed that heay metal levels of lead, cadmium and copper were the highest in summer (0.082± 0.006 ,0.069 ±0.004 and 0.11± 0.008 ppm) respectively. The winter values were generally low for all the metals studied except lead which was the most dominant metal in meat samples through the entire period (0.045±0.003). All heavy metals had maximum concentration in summer season. These results were in agreement with **Naser *et al.*, (2007) and Ijaz *et al.*, (2013).**

The seasonal variations in the concentration of heavy metals, in present study, may be attributed to environmental condition variations and type of feed for animals to increase during summer season which resulted in huge amounts of wastes that must be disposal by burning and industrial activity in the area surrounded by sampling location.

Conclusion and Recommendations

Special attention should be paid, due to environmental pollution and hormone imitators or endocrine disruptors that are able to cause, at concentrations lower than earlier expected, a number of different undesirable responses. So we advice to use natural growth promotor not hormonal growth promotor and measures must be taken to reduce the negative impacts and/or to acclimating cattle to these stressors to the greatest possible degree. Proper management practices, nutrition, and optimum climatic conditions are indispensable for homeostasis and optimum productivity in cattle. Controlling exposure of an animal to adverse climatic conditions is also required for efficient animal production systems, health, and enhanced product quality. In view of present investigation, to avoid heavy metal residues entry to food chain through contaminated food and water, immediate measures must be carried out for the treatment and detoxification of industrial and domestic effluents intended to be used for agricultural purposes. Yet, meat may always contain substances exerting detrimental effects to the consumer's organism. The present study is a literature review of the most important potentially hormonal and heavy metals residiues were found in meat, their ways of getting into the meat and methods for reduction of these responses.

Table 1 Mean hormonal residues in meat samples (µg|kg) in different seasons.

Seasons / Parameters	Summer	Autumn	Spring	Winter
Esteradiol	0. 18± 0.03	0. 15 ± 0.02	0. 11±0.01	0. 13 ±0.02
Progesterone	0.24 ± 0.04	0.26 ±0.03	0.19± 0.02	0.21±0.02
Testesterone	0.17 ± 0.03	0.19 ± 0.02	0.12± 0.02	0.16 ±0.03

Table 2 Mean heavy metals residues in meat samples (ppm) in different seasons.

Season / Parameter	Summer	Autumn	Winter	Spring
Lead	0.082±0.006	0.068± 0.005	0.045± 0.003	0.062± 0.004
Cadmium	0.069± 0.004	0.038± 0.004	0.026± 0.002	0.032± 0.003
Copper	0.11± 0.008	0.017 ± 0.002	0.015± 0.001	0.019 ± 0.004

The hormonal substance used for growth promotion in cattle are either naturally occurring steroids as estradiol or estrogen, progesterone, and testosterone and their synthetic alternatives as zeranol, melengestrol acetate, and trenbolone acetate **(Maia *et al*., 2008).**

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