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PRELIMINARY STUDY ON THE TOXIC EFFECT OF ETHINYLESTRADIOL IN CHICKEN

(With 5 Tables & 2 Fig.)

By

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دراسه أوليه عن التأثير السمى لمادة الايتينيل استراديول فى الدجاج

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فى هذه الدراسه تم معاملة ثلاث مجموعات من الكتاكيت دقى ٤ عمر ١٠ أيام بثلاث جرعات مختلفه من الايثينيل استراديول مقدارها ٣٥ ، ٧٠ ، ١٠٥ ميكروجرام لكل طائر عن طريق الفم على التوالى. اتضح ان هناك زياده فى وزن الطيور المعامله بالمقارنه بمجموعات الضابط وهذه الزياده لها علاقه مضطرده بالجرعه المستخدمه وزمن المعامله.

اتضح من التجربه زيادة الوزن النسبى للقلب ، المبيض ، عضلات الفخذ والصدر فى الطيور المعامله مقارنة بمجموعات الضابط.

بفحص صورة الدم حدث نقص فى الهيموجلوبين والحجم الكلى لخلايا الدم فى الطيور المعامله مقارنة بضابط التجربه .

سجلت نتائج التحليل البيوكيميائى لمصل الدم فى الطيور المعامله زيادة فى البوتاسيوم والكولسترول والبروتين الكلى بينما لوحظ حدوث نقص فى كل من الكلوريد ، الصوديوم ، الجلوكوز فى كل المجموعات ذات الجرعات والأزمنه المختلفه .

كان تركيز الايثينيل استراديول فى مصل الدم فى مجموعات الطيور ذات المعاملات المختلفه كالأتى : ٧ر ١٥٦ ، ٣٠ر ١٧٣ ، ٧٠ر ٢٢٦ نانوجرام / سم٣ بينما سجلت مجموعات الضابط ٥٠ ، ٥٥.٩٠ ، ٦٥.٥٠ نانوجرام / سم٣ على التوالى .

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SUMMARY

In this experiment, three groups of 10 day-old chicks were treated with different doses of the anabolic preparation of ethinylestradiol. The application of ethinylestradiol to chicks caused a significant increase in the body weight gain in comparison to the control birds. This increase in body weight was dose-related in the three treated groups of birds. Organ or tissue-body weight ratio of liver. Kidney, spleen, heart, ovary, breast or thigh muscles was affected by the administration of ethinylestradiol. The effect of ethinylestradiol on haemogram of tested birds was also taken into consideration. Some biochemical blood parameters were investigated parallelly. The concentration of ethinylestradiol in the serum of treated birds was 156.7, 173.3 and 226.7 ng/ml, meanwhile that of control birds was 50.0, 55.9 and 65.5 ng/ml.

Keywords: Ethinylestradiol in chicken.

INTRODUCTION

In several countries, ethinylestradiol is used illegally as a feed additive in chicken production. Objections have been raised against use of substances contain estrogen or estrogen like substances due to its carcinogenic or cocarcinogenic effect for the consumer *(HAPKE, 1988 and JANSEN* et al., *1989).* In spite of the illegal use of anabolic hormones. there are many private poultry farms far away from control measures administered doubtless these substances for fattening purposes.

The use of anabolic hormones in the fattening animals leads to an increase in body weight (10-20%) mainly musculature *(BERENDE and RUITENBERG. 1983).* Most frequently used hormones are diethyl-stilbesterol (DES) and its esters. The application of DES as an anabolic agent had been studied in chicken and laying hens to determine its distribution in liver. Kidney and muscles *(BENGTSSON 1978).* Ethinylestradiol is a synthetic estrogen which when given orally is 50 times effective as water soluble estrogenic preparations or 30 times effective as estradiol benzoate injected intramuscularly. The aim of our work was designed to estimate the growth rate and the toxic effects of ethinylestradiol on poultry and its level in sera of birds after increasing of dose and time of exposure.

MATERIAL AND METHODS

Anabolic preparation:

Norminest tablets (each tablet contains 0.035 mg ethinylestradiol) were manufactured by SYNTEX laboratories Inc. USA. Registered at the Ministry of health under No. 14637.

Experimental birds and procedure:

Sixty. One-day-old Dokki$_4$ female chicks were purchased from the poultry farm of faculty of agriculture. Assiut University, chicks were submitted to routine vaccination programs. Food and water were given ad libitum. At 10 days-old. chicks were classified into 4 equal groups, each of 15 chicks. The first three groups fed 35. 70 and 105 ug ethinylestradiol per bird orally for 10, 20 and 30 days respectively. Birds in the fourth group were kept as control.

Weighing and sampling:

Birds of the four groups were weighed after 10, 20 and 30 days from the beginning of the experiment. Every 10 days. 15 birds from treated group and 5 birds from control group were sacrified.

Two blood samples were taken from each bird, the first anticoagulated blood sample was used for estimation of total erythrocytic count (RBCs). Total leucocytic count (WBCs) and haemoglobin (Hb) concentration according to the standard methods of *CAMPBELL (1988)*. Meanwhile packed cell volum (MCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were estimated according to the standard methods of haematology *(COLES, 1986)*. The second coagulated blood sample was used for biochemical analysis after obtaining clear non haemolysed sera, blood serum Na and K levels were estimated using flame-photometer (Corning 400) while chloride level was estimated using chloride analyzer model 925. Blood serum total protein. glucose and cholesterol level were estimated using test kits supplied from Biomerieux (Bains and france) and after the methods of *Weich-selbaum (1946). Trinder (1969)* and *Zollner and Kirsch (1962)* respectively.

Ethinylestadiol concentration in the sera:

The concentration of ethinylestradiol in the sera of treated and control birds was quantitatively measured by radioimmunoassay *(XING* et al., *1983)* using coata-A-count estradiol kits provided by diagnostic products corporation

(DPC, 5700 west 96th street. Los Angeles. CA 90045, USA Estradiol), approximate sensitivity was 8 pg/ml.

Statistical analysis:

Statistical analysis of data were performed according to Kalton (1967).

RESULTS

It is clear from these experiments that the administration of low. moderate or high doses of ethinylestradiol caused an increase in the body weight of chicks compared with the control one. The difference was more clear in chicks treated with moderate and high doses respectively (Table 1 and fig. 1).

Heart, ovary and muscles (thigh or breast) body weight ratio in all treated birds were higher in comparison with the control birds. On the other hand liver and spleen were higher after 1st treatment and become lower after the 3rd dose. On contrary kidney showed lower kidney/body weight ratio after all treatments (table 3).

Haematological picture in all treated birds revealed a decrease in Hb and PCV, meanwhile RBCs and WBCs counts were increased after 1st and 3rd treatment but decreased after 2nd one (table 4).

Biochemical analysis of blood serum revealed a decrease in blood serum glucose in all treated birds, meanwhile there was an increase in both total protein and cholesterol. Blood serum electrolytes (sodium. potassium and chloride) levels showed no significant difference (table 5).

Serum ethinylestradiol concentration of treated birds with 35. 70 or 105 ug ethinylestradiol per bird after 10, 20 and days of exposure was higher than that in control one. The highest concentration was observed in birds received 105 ug ethinylestradiol while the lowest concentration was reported in chicks received 35 ug of this synthetic hormone. Between these two groups a moderate serum concentration was noticed in birds received 70 ug (table 2 and fig. 2).

DISCUSSION

Administrable contraceptive preparations have been used in poultry since several years ago. There is no available published data on the effect of these preparations in chicks specially after repeated doses. The primary objective of our study was undertaken to develop an effective method for

detecting the illegal use of contraceptives in private poultry farms. data in our study demonstrates that the application of low, moderate or high doses of ethinylestradiol effectively causing an increase in body weight of treated birds compared with the control groups. The increase in the body weight of treated groups was related to the dose and time of exposure which is mainly due to retention of water throughout the cells, in addition to growth and thickening of the cells due to cell proliferation and oedema or through rise in the retention of nitrogen in protein synthesis which causes an increase in body weight *(BAMBERG and NECHANSKY, 1983 and PINEDA, 1989)*. All treated birds with varying doses of ethinylestradiol demonstrate higher levels of this hormone in their sera than in control birds which is time and dose correlated.

There is no available data dealing with hemogram and biochemical parameters in poultry after administration of doping agents. It is well known that the concentrations of the different components of serum proteins are regulated by the liver, and there has been considerable interest in the hepatic dysfunction caused by oral contraceptives *(SMITH, 1974)*. It is now well documented that contraceptive drugs containing estrogen or estrogen combined with gestagens induce marked alterations in the pattern of plasma proteins. The significant decrease in blood serum glucose and the significant elevation in the blood serum cholesterol (table 5) may be due to disturbance in hepatic function. The obtained results can be attributed to the explanation of *KARLSON (1980)* who mentioned that the synthesis of fatty acids and cholesterin needs a large amount of NADPH which in turn needs direct oxidation of glucose-6-phosphate with NADP and this reaction use the glucose-6-phosphate in the cytosol of the cell. The cell takes the free form of the glucose from the blood which leads to hypoglycaemia. Also. *HASSAN (1980)* reported an increase in cholesterol levels in womens received ethinylestradiol.

Finally we can conclude that serum analysis and over growth can be used as a routine screening test to detect the illegal administration of oral contraceptives.

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Table 1. Daily body weight gain in grams per bird in both treated and control birds.

Birds	Time after treatments (days)		
	10	20	30
Treated (n=45)	4.20***	6.90***	11.10***
Control (n=15)	2.70	3.00	5.10

*** Highly significance at P<0.001

Table 2. Mean values ± S.E. of estradiol in the serum.

Birds	concentration (ng/ml serum)		
	after 10 days	after 20 days	after 30 days
Treated	156.66±5.30***	173.33±8.53***	226.66±10.13***
Control	50.00±1.13	55.85±2.33	65.50± 1.75

*** Highly significance at P<0.001

Table 3. Mean ± S.E. of organ-body weight ratio in treated and control birds

Time of exposure		Liver	Kidney	Spleen	Heart	Ovary	Thigh	Breast
10 days	Treated (n=15)	3.07* ±0.16	1.00 ±0.08	0.31* ±0.01	0.57* ±0.03	0.06* ±0.00	8.15 ±0.66	12.89 ± 1.05
	Control (n=5)	2.90 ±0.07	1.25 ±0.03	0.25 ±0.00	0.48 ±0.02	0.04 ±0.01	8.04 ±0.77	12.15 ± 1.16
20 days	Treated (n=15)	3.34* ±0.22	0.99 ±0.07	0.32* ±0.00	0.43 ±0.02	0.06 ±0.01	8.12 ±0.33	13.49 ± 0.95
	Control (n=5)	3.89 ±0.15	1.08 ±0.08	0.26 ±0.01	0.42 ±0.04	0.05 ±0.00	8.10 ±0.18	12.04 ± 1.15
30 days	Treated (n=15)	3.20* ±0.18	0.89 ±0.06	0.34* ±0.03	0.48 ±0.03	0.06 ±0.00	13.75 ±0.35	17.73 ± 1.01
	Control (n=5)	3.69 ±0.22	0.90 ±0.07	0.40 ±0.02	0.40 ±0.02	0.05 ±0.00	12.03 ±0.16	16.36 ± 1.38

* Significance at P<0.05

Table 4. Effect of ethinylestradiol on the haemogram of chickens

Time of exposure		RBCs (10^6/mm^3)	WBCs (10^3/mm^3)	Hb g%	PCV (%)	MCV (Fl)	MCH (Pg)	MCHC (g/dl)
10 days	Treated	2.62* ±0.19	23.43** ±1.55	6.83** ±0.51	22.67* ±2.15	90.40 ±3.88	27.24 ±1.56	30.15 ±2.42
	Control	2.42 ±0.15	19.60 ±1.88	8.00 ±0.75	23.00 ±2.18	95.04 ±5.56	33.06 ±2.85	34.78 ±3.13
20 days	Treated	1.96** ±0.17	23.57 ±2.05	6.97** ±0.55	21.67** ±1.95	114.46 ±5.75	37.77 ±2.13	32.09 ±2.75
	Control	2.14 ±0.13	24.20 ±1.97	8.40 ±0.68	25.00 ±1.55	116.82 ±8.33	39.25 ±3.13	33.60 ±2.13
30 days	Treated	2.65 ±0.21	25.01 ±2.13	8.13 ±0.75	26.67* ±2.35	100.87 ±5.61	30.70 ±2.85	30.61 ±3.18
	Control	2.56 ±0.18	24.50 ±1.92	8.30 ±0.83	28.00 ±2.50	109.37 ±6.13	32.42 ±2.32	29.64 ±1.95

* Significant at P<0.05
** Significant at P<0.01

Table 5. Some biochemical parameters related to the effect of ethinylestradiol in chickens

Time of exposure		Cl (mmol/L)	Na (mmol/L)	K (mmol/L)	Glucose (mg/100ml)	T.protein (g/100ml)	Cholesterol (mg/100ml)
10 days	Treated	102.67 ± 7.55	139.07 ±10.10	7.07 ±0.53	149.01** ±12.13	2.02* ±0.15	32.99** ±2.16
	Control	104.00 ± 5.85	142.40 ±10.85	6.30 ±0.72	181.11 ±10.85	1.98 ±0.13	28.91 ±1.33
20 days	Treated	98.67 ± 6.12	145.87 ± 9.35	5.80 ±0.33	146.17** ±10.65	2.86* ±0.17	37.41** ±2.85
	Control	107.00 ± 7.13	148.40 ±10.76	5.80 ±0.21	152.22 ±12.17	2.48 ±0.21	32.65 ±3.13
30 days	Treated	104.33 ± 5.18	167.07 ±5.33	7.27 ±0.55	162.34*** ±14.44	2.24 ±0.11	29.38* ±1.78
	Control	108.00 ± 4.65	173.60 ± 6.34	6.7 ±0.47	200.37 ±15.12	2.23 ±0.17	27.21 ±1.15

* Significant at P<0.05
** Significant at P<0.01
*** Highly significant at P<0.001

Fig.1. Daily body weight gain in gram per bird of treated and control chicks

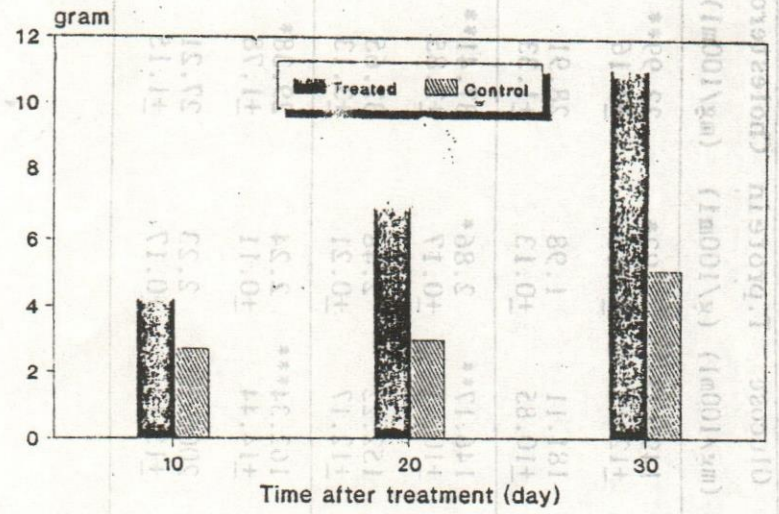

Fig.2. Mean values of estradiol in the sera of treated and control chicks