Effect of Method of Administration Of 17β-Estradiol On Hormonal Residues in Chicken Meat, Livers and Kidneys

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A B S T R A C T

Administration of hormones to broiler chickens for performance- enhancing purposes may lead to deposit of residues in their carcasses. This could be a potential human health hazard up on exposure to these residues. Therefore this study was performed on sixty chicks (one day old) were reared under the same condition. At 10 day old age the chicks were divided into 4 groups (control, I/M administrated group, oral administrated group and withdrawal group). Then hormonal residues were detected in chick's carcasses and their livers and kidneys using Enzyme-linked immune sorbent assay (ELISA) method. The results obtained that mean values of estradiol 14.2±0.20, 20.20±0.30 and 22.29±0.70 ppb in muscle, liver and kidneys of chicken, respectively. .The public health significance and recommendations for the use of hormones were discussed.

Key words: 17β-Estradiol, ELISA, Chicken anabolic hormones.

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1. INTRODUCTION

Broilers are some of the most economical meat protein sources available to consumers. Currently, chicken prices remain a bargain for the nutritional value, and this has held true for the last 40 years. The ability to efficiently use foodstuffs with minimal time to market size is the primary reason for chickens to be excellent meat sources. This efficiency is misinterpreted as unsafe because broilers are given hormones to achieve the growth rate with so little feed. (Marzouk, *et al.*, 2016)

Hormones produced by the bodies of humans and animals are called endogenous or natural hormones. Compounds chemically synthesized to mimic the effect of natural hormones are called synthetic or xenobiotic hormones. Hormones are vital in normal development, maturation and physiological functioning of many vital organs and processes in the body (Platter *et al.,* 2003).

17 β- Estradiol hormone used as growth promoter for poultry but it has great side effect on human who consume meat contain these hormone which made hepatocellular carcinoma and consider hepatotumorigenic agent also, it effect on gonadotrophins of lactating mother.

The administration of health-risk related substances such as growth promoting agents and hormones is a recurring problem in animal production where these compounds

are often used to increase the productivity and to reduce breeding costs

(Toffolatti *et al.,* 2006).

Most of researches concluded that 17β-estradiol usually used in chicken farms and given either orally as contraceptive tablets or intramuscular administration to increase the body weight in short time so these study performed to showed the effect of these hormone on chicks and detect the amount of residue for each method and also detect the withdrawal time for degradation of hormone from the body of chicks.

2. MATERIALS AND METHODS

2.1. Collection of samples:

Sixty (60) chicks (One day old) were kept under the same environment conditions and were given the same ration, vaccination and prophylactic program. They were reared at Animal Health Research Institute – Dokki – Giza. The birds were divided into 4 groups after 10 days into: control group which were fed on hormone free ration. The second group which was given I/M injection of folone (5mg estradiol) repeated every 10 days for 3 times. The third group which were given one tablets Microcept (0.03 mg estradiol) repeated every 10 days for 3 times and the fourth group which were given I/M injection of folone (5mg estradiol) only one and the residues were detected every week to estimate the withdrawal time, for detection of hormonal residues according to manual kits ELISA R-BiopharmaAG, Darmstadt, Germany.

1.2. Preparation of samples

1.2.1. Detection of 17 β- Estradiol residues:-

1) Skin and fat were removed from the muscle.
2) Ten grams of the ground muscle were homogenized with 10 mL of 67mMPBS (phosphate buffer saline) by mixer for 5min.
2) Two grams of homogenized sample were mixed with 5mL of tertiary butyl methyl ether (TBME) in a centrifugal screw cap vial and shaken vigorously by shaker for 30-60min.
3) The contents were centrifuged at 3000rpm for 10min.
4) The supernatant was kept and the extraction with TBME was repeated.
5) The supernatants were combined and evaporated then the dried extract was dissolved in 1mL of 40% methanol for MT and 80% methanol for TBA.
6) The methanolic solution was diluted with 2mL of 20mM PBS-buffer and applied to a RIDA C18 column (solid phase extraction column with C18 end-capped sorbent of an average particle size of 50µm) (Art. No. R2002); flow rate: 1 drop / sec.

- Column was rinsed by flowing of 3mL methanol (100%).

- Column was equilibrated by injection of 2mL PBS – Buffer (20mn).

- 3mL of sample was loaded on column.

- Column was rinsed by injection of 2mL methanol (40%).

- Column was dried by pressing nitrogen through it for 3min.

- Sample was eluted slowly by injection of 1mL methanol (80 %) An aliquot of the eluate was diluted with water, then 20µL per well of resulting solution was used in the test.

2.3. Test procedures

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

3. RESULTS

The mean value of estradiol in the second group (I/M administrated group) 14.2±0.20, 20.20±0.30 and 22.29±0.70 ppb in muscle, liver and kidneys of chicken, respectively.

The mean value of estradiol in third group (oral administrated) was 3.48±0.50, 6.280±0.42 and 8.64±0.40 ppb in muscle, liver and kidneys of chicken, respectively.

The mean value of estradiol in the fourth group in the 1st week were 12.01±0.01, 17.40±0.06 and 19.85±0.04 ppb and the results obtained marked decrease in the amount of hormone until 5th week the mean values were 2.46±0.04, 3.82±0.03 and 6.63±0.04 ppb in muscle , liver and kidneys of chickens respectively.

The results were calculated by this equation:

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

In order to obtain the 17β-Estradiol concentration in ppt actually contained in the samples. The concentrations were read from the calibration standard curve. {For 17β-Estradiol (Fig .A)}.

Table(1):- Statistical analytical results of 17β- estradiol residues (ppb) in the examined Chickens samples by ELISA method after I/M administration of (5mg)Folone repeated every 10 days for 3 times.

Examined tissues	Min.	Max.	Mean ±SE
Muscle	13.89	14.51	14.2±0.20
Liver	19.74	20.67	20.20±0.30
kidneys	21.07	23.45	22.29±0.70

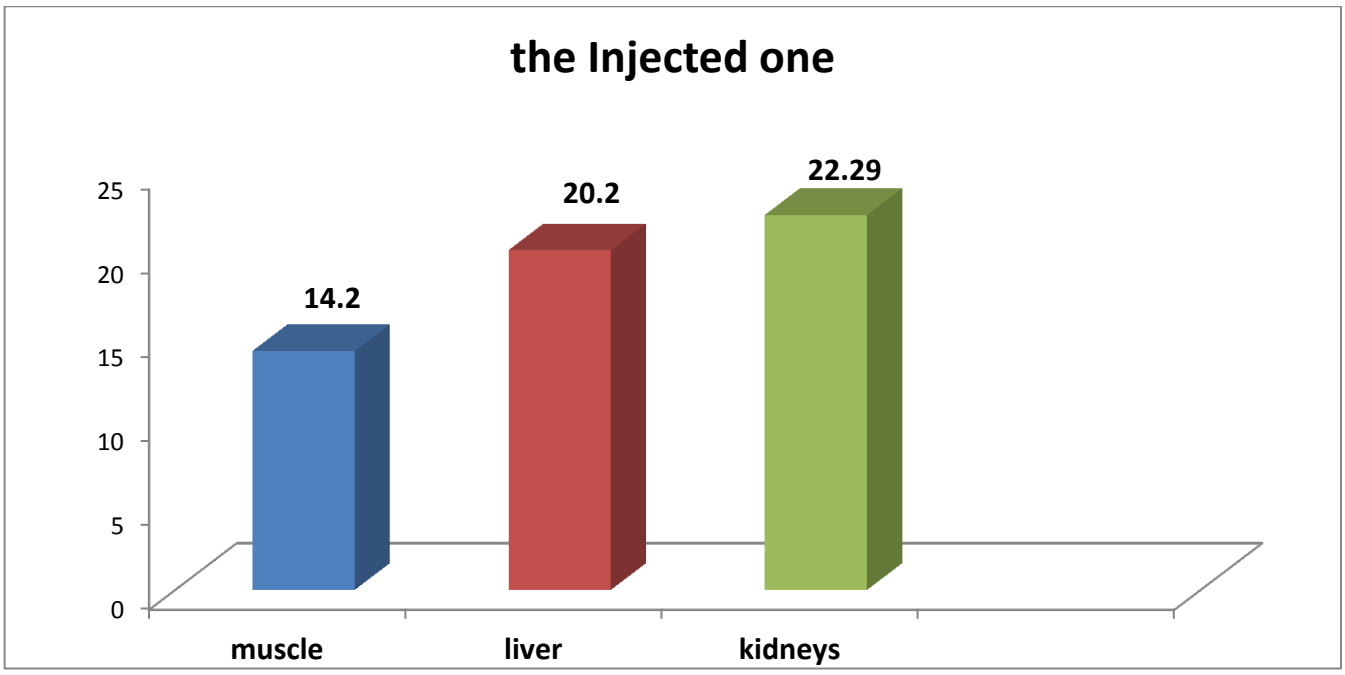


Fig (1): Mean values of 17β- estradiol residues in the examined chickens samples by ELISA method after I/M administration of (5mg)Folone repeated every 10 days for 3 times:

Table(2):- Statistical analytical results of 17β- estradiol residues (ppb) in the examined Chickens samples by ELISA method after oral administration of (0.03mg)Microsept repeated every 10 days for 3 times.

Examined tissues	Min.	Max.	Mean ±SE
Muscle	2.65	4.31	3.48±0.50
Liver	5.56	7.01	6.280±0.42
kidneys	7.93	9.35	8.64±0.40

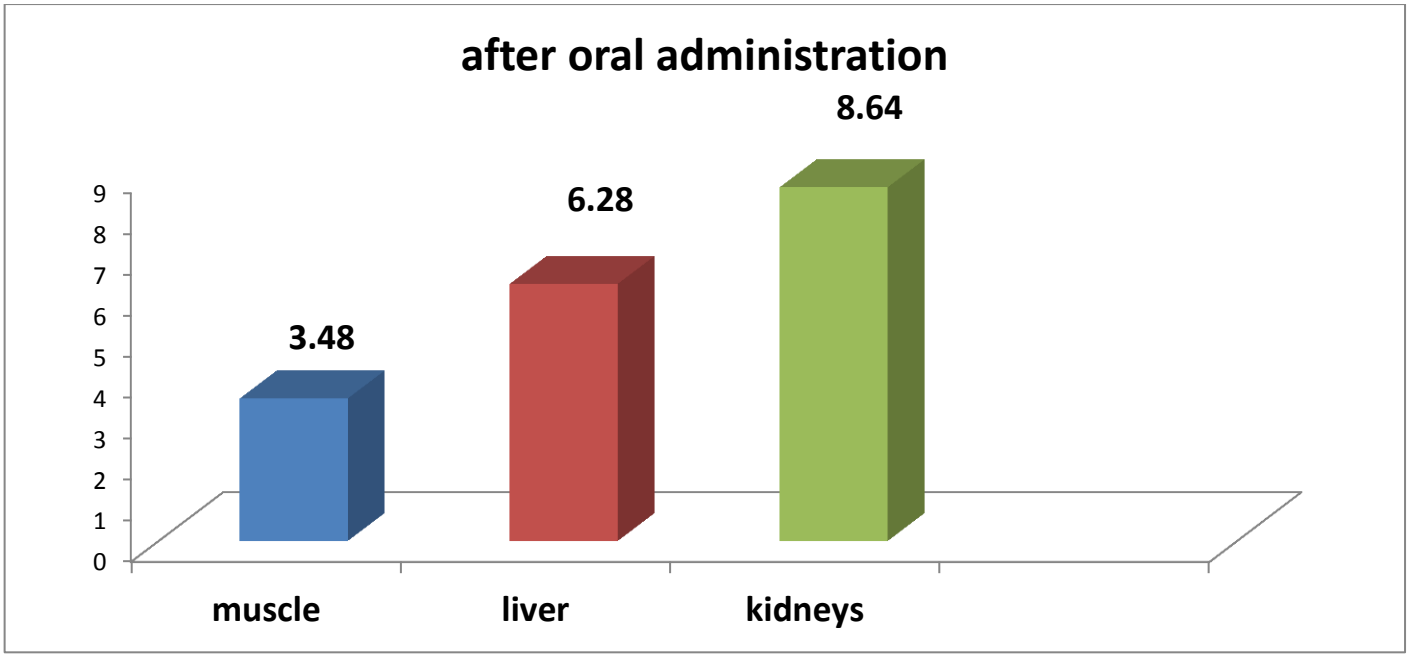


Fig (2): Mean values of 17β- estradiol residues in the examined chickens samples by ELISA method after oral administration of (0.03mg)Microsept repeated every 10 days for 3 times.

Table (3):- significance difference between means values of the control, injected and oral administrated chickens:-

	Muscle	Liver	kidneys
Control	0.02±0.0005 [a]	0.03±0.005 [a]	0.04±0.006 [a]
Injected	14.20±0.20 [b]	20.20±0.30 [b]	22.29±0.70 [b]
Feeding	3.48±0.50 [c]	6.280.42 [c]	8.64±0.40 [c]

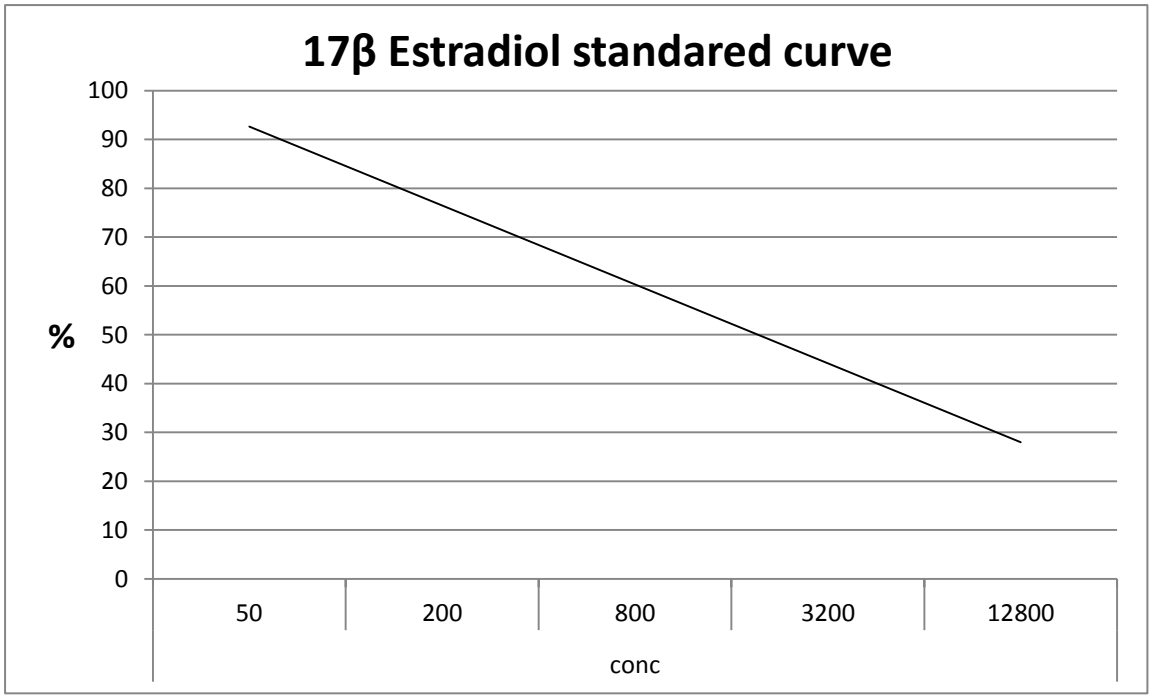
No significant difference (P<0.05) between cells contain same letter in the same column.

Table (4): Statistical analytical results of 17β- estradiol residues (ppb) in the examined Chickens samples by ELISA method after I/M administration of (5mg)Folone only one time then detection the withdrawal time every week:-

Withdrawal time	Muscle	Liver	kidney
1 week	12.01±0.01 $^{a\ I}$	17.40±0.06 $^{b\ I}$	19.85±0.04 $^{c\ I}$
2 week	9.96±0.07 $^{a\ II}$	13.68±0.06 b ^{II}	15.43±0.03 $^{c\ II}$
3 week	6.76±0.04 $^{a\ III}$	10.58±0.06 b ^{III}	12.01±0.01 $^{c\ III}$
4 week	4.37±0.08 a ^{IV}	7.52±0.02 b ^{IV}	9.26±0.03 c ^{IV}
5 week	2.46±0.04 a ^{V}	3.82±0.03 b ^{V}	6.63±0.04 c ^{V}

No significant difference (P<0.05) between cells contain same letter in the same row.

No significant difference (P<0.05) between cells contain same Roman letter in the same column.



(Fig.A): standard curve of 17β Estradiol S.

4. DISCUSSION

Recently, hormones and hormone like substances have been used in livestock production to obtain a high yield performance in shorter period of time. These agents are used to increase weight gain, to improve the food efficiency, storing protein and to decrease fatness. (Asia and Akzira, 2016)

According to the results that obtained by ELISA in table (1) and Fig(1) the mean values of 17β- estradiol were 0.782±0.07,

1.53±0.09 and 2.077±0.08 ppb with minimum values of 0.043, 0.379, 0.985ppb while maximum values of 0.621, 2.957, 3.377ppb in muscle, liver and kidneys, respectively

Lower findings were obtained by Doyle (2000) who found 17β- estradiol in chicken meat was ranged from < 0.03- 0.02 ppb. While higher findings were obtained by Sadek *et al*.,(1998) who found 17β- estradiol in chicken meat and Liver were 0.865, 4.216 ppb respectively.

The acceptability of samples according to permissible limit (>2ppb) stipulated by the Egyptian Organization for standardization and quality control (EOS),(1992)is shown in table (1). Accurately, 100 (100%), 29(29%) and 18(18%) samples in muscle, livers and kidneys, respectively. No samples were exceeding the permissible limit stated by Gracey (1986). While EL-Neklawy (1989) and Doyle (2000) found 46,45samples in muscle and fat respectively within the physiological level.

The presence of natural steroid hormones in chicken meat may be attributed to the fact that the natural steroid hormones are secreted by the gonads and adrenals (Rico *et al.*, 1981).

Table (2) and Fig (2) revealed that the mean values of zeranol residues 0.1065±0.01، 0.1235±0.01 and 0.262±0.02 ppb with minimum values of 0.011, 0.106 and 0.122 while maximum values of 0.143, 0.192 and 0.389 ppb in muscle, livers and kidneys, respectively.

Lower findings were obtained by Sadek *et al*.,(1998) who not found any residues of zeranol in chicken muscle .While higher findings were obtained by Xiamong *et al* .,(2002) who found zeranol residues (2.5 ppb) in liver samples of chickens.

Table (2) indicated that there were no samples above the maximum residue limit (MRL) 2ppb in muscle while, 10 ppb in liver according to Codex Alimentarius Commission (2017)

Accordingly, it seems that the present status of these anabolic hormones in market is not at risk but on the other hand, these results do not exclude the possibility of misuse of these anabolic hormones in the future and significantly increase exposure of humans, particularly children, to trenbolone which may adversely affect health. Therefore, there is need to routinely monitor these chemical residues as a food quality control measure.

Using hormones in broiler chicken farms for performance-enhancing purposes may lead to deposit of residues in their carcasses, particularly when the birds are slaughtered without the observance of withdrawal period of the hormones. Ignorance of observation of withdrawal period leads to a serious threat to human health upon exposure to these residues. Therefore, residues monitoring are required in detecting anabolic hormones for the safety of consumers (Donoghue and Hairston, 2000).

The results obtained by ELISA in table (1) and Fig(1) discussed the values of 17 β estradiol in chickens which I/M administrated of (5mg) Folone repeated every 10 days for 3 times beginning fromage10 days .the mean values were14.2±0.20, 20.20±0.30 and 22.29±0.70 ppb and with minimum values of 13.89 , 19.74 and 21.07 ppb while maximum values of 14.51, 20.67 and 23.45 ppbin muscle, liver and kidneys of chicken, respectively.

Nearly similar results were obtained by Hala (2009) who gave results with mean values 13.83±0.14, 20.66±0.16 and 23.06±0.14ppb and with minimum values of

13.01, 19.9 and 22.43 ppb while maximum values of 14.38 , 21.22 and 23.68 ppb in muscle, liver and fat of chicken, respectively.

Table (2) and Fig (2)discussed 17 β estradiol residues after oral administration of (0.03mg) Microsept tablets repeated every 10 days for 3 times beginning fromage10 days. The mean values were 3.48±0.50, 6.280±0.42 and 8.64±0.40 ppb with minimum values of 2.65, 5.56 and 7.93 ppb while maximum values of 4.31, 7.01and 9.35 ppb in muscle, liver and kidneys of chicken, respectively.

Nearly similar results were obtained by Hala (2009) who gave results with mean values3.55±0.12, 6.23±0.11 and 8.49±0.11 ppb and with minimum values of 2.63 , 5.68 and 7.78 ppb while maximum values of 4.28 , 6.83 and 9.17 ppb in muscle, liver and fat of chicken, respectively.

Also higher results were obtained by Abu- Taleb (2003) who examined the concentration of 17 β estradiol in chickens after treatment for 28 days with Microvlar 30(each tablet contain 0.03mg Ethinyl-Estradiol) and detected results with mean values 7.49, 17.82 and 18.38 ppb while the minimum values were 6.27, 16.12and 15.67 ppb with maximum values of 8.45, 19.45 and 19.66 ppb in muscle, liver and fat of chicken respectively. While lower results were reported by El-Neklawy(1989).

These differences may be due to the method of detection, the rout of hormone administration, the place where the sample was collected and the chicken breed.

Table (3) revealed that there were significant difference between muscles in control, injected and oral administrated chicks at ($P<0.05$) and also between liver and kidneys of all of them.

Table (4) detected the withdrawal time of 17 β estradiol in chickens after treatment only one with I/M administrated of (5mg) Folone then detect residue every week , in the 1st week the mean values were 12.01±0.01,17.40±0.06 and 19.85±0.04 ppb .

In 2ndweek the mean values were 9.96±0.07, 13.68±0.06 and 15.43±0.03ppb.

In 3rd week the mean values were 6.76±0.04, 10.58±0.06 and 12.01±0.01ppb
In 4th week the mean values were 4.37±0.08, 7.52±0.02 and 9.26±0.03 ppb.

In 5th week the mean values were 2.46±0.04,3.82±0.03 and 6.63±0.04 ppb in muscle, Liver and kidneys of chicken respectively. That's mean not occur complete degradation of hormones from the body of chickens till the time of marketing so it is forbidden to give hormones as growth promoters to chicken. Although Roushdy *et al.*, (1992) mentioned that a hormone withdrawal time of 20 days before marketing of treated chickens is recommended.

Nearly similar results after 3rd week were obtained by El-Neklawy(1989), El-Shorbagy(1997) and Hala(2009). On the other hand Richou-Bac *et al*.,(1978) said that the residues disappeared at 8 days. In this respect, Biondi *et al.,* (1992) and El-bayomy (1993) reported that the hormonal residues were persisted for a month to three months. It referred to the dose of the hormone applied and the tissue examined.

Also in table (4) there were significant differences at ($P<0.05$) between results in muscle, liver and kidneys in the same week, but no significant difference between results with other weeks.

Endogenous estrogens when given orally are largely metabolized during their first passage through the liver as well as diethylstilbestrol is resistant to hepatic metabolizes and when administered orally showed high oestrogenic activity this in-

accordance with the hypothesis of preston (1975) and Page (1991).

From these result concluded that by I/M injection of 17- β estradiol to broilers was indicated the presence of high level of hormonal residues in different tissues with the highest concentration in kidneys (organ of excretion) followed by liver then muscles but these residues were lower by oral administration .

The withdrawal time of hormone from the body of chicks need more time so it is forbidden to give hormones as growth promoters to chickens to avoid exposure to hormonal residues in poultry meat and its public health hazard.

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