

EFFECT OF DIET DENSITY ON SERUM AND EGG YOLK CHOLESTEROL IN RELATION TO SOME BLOOD CONSTITUENTS OF LAYING HENS

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ABSTRACT

Fifty four laying hens at 24 weeks of age, from both of Fayoumi and Hy-line were used to study the relationship among cholesterol content in serum and egg yolk and some serum constituents. Birds of each strain were divided into three groups. The groups of each strain were assigned to different diet density of metabolisable energy level. Those of Fayoumi were of 2615, 2794 and 2987 Kcal ME/Kg diet. While those of Hy-line were of 2682, 2888 and 3076 KCal ME/Kg diet. Diets were isonitrogenous being 13% and 16% of crude protein for Fayoumi and Hy-line, respectively. Poultry fat by-product was added to the basal diet by levels of 0.5, 3.5 and 5.5%; and 1, 3.5 and 6.5% to adjust the applied ME for Fayoumi and Hy-line examined diets, respectively. The experiment was extended up to 40 weeks of age.

Results indicated the superiority of Hy-line hens in egg production (number and weight) and feed conversion over Fayoumi hens. In both strains, high levels of ME recorded best egg production scoers ($P < 0.05$) and less egg cholesterol than other dietary metabolisable energy level.

Fayoumi hens had ($P < 0.05$) higher serum cholesterol and egg yolk cholesterol than Hy-line hens.

Results revealed that there are positive and significant correlations among serum cholesterol, egg yolk cholesterol and each of glucosæ, total lipids, thyroxine, progesterone and estradiol in blood serum. Also, there are negative and significant correlation among triiodothyronine and gonadal steroid hormones studied.

INTRODUCTION

The evidence correlating serum cholesterol level with coronary heart disease was established. It is well known that cholesterol is a major component of the atherosclerotic plaque and that the induction of hypercholesterolemia is a prerequisite for the production of atherosclerosis in varieties of animal species. Cholesterol synthesis increases in hens as they mature, and this may be due to an increased demand for egg production (Mady, 1990). The developing oocyte utilises blood cholesterol carried by triacylglycerol-rich lipoprotein and vitellogenin (Hall and Mckay, 1994).

The levels of several serum constituents are quite different in laying hens when various reproductive states are compared. Because cholesterol is a major precursor in the biosynthesis of steroids, it seems important to investigate the ability of producing low yolk cholesterol content. The levels of these constituents are under the control of circulating hormones that are related to metabolism.

Apart from storing and transporting forms of metabolic fuel, the fatty acid portion of dietary fats serves several other important functions in the body (Sakomura, et al., 2003).

In the present study the effects of diet density of ME using (poultry fat by-product) on some blood serum profiles in relation to egg yolk cholesterol of Fayoumi and Hy-line layers were determined.

MATERIALS AND METHODS

This study was carried out during January to April. Two strains of laying hens, Fayoumi (F) and Hy-line (H), at 24 weeks of age were used. Fifty four hens of each strain were divided randomly into three groups. Birds in each group were subdivided into three replicates. All hens were allocated in slatted floor iron cages, each replicate in one cage of 70 x 70 x 75cm of length, width and height, respectively. The cages were kept inside a room of 8 x 3 x 2.5m for length, width and height, respectively. There were two windows covered with wire ganze while the ceiling was made from wooden logs. The experiment extended up to 40 weeks of age.

Three diet densities of metabolisable energy levels for Fayoumi 2615, 2794 and 2987 Kcal ME/Kg and three others for Hy-line 2682, 2888 and 3076 Kcal ME/Kg were formulated using poultry fat by product in level of 0.5, 3.5 and 5.5% for Fayoumi and 1, 3.5 and 6.5% for Hy-line diets. Diets were isonitrogenous being 13% and 16% of crude protein for Fayoumi and Hy-line, respectively. These diets were considered as low, medium and high metabolisable energy (ME). The medium level is the recommended requirement of the NRC, (1994). Food and water were available all time.

Egg production (number and weight) and feed intake were daily recorded for each replicate and the data was expressed for each eight weeks interval. Feed conversion (gm feed/gm egg) was then calculated.

At 24, 32 and 40 weeks of age, blood samples from five hens of each group within each strain were collected from the brachial vein, immediately centrifuged at 3000rpm for 20min and then serum was stored at -20°C till analysis. Serum glucose (Trinder, 1969), cholesterol (Watson, 1960) and total lipids (Frings et al., 1972) were determined by calorimetric method using commercial kits purchased from Bio-Merieux (France).

Direct radioimmunoassay (RIA) technique was performed to determine the serum hormones. Ready antibody coated tube kits for chickens (Diagnostic product corporation, Los Angelos) were used according to the procedure outlined by the manufacturer. Serum thyroxine (T_4) and triiodothyronine (T_3) were determined according to May (1978). The means of intra-assay coefficient of variations were 6.6% and 3.4% for T_4 and T_3 , respectively. The T_4/T_3 ratio was then calculated. Serum progesterone (P_4) and estradiol (E_2) were determined according to Etches et al (1981). The means of intra-assay coefficient of variations were 3.2% and 4.4% for P_4 and E_2 , respectively.

Five eggs from each group within each strain were collected at 24, 32 and 40 weeks of age. Yolk of each egg was separated, weighed and mixed. One gram from each yolk was mixed with 15ml of 2:1 chloroform-methanol, well shaken and then 5ml of distilled water was added to each sample and reshaked. Thereafter, samples were centrifuged at 2500rpm for ten min. The

aqueous methanol layer was removed by suction and discarded, while the chloroform layer was filtered through fiber glass filter paper into a test tube.

The tube was stoppered by rubber cork, stored at -5°C for assay and expressed as mg cholesterol/gm yolk (Washburn and Nix, 1974). Yolk cholesterol was determined according to Courchaine et al (1959).

Data were statistically analysed by the analysis of variance with the general linear model (GLM) procedure of the SAS institute (SAS, 1992), with strain and energy level as main factors. One way interaction was done and dropped from the model when it was not significant ($P > 0.05$). Significant differences among groups were analysed using Duncan's multiple range test (1955). Correlation coefficients among yolk cholesterol and some serum constituents were calculated (SAS, 1992).

RESULTS AND DISCUSSION

1 - Productive Performance:

Data of egg production (number and weight), feed intake and feed conversion are listed in Table 1. Obtained data revealed that Hy-line hens laid significantly ($P < 0.05$) more and heavier eggs than Fayoumi hens within each energy level. The strain differences could be attributed to genetic background.

Table 1: Means (\pm SE) of productive performance as affected by dietary energy level of Fayoumi and Hy-line layers

	Age in weeks	Fayoumi			Hy-line		
		L	M	H	L	M	H
Egg No.	24-32	17.9 \pm 1.6 ^a	18.8 \pm 1.7 ^a	20.4 \pm 1.8 ^d	25.8 \pm 1.1 ^c	29.7 \pm 1.0 ^b	37.8 \pm 1.2 ^a
	32-40	30.6 \pm 1.5 ^d	32.7 \pm 1.7 ^d	34.0 \pm 1.9 ^c	36.7 \pm 1.3 ^b	38.8 \pm 1.2 ^{ab}	41.5 \pm 1.8 ^a
Egg Wt. (gm)	24-32	35.5 \pm 2.4 ^d	38.7 \pm 2.3 ^c	38.8 \pm 2.1 ^c	63.4 \pm 2.6 ^b	63.8 \pm 2.5 ^b	66.7 \pm 2.7 ^a
	32-40	39.5 \pm 3.0 ^d	41.5 \pm 3.1 ^c	41.5 \pm 3.4 ^c	64.7 \pm 4.4 ^b	65.8 \pm 4.2 ^b	68.2 \pm 3.8 ^a
F.I. (gm/hen/day)	24-32	93.1 \pm 5.4 ^c	88.6 \pm 6.7 ^d	85.6 \pm 6.4 ^d	107.5 \pm 10.2 ^a	102.8 \pm 9.7 ^b	100.3 \pm 9.7 ^b
	32-40	114.5 \pm 9.3 ^c	108.2 \pm 8.9 ^c	98.7 \pm 10.2 ^d	121.4 \pm 11.2 ^a	111.2 \pm 10.2 ^b	107.4 \pm 9.9 ^b
F.C. (gm feed/gm egg)	24-32	6.2 \pm 0.8 ^a	5.7 \pm 0.6 ^b	5.2 \pm 0.8 ^b	5.2 \pm 0.5 ^b	4.5 \pm 0.4 ^c	3.7 \pm 0.4 ^d
	32-40	4.7 \pm 0.7 ^a	4.3 \pm 0.7 ^b	3.9 \pm 0.9 ^c	4.3 \pm 0.4 ^b	3.9 \pm 0.3 ^c	3.6 \pm 0.2 ^d

Means in the same row with no common superscripts differ significantly ($p < 0.05$).

L: Low energy diet M: Medium energy diet H: High energy diet.

F.I.= Feed intake F.C.= Feed conversion. n=54

Obtained results for Fayoumi and Hy-line hens revealed that egg production was significantly ($P < 0.05$) affected by energy level in the diet. Moreover, the results showed that high energy level recorded highest values of egg production (number and weight) for Fayoumi and Hy-line layers when compared with other energy levels. These results are in agreement with those obtained by Reddy et al (1980).

In both strains, birds consumed more feed with low energy diet as compared with medium or high energy diets. Boulos (1983) found that feed consumption decreased as the dietary energy level increased. Therefore, with isonitrogenous diets, the daily protein intake increases as the dietary energy level decreases.

Also, it could be observed that averages of feed intake of Fayoumi were lower than those recorded by Hy-line. This could be attributed to the relatively lower body weight of Fayoumi than Hy-line. In addition, Hy-line strain is a specialized for egg production which needs higher requirements for maintenance and production than Fayoumi. Similar results were found by Kondra et al (1968); and Bean and Leeson (2003).

Data of feed conversion revealed that Hy-line had significantly ($p < 0.05$) better feed conversion than Fayoumi. This could be related to genetic differences and strain effect. Energy level within each strain had significant effect ($p < 0.05$) on feed conversion. Moreover, birds fed high energy diet converted food to produce egg ($p < 0.05$) better than other energy levels. Attia (1986) and Thomas et al (1996); Swennen, et al., (2004) concluded that feed conversion to eggs improved ($P < 0.05$) by increasing the dietary energy level.

2 - Serum metabolites and yolk cholesterol:

Data of serum glucose, cholesterol, total lipids and yolk cholesterol are presented in Table 2. The obtained data revealed that Hy-line hens had significantly higher serum concentrations of glucose and total lipids than Fayoumi. On the other hand, they had significantly lower values of serum and yolk cholesterol than Fayoumi. Jiang and Sim (1991) and Hall and Mckay (1994); and Kim, et al., (2004) found negative relationships between yolk cholesterol and yolk weight and egg production capacity.

Obtained results indicated that the high energy level caused ($p < 0.05$) higher level of glucose, total lipids and cholesterol in blood serum for both strains when compared with other levels. While the high level of energy caused significantly lower values of yolk cholesterol when compared with other levels. This could be related to increase the production level occurred and/or decrease the feed consumption. Mady (1990) and Hall and Mckay (1994) came to similar observations. Edward and Savage (1992); and Grimes, et al., (1996); and Ponte, et al (2004) speculated that metabolic rate of glucose, total lipids and cholesterol increases by increasing dietary fat.

3 - Hormonal changes:

Obtained data on serum thyroxine (T_4), triiodothyronine (T_3), T_4/T_3 ratio, progesterone (P_4) and estradiol (E_2) are presented in Table 3. Obtained data revealed that Hy-line hens had higher values than Fayoumi in their serum of the studied hormones. Generally, strain differences might be dependent on and reflecting the strains distance and genetic background. Moreover, high levels of energy caused ($p < 0.05$) an increase in serum T_4 , P_4 and E_2 and ($p < 0.05$) decrease T_4/T_3 in both strains. From the results of T_4/T_3 ratio, it could be observed that T_4 converted more to T_3 by increasing the energy level, while the conversion patterns were similar in both strains.

Table 2: Means (\pm SE) of serum glucose, cholesterol, total lipids and yolk cholesterol as affected by dietary energy of Fayoumi and Hy-line layers.

Items	Age in weeks	Fayoumi			Hy-line		
		L	M	H	L	M	H
Gl. (mg/dl)	24	172.2 \pm 3.4 ^d	180.7 \pm 5.2 ^c	200.6 \pm 5.1 ^b	170.5 \pm 5.2 ^d	185.7 \pm 4.7 ^c	210.7 \pm 4.5 ^a
	32	167.6 \pm 5.2 ^d	189.5 \pm 4.2 ^c	215.2 \pm 5.3 ^b	200.3 \pm 5.2 ^b	210.6 \pm 5.3 ^b	248.4 \pm 5.5 ^a
	40	212.4 \pm 3.1 ^d	221.3 \pm 4.7 ^c	243.7 \pm 4.8 ^b	232.4 \pm 4.4 ^c	245.5 \pm 6.2 ^b	289.2 \pm 7.1 ^a
Ch. (mg/dl)	24	315.5 \pm 6.2 ^c	350.3 \pm 5.4 ^b	394.4 \pm 5.5 ^a	310.3 \pm 5.4 ^c	319.5 \pm 6.0 ^c	313.4 \pm 6.2 ^c
	32	325.7 \pm 7.0 ^c	360.5 \pm 6.4 ^b	382.2 \pm 6.2 ^a	320.7 \pm 6.5 ^c	339.8 \pm 7.2 ^c	346.5 \pm 7.4 ^c
	40	284.4 \pm 8.0 ^b	321.7 \pm 8.2 ^a	332.3 \pm 7.9 ^a	290.8 \pm 7.7 ^b	268.7 \pm 8.6 ^c	280.6 \pm 8.0 ^b
T.L. (mg/dl)	24	557.1 \pm 5.7 ^d	575.2 \pm 6.8 ^d	637.7 \pm 6.7 ^b	605.3 \pm 6.6 ^c	620.9 \pm 7.1 ^b	654.4 \pm 7.8 ^a
	32	565.9 \pm 6.2 ^d	581.5 \pm 7.1 ^d	648.4 \pm 7.0 ^b	630.5 \pm 6.4 ^c	647.2 \pm 8.0 ^b	732.3 \pm 8.3 ^a
	40	598.7 \pm 6.6 ^d	640.4 \pm 8.3 ^c	688.3 \pm 8.4 ^b	670.2 \pm 7.2 ^b	689.6 \pm 8.4 ^b	749.5 \pm 9.8 ^a
Y. Ch. (mg/gm)	24	15.3 \pm 1.3 ^a	14.4 \pm 1.5 ^a	14.2 \pm 1.1 ^a	13.4 \pm 1.2 ^b	13.4 \pm 1.2 ^b	12.4 \pm 0.8 ^c
	32	14.0 \pm 1.2 ^a	14.1 \pm 1.4 ^a	13.5 \pm 0.8 ^b	12.3 \pm 0.7 ^c	12.7 \pm 0.9 ^c	11.5 \pm 0.9 ^d
	40	13.8 \pm 0.9 ^a	13.8 \pm 0.8 ^a	13.0 \pm 0.7 ^b	12.1 \pm 0.8 ^c	12.3 \pm 0.8 ^c	11.2 \pm 0.6 ^d

Means in the same row with no common superscripts differ significantly ($p < 0.05$).

L: Low energy diet

M: Medium energy diet

H: High energy diet.

Gl.: Serum glucose

Ch.: Serum cholesterol

T.L.: Serum total lipids

Y.Ch.: Yolk cholesterol

n=5

Table 3: Means (\pm SE) of serum thyroxine (T_4), triiodothyronine (T_3), T_4/T_3 ratio, progesterone (P_4) and estradiol (E_2) as affected by dietary energy level of Fayoumi and Hy-line layers:

Items	Age in weeks	Fayoumi			Hy-line		
		L	M	H	L	M	H
T_4 (ng/ml)	24	9.6 \pm 0.2 ^a	10.4 \pm 0.2 ^c	11.4 \pm 0.1 ^b	10.6 \pm 0.2 ^c	11.8 \pm 0.2 ^b	12.4 \pm 0.3 ^a
	32	10.5 \pm 0.2 ^d	11.1 \pm 0.2 ^c	12.2 \pm 0.2 ^b	11.5 \pm 0.2 ^c	12.4 \pm 0.3 ^b	14.9 \pm 0.3 ^a
	40	11.6 \pm 0.3 ^d	12.2 \pm 0.3 ^c	12.8 \pm 0.3 ^c	12.4 \pm 0.3 ^c	13.9 \pm 0.4 ^b	15.8 \pm 0.4 ^a
T_3 (ng/ml)	24	1.8 \pm 0.1 ^b	1.4 \pm 0.1 ^c	1.2 \pm 0.2 ^d	2.3 \pm 0.1 ^a	1.7 \pm 0.2 ^b	1.3 \pm 0.2 ^c
	32	2.1 \pm 0.1 ^b	1.6 \pm 0.1 ^c	1.4 \pm 0.2 ^c	3.5 \pm 0.2 ^a	2.4 \pm 0.3 ^b	2.0 \pm 0.3 ^b
	40	2.4 \pm 0.2 ^b	2.6 \pm 0.2 ^b	2.8 \pm 0.2 ^b	5.6 \pm 0.3 ^a	4.8 \pm 0.3 ^b	3.6 \pm 0.4 ^c
T_4/T_3	24	5.3 \pm 0.6 ^c	7.4 \pm 0.8 ^b	9.5 \pm 1.0 ^a	4.6 \pm 0.5 ^c	6.9 \pm 0.8 ^b	9.5 \pm 1.0 ^a
	32	5.0 \pm 0.6 ^c	6.9 \pm 0.7 ^b	8.7 \pm 1.0 ^a	3.3 \pm 0.4 ^d	5.2 \pm 0.6 ^c	7.5 \pm 0.8 ^b
	40	4.8 \pm 0.5 ^a	4.7 \pm 0.5 ^a	4.6 \pm 0.4 ^a	2.2 \pm 0.3 ^b	2.9 \pm 0.3 ^b	4.4 \pm 0.4 ^a
P_4 (ng/ml)	24	0.89 \pm 0.01 ^d	0.96 \pm 0.01 ^c	0.109 \pm 0.02 ^b	0.104 \pm 0.02 ^b	0.111 \pm 0.02 ^b	0.132 \pm 0.02 ^a
	32	0.116 \pm 0.01 ^c	0.118 \pm 0.01 ^c	0.142 \pm 0.02 ^b	0.125 \pm 0.04 ^c	0.143 \pm 0.05 ^b	0.174 \pm 0.04 ^a
	40	0.132 \pm 0.01 ^c	0.130 \pm 0.01 ^c	0.167 \pm 0.03 ^b	0.149 \pm 0.04 ^c	0.172 \pm 0.05 ^b	0.198 \pm 0.06 ^a
E_2 (pg/ml)	24	36.4 \pm 2.3 ^d	40.3 \pm 2.4 ^c	43.4 \pm 2.3 ^b	39.8 \pm 2.2 ^c	42.4 \pm 2.1 ^b	48.8 \pm 2.4 ^a
	32	40.1 \pm 2.7 ^d	45.4 \pm 2.2 ^b	45.4 \pm 2.5 ^b	43.7 \pm 2.8 ^c	47.7 \pm 2.3 ^b	53.6 \pm 3.0 ^a
	40	48.8 \pm 2.6 ^b	48.8 \pm 2.4 ^b	48.6 \pm 2.4 ^b	50.6 \pm 3.0 ^b	56.9 \pm 2.7 ^a	63.7 \pm 3.1 ^a

Means in the same row with no common superscripts differ significantly ($p < 0.05$).

L: Low energy diet

M: Medium energy diet

H: High energy diet

n=5

The increase in serum P₄ concentration might be related to the increased release of gonadotropin hormones from pituitary gland. Etches et al (1981) reported a relationship between the release of gonadotropin hormones and its stimulation of ovarian follicles for progesterone secretion. While the significant increase in serum E₂ concentration at peak of egg production (32-40) weeks of age may be due to the association between estradiol level and yolk protein formation. Tixier-Biochard et al (1990) and Herber and Van Elswyk, (1996) came to similar observations. Moreover, the significant decrease of T₃ is confirmed by the result obtained by Sharp and Klandorf (1981); and Schreiner, et al., (2004) who attributed the decrease in T₃ hormone to the inverse relationship between gonadal steroid hormones and thyroid functions.

4 - Correlation coefficients:

The correlation coefficients among yolk cholesterol and some serum constituents are presented in Table 4. Obtained values of the correlations indicated that there are positive and significant correlation coefficients among yolk cholesterol and metabolic parameters studied, while it recorded a negative and significant correlation coefficient with serum T₃. The gonadal hormones P₄ and E₂ showed positive and significant correlation with the metabolic parameters. Serum concentration of T₃ showed negative and significant correlation with gonadal hormones. These results are in agreement with those observed by Etches et al (1981); Sharp and Klandorf (1981); Tixier-Biochard et al (1990) and Edward and Savage (1992) and Leeson, et al., (2001).

From these results it could be concluded that the metabolic activities increased due to increased dietary metabolisable energy, and positively affected serum steroid hormones which in turn augmented egg production. Moreover, hens fed high ME level used the cholesterol to biosynthesis more steroid hormones and led to produce eggs lower in yolk cholesterol than controls.

Table 4: Phenotypic correlation coefficients among yolk cholesterol and some serum constituents:

Items	Y.CH	Serum constituents						
		S. CH.	Gl.	T.L	T ₃	T ₄	P ₄	E ₂
Y.CH	1.00	0.615**	0.301*	0.455*	-0.312*	0.281*	0.281*	0.265*
S.CH		1.00	0.216*	0.356*	-0.018 ^{NS}	0.211*	0.310*	0.372*
Gl			1.00	0.281*	0.019 ^{NS}	0.312*	0.118 ^{NS}	0.105 ^{NS}
T.L				1.00	0.012 ^{NS}	0.186 ^{NS}	0.218*	0.226*
T ₃					1.00	0.318*	-0.411*	-0.382*
T ₄						1.00	0.512*	0.318*
P ₄							1.00	0.741*
E ₂								1.00

NS: No significant (P > 0.05) * : Significant (P < 0.05) ** : Significant (P < 0.01).

Y.CH: Yolk cholesterol

S.CH : Serum cholesterol

Gl : Serum glucose

T.L: Serum total lipids

T₄ : Serum thyroxine

T₃: Serum triiodothyronine

P₄: Serum progesterone

E₂: Serum estradiol

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تأثير كثافته العليقه على كولستيرول سيرم الدم وصفار البيض وعلاقته ببعض
مكونات الدم للدجاج البياض
فاتن عبد الفتاح احمد ابراهيم
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استخدم فى هذه التجربة عدد ٥٤ دجاجة عمر ٢٤ أسبوع من كلا من الفيومى والهاى لاين. قسمت دجاجات كسل سلالة إلى ثلاث مجموعات وقسمت دجاجات كل مجموعة إلى ثلاثة مكررات. استمرت التجربة حتى عمر ٤٠ أسبوع.

غذيت المجموعات الثلاثة لكل سلالة على ثلاثة مستويات من الطاقة الممتلئة باستخدام دهن الدواجن . بالنسبة لدجاجات الفيومى ضبطت العلائق لتحتوى على ٢٦١٥ و ٢٧٩٤ و ٢٩٨٧ كيلو كالورى طاقة ممتلئة / كجم عليقة. أما بالنسبة لدجاجات الهاى لاين فكانت العلائق تحتوى على ٢٦٨٢ و ٢٨٨٨ و ٣٠٧٦ كيلو كالورى طاقة ممتلئة / كجم عليقة. بينما البروتين الخام كان بنسبة ١٣% فى علائق الفيومى الثلاث و ١٦% بالنسبة لعلائق الهاى لاين.

ولقد أوضحت النتائج ارتفاع إنتاج البيض فى دجاجات الهاى لاين عن الفيومى بالنسبة لعدد ووزن البيض وكذلك لمعدل التحويل الغذائى. كما كان كولسترول السيرم وكولسترول صفار البيض أعلى معنوياً فى دجاجات الفيومى عن دجاجات الهاى لاين.

وكان أفضل إنتاج (عدد ووزن البيض) ومعدل التحويل الغذائى قد تحقق مع العلائق العالية فى الطاقة فى كلا من الفيومى والهاى لاين وحققت أقل مستوى لكولسترول صفار البيض فى كلا السلالتين. أوضحت النتائج وجود ارتباط إيجابى معنوى بين كولسترول صفار البيض وكولسترول السيرم وكلا من الجلوكوز، الليبيدات الكلية، والنثروكسين والبروجسترون والاستراديول فى سيرم الدم. بينما أوضحت النتائج وجود ارتباط سلبى معنوى بين الترايودثيرونين وهرمونات الجنس التى درست.