# EFFECT OF VITAMIN E AND THYROXINE ON SOME BIOCHEMICAL AND PHYSIOLOGICAL MARKERS OF LAYING HENS (ISA BROWN STRAIN).

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## ABSTRACT

The present work was conducted to evaluate the effect of dietary two levels of vitamin E (250 and 500 mg/Kg diet) with/or without single dose of thyroid hormone Thyroxin (0.25 mg/Kg diet), on the biochemical and physiological markers of Laying hens (ISA Brown Strain). A total of 120, 29 weeks-old layer, were housed in 120 pens and assigned to 6 diets groups, first group (control, receiving basal diet), the second and third groups received basal diet supplemented with vit-E 250 and 500 mg/kg diet respectively, the forth group kept on the basal diet supplemented with a single level of thyroid hormone 0.25mg/kg diet; the fifth and sixth groups received basal diet supplemented with the same level of thyroid hormone combined with the two levels of vit-E/kg diet. The study was accomplished and still for 8 weeks. Finally, dietary vit-E in second level and thyroid hormone improved physiological and antioxidant parameters and decreased lipid peroxidation as confirmed from lowering in malonic dialdehyde (MDA) and enhanced GPx activity, B and Gamma globulin. Moreover, it increased HDL-C, decreased triglycerides, LDL-c and risk ratio TC/HDL-C in laying hens (ISA Brown Strain). Combining both of thyroxine and V. E had a more pronounced effect than adding each solely in comparison to the control group. Keywords: Laying hens (ISA brown strain), plasma lipids, Antioxidant enzymes

(Catalase (CAT) & Glutathione peroxidase (GPx), MDA, Protein electrophoresis, gamma and beta plasma proteins.

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# INTRODUCTION

Enzymatic and non-enzymatic antioxidants are engaged in scavenging free radicals produced during cellular metabolism, of which superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), vitamin C and E, reduced glutathione (GSH), and total sulfhydryl groups (TSH) are of important concern (Chiu, *et al.*, 1982). During aging, antioxidant functions declined in almost all mammals (Harman, 1992). Also, high levels of free radicals have been reported (Sawada, and Crlson, 1987). A rise in free-radical level may be attributed either to its enhanced generation or to the reduction in antioxidant level. The mRNA level of the enzymatic antioxidants such as SOD, CAT, and GPx were quantified in order animals and found to be diminished Rao *et al.*, 1990).

Protection rendered by antioxidants in free radical-mediated pathological conditions has been reviewed (Halliwell, *et.al.*, 1992). Many studies have shown that the supplementation of an antioxidant vitamin C has normalized the levels of lipid peroxidation (LPO), GSH, and GPx (Jaychandran, *et al.*1996). Free radical attack on polyunsaturated fatty acids (PUFA) may result in the loss of PUFA (Sugiyama, *et.al.*, 1987) which plays

an important role in cell membrane structure, and the formation of lipid peroxides and related compounds which can cause damage to other cellular constituents. These are precisely controlled by several antioxidants which serve as a protective mechanism (Sugiyama, and Muramatsu, 1987). Supplementation with vitamin E prevents the elevation in plasma thiobarbituric acid reactive substances (TBARS) (Yutaka *et al.*, 1996).

The involvement of lipid peroxidation in liver injury in mammalian systems has been reviewed by Comporti, 1985. However, the degree of pathological tissue damage that can be linked directly to lipid peroxidation invivo is usually quite low. On the contrary, egg-producing chickens can suffer from a condition known as fatty liver hemorrhagic syndrome (FLHS), which is characterized by the accumulation of large amounts of fat in the liver with the subsequent development of liver hemorrhages (Couch, 1956 & Wolford and Polin,1974)[as quoted from Wu and Squires, 1997)]. In addition, increased lipid peroxidation is responsible for the development of hemorrhagic lesions in chicken liver as well as Squires and Leeson, 1988; and Squires and Wu (1992). Lipid synthesized in the liver are transported to the growing oocyte as components of lipoproteins, primarily very low density lipoprotein (VLDL), to form the egg yolk. The activity of fatty acid synthase in avian liver increases dramatically in response to increase circulating levels of estrogen as the female reaches sexual maturity. In some conditions, such as the excess consumption of high energy diets or insufficient production of lipoproteins, excess lipid accumulates in the liver of birds in active egg production (Squires, and Leeson, 1988).

High levels of polyunsaturated fatty acids (PUFA) in the diet result in decrease the levels of vitamin E in the body (Kaasgaard *et al.*, 1992), and led to increase lipid peroxidation *in-vivo* (Kaasgaard *et al.*,1992; and Hu *et al.*, 1990). On the other hand, vitamin E breaks free radical chain reactions and thus inhibits lipid peroxidation and led to decrease the formation of MDA (Haglund *et al.*1991), and consequently may reduce the incidence of FLHS.(Jensen, 1974).

It has been proved that vitamin E plays a major role in the development and the maintenance of the defense systems (Bird and Boren, 1999). He, *et al.* (2006), reported that dietary thyroid hormone improves the growth and the muscle protein accumulation of black-boned chickens. Also, adequate dietary levels of vitamin E are important; not only to prevent their deficiency signs, but also to preserve the organelles responsible for building antibodies and defense mechanisms against diseases and other stresses (Yu, 1994).

Free radical and non free radical oxidants can produce damaging effects in animal tissues if antioxidants are deficient. These oxidants are produced during the metabolism, and they may be substantially increased by aerobic exercise, stress tissue injury, infection and detoxification of many compounds. Stress may precede an infectious episode in animals by decreasing antioxidants needed later by an active immune response. Antioxidant nutrients such as vitamin E, B-carotene and the trace elements selenium, copper, zinc and manganese in enzymes are very important in protecting the animal's tissues from oxidative destruction. This protective benefit also results in improved immune responses which decrease infectious disease (Nockels, 1996).

Nutritional supplementation with antioxidants has been found to delay lipid peroxidation parallel to higher metabolism and enhance antioxidant capacity of the body. Thus, this study was conducted to investigate the effect of two levels from vitamin E with/or without single level of thyroid hormone Thyroxin supplementation to laying hens (ISA brown strain) given individual or combined in diet of layer hens. To investigate these affects on lipid peroxidation and lipid profile and some antioxidant enzyme GPx to asses some biochemical and physiological markers, as well as the influence of these antioxidants on lipid peroxidation and glutathione peroxidase activity.

## MATERIALS AND METHODS

### Materials:

## Vitamin and Thyroid hormone

Vitamin E was purchased from ...... and Thyroid hormone was purchased from ......

#### Laying Hens (Layers)

The present work was conducted in the farm of Fac.Agric.Ain Shams Univ. in July 2004. The experiment included 120 birds of laying hens (ISA brown strain) divided into 6 treatments (each 20 bird) which represent 20 replicate, every replicate was housed in individual pen. The experiment was continued for eight weeks. The layers were housed in stainless steel experimental animal's cages in air condition room at ambient temperature with a 16 hour light/dark cycle. Layers were kept for one week to adapt the laboratory conditions before starting the experiment and maintained on free access of water and a balanced diet. Layers in all treatments were kept under the same management system.

#### Diets:

Diets and water were offered to layers *ad-libtium* throughout the experimental period. Standard diet was supplemented with two levels of vitamin E (250 and 500 mg/kg diet) and single level of thyroid hormone thyroxin (0.25 mg/kg diet) and double treatments represent combination between the same two levels of vit E and the same level of thyroid hormone thyroxin. The composition of the experimental diets and diets for groups of treatment are shown in Table (1).

#### **Experimental Design and Groups:**

Age-matched 120 Laying hens (ISA Brown Strains) (29 weeks-old) were randomly divided into the following six groups: 1) Control group (untreated), hens kept on basal diet only for 8 weeks; 2) Vit-E250 group, hens kept on basal diet supplemented with lower dose of Vit E (250 mg/Kg diet); 3) Vit-E500 group, hens kept on basal diet supplemented with higher dose of vit E (500 mg/Kg diet) 4) thyroid hormone (H) group, hens kept on basal diet supplemented with certain dose of thyroid hormone thyroxin (0.25 mg/kg diet), 5) H-E250 combined group, hens kept on basal diet supplemented with both the same dose of thyroid hormone and the lower dose of vit E (250 mg/Kg diet); 6) H-E500 combined group, hens kept on basal diet supplemented with both the same dose of thyroid hormone and the lower dose of vit E (250 mg/Kg diet); 6) H-E500 combined group, hens kept on basal diet

supplemented with both thyroid hormone thyroxin and high dose of vit E (500mg/kg diet) for 8 weeks.

		Control	Diet 1	Die	et 2 %	Die	t 3 %	Diet 4	%	Diet 5
Ingredient	1	Diet Final	%							%
-	1	Weight %								
Yellow corn (8.5% CP)		63.0	63.0	6	63.0	6	3.0	63.0		63.0
Soybean meal (44 % CP)		18.0	18.0	1	8.0	1	8.0	18.0		18.0
Corn glutene meal (60% CP)		8.0	8.0	1	8.0	8	3.0	8.0		8.0
Lime stone		7.5	7.5	-	7.5	7	7.5	7.5		7.5
Bone meal		2.5	2.5	1	2.5	2	2.5	2.5		2.5
Vitamin and minerals Premix <sup>1</sup>		0.3	0.3	(	0.3		).3	0.3		0.3
Salt (NaCl)		0.4	0.4	(	0.4	(	).4	0.4		0.4
DL-Methionine		0.15	0.15	C	0.15		.15	0.15		0.15
L-Lysine		0.15	0.15	C	0.15		.15	0.15		0.15
Total		100	100	1	100	1	00	100		100
Calculated Nutrient Values										
Metabolizable Energy (ME)										
Kcal/kg	2810	2810	281	0	281	0	2810			2810
Crude Protein (%)	18.08	18.08	18.0	)8	18.0	08	18.08			18.08
Calories/Protein (C/P) ratio	155.42	155.42	155.	42	155.	42	15	5.42		155.42
DL-Methionine (%)	0.35	0.35	0.3	5	0.3	5	0	.35		0.35
Lysine (%)	0.73	0.73	0.7	3	0.7	3	0	.73		0.73
Calcium	3.67	3.67	3.6		3.6			.67		3.67
Available P %	0.43	0.43	0.4	3	0.4	3	0	.43		0.43
Vitamin & Thyroid hormone Standa		d 250mg E	500mg E		0.25 mg		250 mg E			00 mg E
	1				Thyro	oxin	+Th	vroxin	+	Thvroxir

Table 1. Composition of the experimental diet.

<sup>1</sup> provide per kilogram of diet: vitamin A (as all-trans-retinyl acetate); 5.500 IU; vitamin E (all rac-a tocopheryl acetate); 11 IU; menadione (as menadione sodium bisufite); 1.1 mg; Vit D3, 1.100 ICU; riboflavin, 4.4 mg; Ca pantothenate, 12mg; nicotenic acid, 44 mg; choline chloride, 191 mg vitamin B12, 12.1 ug; vitamin B6, 2.2 mg; thiamin (as thiamin mononitrate); 2.2 mg; folic acid, 0.55mg; d-biotin, 0.11 mg. Trace mineral (mg/kg of diet): Mn, 60; Zn, 50; Fe, 30; Cu, 5; Se.0.07.

## Methods:

#### **Collection of blood samples:**

At the end of experimental period (8 weeks), thirty six individual blood samples were randomly taken from the slaughtered layers (6 samples/group). Blood samples were collected from layers after slaughtered by cutting the throat jugular vein with a sharp knife. Blood was taken on heparin as anticoagulant then centrifuged at 3000 r.p.m. for 15 min. Plasma samples were carefully separated and stored frozen for different biochemical analysis. **Biochemical analysis:** 

## Determination of Blood Parameters:

Plasma albumin and total protein were determined according to Doumas (1971) and (1975). Plasma globulin was calculated by subtraction of albumin from total protein. Plasma total lipids; total cholesterol; high density lipoprotein-cholesterol (HDL-C) were determined according to the method of knight *et al.* (1972); Flegg (1973), Fruchart (1982) respectively; LDL-C and VLDL-C were measured as described by Friedewald *et al.* (1972). Determination of plasma triglycerides was done enzymatically according to the method of Fossati and Prencipe (1982).

#### Determination of Antioxidants markers:

Malondialdehyde (MDA) was determined according to method of Uchiyama and Mihara (1978) and Strove and Makarova (1989). Blood Catalase (CAT) was determined as stated by Sinha (1972), Glutathione peroxidase measured by the method of Paglia & Valentine (1967).

#### Protein Electrophoresis

Plasma protein electrophoresis fractionation was performed according to Henry (1974) and Killingworth *et al.* (1980) according to Helena Laboratories Beaumont, Texas kits.

### **Statistical analysis**

Data were subjected to analysis of variance using general linear model described in SAS User's Guide (SAS Institute, 1998). Differences among means were tested using Duncan's multiple range test (Duncan, 1955).

## **RESULTS AND DISCUSSION**

Data obtained for plasma total protein were presented in Table,1. Data revealed that total protein was insignificantly affected in all groups, except a significant reduction in total protein was observed in thyrooxin groups either individual or combined with vit-E. This lowering in total protein was significantly noticed either by using thyroxin individual or in combined with second dose of vitamin E. In addition, this lowering was observed in H-E250 combined group at first level of vitamin E, but this reduction was insignificant. Meanwhile, significant reduction was found in plasma globulin in Thyroxin group.

On the other hand, plasma Albumin was not significantly affected with all treatments. Meanwhile, albumin/globulin (A/G) ratio was significantly affected. Data revealed that thyroxin resulted in A/G ratio elevation. In contrary, vit-E reduced this ratio in all treatments either individual or in combined with thyroxin. However, this lowering effect was markedly shown in the second level of vit-E (500 mg/kg diet). While, adding thyroxin to that level of vitamin E resulted in elevation of A/G ratio as shown in combined group; whereas the ratio was raised from 0.33 to 0.47. Generally, lowering A/G ratio mainly attributed to decrease of plasma globulin content not to plasma albumin which was not significantly affected, as it is shown in Table (2).

or/and sin	gle dose (	of thyrox	n (0.25m)	g/kg diet)		
Groups	Control	E250	E500	Thyroxin	Thyroxin-	Thyroxin-
Parameter				-	É250	É500
Total Protein**(g/dl)	5.57 <sup>a</sup>	5.63 <sup>a</sup>	5.69 <sup>a</sup>	3.71 <sup>b</sup>	5.26 <sup>a</sup>	4.68 <sup>ab</sup>
	±0.55	±0.24	±0.27	±0.30	±0.19	±0.33
Albumin (g/dl)	1.78	2.07	1.75	1.70	1.70	1.44
	±0.15	±0.11	±0.37	±0.11	±0.19	±0.05
Globulin*(g/dl)	3.78 <sup>a</sup>	3.56 <sup>a</sup>	3.93 <sup>a</sup>	2.00 <sup>b</sup>	3.55 <sup>a</sup>	3.23 <sup>a</sup>
	±0.52	±0.34	±0.37	±0.37	±0.22	±0.37
A/G Ratio*	0.55 <sup>ab</sup>	0.53 <sup>b</sup>	0.33 <sup>b</sup>	0.78 <sup>a</sup>	0.49 <sup>b</sup>	0.47 <sup>b</sup>
	±0.08	±0.07	±0.04	±0.14	±0.07	±0.06

Table 2. Plasma proteins of laying hens (ISA brown strain) after supplemented diet with two levels of vitamin E (250 and 500 mg) or/and single dose of thyroxin (0.25mg/kg diet)

<sup>a-d</sup> Means within a row with different superscripts are significantly different. \*= p<0.05; \*\*= p< 0.01; \*\*\*= p<0.001.

However, this lowering in plasma total protein may be attributed mainly to thyroxin influence that resulted in higher metabolic rate in this group that may be accelerated the metabolic process which is given this significant reduction in plasma total proteins. Despite, albumin was not affected in all treated groups, but low A/G ratio indicates more disease resistance and immune response as mentioned by Griminger, 1986.Protein electrophoresis of plasma of lying hens (ISA Brown Strain)

Proteins of plasma of laying hens were fractionated on gel electrophoresis and illustrated in Fig.1a and the peak areas chromatograms were present in Figs (1b).

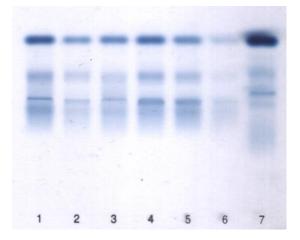
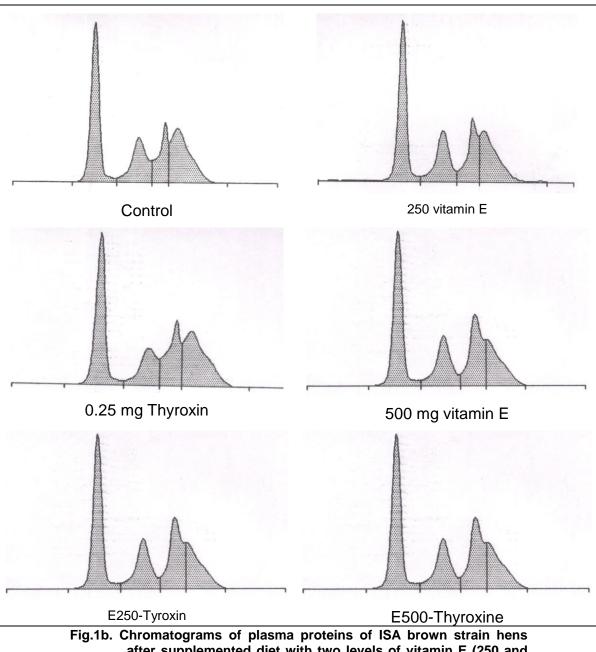
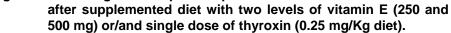


Fig.1a. Electrophoretic bands of Plasma proteins of ISA brown strain hens after supplemented diet with two levels of vitamin E (250 and 500 mg) or/and single dose of thyroxin (0.25 mg/Kg diet, (Standard proteins No.7).





# Influence of vitamin E and Thyroxin on Alpha2, beta and gamma globulins of laying hens

Plasma alpha2, beta and gamma globulins were illustrated in Fig.2. Data revealed that beta globulin was higher due to both vitamin E and thyroxin either individual or combined than control group. This influence was markedly shown in the second level of vit-E500. Meanwhile, second level of vit E or thyroxin resulted in lowering plasma alpha2 globulin compared to control group. Moreover, the second level of vit E increase gamma globulin percentage compared to all treatment. These results are in a harmony with Lawrence *et al.* (1985) who reported that vitamin E aids the immune response by reducing the production of the immunosuppressive prostaglandin (PGE<sub>2</sub>).

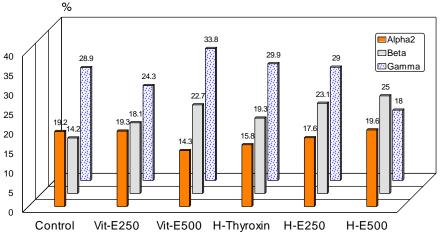


Fig.1. Variability in protein fractions % in plasma laying hens (ISA brown strain) after supplemented diet with two levels of vitamin E (250 and 500 mg) or/and single dose of thyroxin (0.25 mg/Kg diet).

## Lipids profile

Data of lipid profile and lipoproteins of laying hens were presented in Table (2). Data obtained revealed that plasma total lipids were significantly affected by both vit-E and thyroxin solely or in combined treatments. Supplemented diet with vit-E and Thyroid hormone resulted in significant decrease in plasma total lipids, and this significant decrease was markedly shown in second level of vit-E treatment comparison to all other groups and treatments.

Regarding to data tabulated in (Table,2), it is obviously shown significant reduction in plasma triglycerides as a result of supplementation diets with vitamin E and thyroid hormone either single or associated with each other. Lowering of plasma triglycerides level was markedly observed in all treatments especially in the thyroid hormone and mixed diet at first level of vit-E250 with thyroid hormone.

Respecting plasma total cholesterol (Table, 3) significant decrease was observed as a result of supplementation diet with vitamin E or thyroid hormone individuals or combined. Despite little raising was noticed in mixed

groups, but it is still significantly lowered than control group. Mezes,(1994); and Mezes and Salyi, (1994) reported that vitamin E have been shown to play a major role in the lipid metabolism.

#### Lipoproteins profile

Lipoproteins for plasma laying hens were shown in Table (2). Data indicated that lipoproteins were significantly affected due to all treatments. However high density lipoprotein cholesterol was significantly increased due to vit-E or thyroid hormone supplementation, but this significant increase was more pronounced in both the second level of vit-E500 and the first level of vit-E250 combined with thyroid hormone, while thyroid hormone treatment alone showed lowest value in HDL-C, but it is still significantly higher than control group.

Moreover, low density lipoprotein cholesterol presented in Table (2) also revealed significant reduction in LDL-C after diet supplementation either with vitamin E or thyroid hormone individual or combined with each other. The best value in this state was recorded to the second level of vit-E500 which gave lowest LDL-C (113.34 mg/dl) among all supplemented diets and control groups. Despite, thyroid hormones resulted in significant reduction in LDL-C, but the level was more than vit-E. Therefore, the elevation appeared in LDL-C in combined groups may be attributed mainly to thyroid hormone in combined groups not for vit-E.

In Addition, very low density lipoprotein cholesterol (VLDL-C) values presented in Table (2), showed significant reduction in all values due to applying both of vit-E or thyroid hormone individual or combined in diets.

Generally, lipid profile and lipoproteins were affected with vit-E and the change was towards the health effect. Nevertheless, also the high density lipoprotein cholesterol/total cholesterol (HDL-C/TC) ratio was converted to the lowered ratio which means best quality and good healthy growth with little cholesterol and good lipoprotein.

Table 3. Plasma lipids profile of ISA brown strain hens after supplementation diet with two levels of vitamin E (250 & 500 mg) or/and single dose of thyroxin (0.25 mg/Kg diet).

mg) or/and single dose of thyroxin (0.25 mg/Kg diet).										
Groups Parameter (mg/dl)	Control	E250	E500	Thyroxin	Thyroxin- E250	Thyroxin- E500				
Total Lipids**	1096.88 <sup>a</sup>	948.16 <sup>b</sup>	782.55 <sup>d</sup>	919.49 <sup>bc</sup>	903.53 <sup>cbd</sup>	820.43 <sup>cd</sup>				
	±26.50	±42.53	±62.15	±44.95	±30.76	±34.53				
Triglycerides**	313.23 <sup>a</sup>	205.88 <sup>b</sup>	206.37 <sup>b</sup>	185.29 <sup>b</sup>	185.78 <sup>b</sup>	215.19 <sup>b</sup>				
	±25.57	±12.82	±20.06	±8.09	±16.42	±21.58				
Total	258.28 <sup>a</sup>	201.57 <sup>d</sup>	196.84 <sup>d</sup>	212.12 <sup>cd</sup>	233.13 <sup>bc</sup>	237.23 <sup>ab</sup>				
Cholesterol**	±7.28	± 6.38	±8.60	±7.15	±9.679	±2.956				
HDL-C**	26.07 <sup>d</sup>	31.50 <sup>b</sup>	35.69 <sup>a</sup>	29.15 <sup>bc</sup>	38.31 <sup>a</sup>	31.68 <sup>b</sup>				
	±0.97	±1.56	±1.37	±1.26	±1.19	±0.20				
LDL-C**	175.6 <sup>a</sup>	130.12 <sup>cd</sup>	113.34 <sup>d</sup>	145.78 <sup>bc</sup>	164.17 <sup>ab</sup>	165.10 <sup>ab</sup>				
	±2.56	± 5.21	±8.70	±7.49	±13.78	±2.96				
VLDL_C**	62.64 <sup>a</sup>	41.17 <sup>b</sup>	41.27 <sup>b</sup>	37.05 <sup>b</sup>	37.15 <sup>b</sup>	43.03 <sup>b</sup>				
	±5.11	±2.56	±4.01	±1.61	±3.28	±4.31				
TC/HDL-C	9.90	6.39	5.51	7.27	6.08	7.48				

<sup>a-d</sup> Means within a row with different letters are significantly different.

\*= p < 0.05; \*\*= p < 0.01; \*\*\*= p < 0.001.

#### Lipid Peroxidation Malonaldehyde (MDA)

Lipid peroxidation was measured in all groups of laying hens and illustrated in Fig.3. Data depicted in Figure, 3 revealed that lipid peroxidation was increased significantly in thyroid groups compared to control group. Meanwhile, vit-E treatment resulted in remarkable lowering in lipid peroxidation either solely or combined with thyroxin in treated groups. As shown in Figure 3, it is obviously revealed that vit-E individually was highly effective on reduce lipid peroxidation in the second level more than first level as confirmed from single treatment or combined treatments. These data are coincided with Sahin et al. (2001) who found that MDA concentration was decreased in broilers reared due to increase supplementation of vit E in the diet.

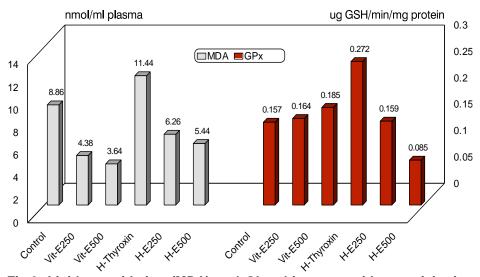


Fig.2. Lipid peroxidation (MDA) and Glutathione peroxidase activity in plasma of laying hens (ISA Brown Strain) after supplementation by tow levels of vitamin E (250 & 500 mg) with/or without single level of thyroid hormone thyroxin.

## Antioxidant enzymes Glutathione Peroxidase (GPx)

As shown in Fig.3 GPx activity in vit-E groups was similar to control group. Meanwhile, glutathione peroxidase activity was increased in the thyroxin group compared to control. This increase may be attributed to hormonal effect that resulted in higher elevation in metabolism consequently yields more hydrogen peroxide in metabolic pathway. Where peroxides produced are converted to water and oxygen by glutathione peroxidase, and so, the removing of this toxic peroxide needs more activity of glutathione peroxide from the metabolic pathway. On the other hand combined thyroxin with vit-E

lowered this higher activity of GPx which may be due to the scavenge potential of vit E could remove the radicals from metabolic pathway. This could be interpret on the fact that superoxide dismutase convert superoxide anion to hydrogen peroxide consequently may be vitamin E scavenge this radical and so the yield of hydrogen peroxide will decrease, and consequently GPx decreased as it is observed in the vit-E group.

Abaza, (2002) reported that glutathione peroxidase activity in blood, kidney and liver homogenates was increased significantly (p<0.05) with increasing selenium and vitamin E levels which was accompanied by raising of lipid content in liver. He also added that a pronounced decrease in blood and liver homogenates malondialdehyde content was found due to both selenium and vit-E. He concluded that, selenium and/or vitamin E (200 IU) supplementation to Japanese quail's basal diet was effective in improving GSH-Px activity to act as antioxidant, reducing free radicals and elevating the immune system response.

#### Catalase (CAT)

Data obtained in Fig.4 illustrated plasma catalase activity of laying hens in all treatments. Plasma catalase activity was affected due to vit E supplementation which led to decrease catalase activity in groups supplemented with vitamin solely or combined, while thyroxin-treated group catalase activity was close to control group.

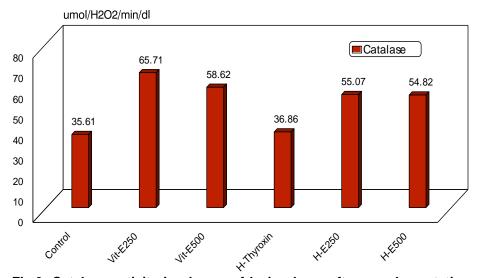


Fig.3. Catalase activity in plasma of laying hens after supplementation by tow levels of vitamin E (250 & 500 mg) with/or without single level of thyroxin.

#### **Glutathione Peroxidase, Catalase and MDA Interaction**

It is point out that the relation between GPx and MDA was clearly appeared from Figures (2 and 3). Increased supplementation vit E caused a decrease (p<0.05) in MDA level. There was a significant (P< 0.05) increase in

the activities of antioxidant enzymes in plasma such as catalase (CAT) as well as glutathione peroxidase (GPX) in hens treated with vit E in comparison with untreated controls. A great deal of significant (P < 0.05) variations were seen in reduced plasma MDA concentration in treated and comparison with untreated hens diet. Plasma lipid peroxidation levels were found to be significantly (P < 0.05) higher in non treated hens with vit E when they compared with other groups. Thus Vit E supplementation enhanced action of enzymatic and non-enzymatic antioxidants, which nullified the undesirable effects of free radicals that are generated during gowth.

#### Conclusion

From the data of the herein results, revealed that the supplementation of thyroxin and vitamin E alone or altogether decreased lipid peroxidation and led to increase HDL-C. Nevertheless Thyroxin led to increase the activity of GSH-Px in laying hens (ISA brown strain). Moreover, these effects were greater when both of them were supplemented together.

# REFERENCES

- Abaza, M. (2002). Immune system and some physiological aspects in Japanèse Quail affected by antioxidants
- Bird-JN; and Boren, B. (1999). Vitamin E and immunity in commercial broiler production. World Poultry.15: 7, 20-21.
- Chiu, D. Lubin, B. and Shoket, S.B. (1982). Peroxidative reactions in red cell biology. In *free radicals in Bio;ogy* (A.W. Pryor, eds.). pp. 115-153, Academic Press, New York, NY USA.

Comporti, M. (1985). Lipid peroxidation and cellular damage in toxic liver injury. *Lab. Invest.* 53, 599-623.
Doumas, B. T. (1975). Standard methods of protein determination. *Clin.*

Chem. 7 pp. 175 - 188.

Doumas, B. T.; W.Watson and H.G. Biggs (1971). Albumin standard and the measurement of serum albumin with bromocresol green. Clin. Chem. Acta. vol. 31 pp 87-96. Duncan, D. B. (1955). Multiple range and multiple F test. Biometrics, 11:1-42.

Flegg, H.M. (1973). An investigation of the determination of serum cholesterol by an enzymatic method. Ann. Clin. Biochem., 10: 79-84.

Fossati, P. and Prencipe, L. (1982). Serum triglycerides determined colomtrically with an enzyme that produces hydrogen peroxide. *Clin.* Chem., 28 (10): 2077-80.

Friedewaled W.T.; Leveey R.I and Fredrickson D.S. (1972). Estimation of plasma low density lipoprotein chollesterol concentration without use of the preparative ultracentrifuge. Clin. Chem. 18:499-509.

Fruchart, J.C. (1982). Rev Fr. Des laboratories, 105: 7-17, as quoted from pamphlet of bio Merieux.

Griminger. P. (1986). Lipid Metabolism in "Avian Physiology" edited by P. D Sturkie. 4th ed. Springer-Verlag, New York, Inc. USA.

Haglund O., Luostarinen, R.; Willin, R.' Wibell, L. and Saldeen, T. (1991). The effects of fish oil on triglycerides, cholesterol, fibrinogen and malonaldehyde in humans supplemented with vitamin E. J.Nutr. 121, 165-169.

Halliwell, B.; Gutteridge, J.M.C. and Cross, C. (1992). Free radicals, antioxidants and human disease; where are we now? J.Lab. Clin. Med. 119, 598-620

Harman, D. (1992) Free radical theory of aging, Mutat. Res. 275, 257-266.

- He, J.H.; M.H. Cao, F.X. Gao, J.H. Wang, K. Hayashi (2006). Dietary thyroid hormone improves growth and muscle protein accumulation of blackboned chickens. British Poultry Science 47,(5), 567 - 571
- Hu,M.-L.; Frankel,E.N. and Tappel, A.L. (1990). Effect of menhaden oil and vitamin E on in-vivo lipid peroxidation induced by iron. Lipids. 25,194-198.
- Henry, R.J.; D. C. Canon and J. W. Wnkelman (1974). Clinical Chemistry:
- Principals and Techniques. p437, Harper and Row, Hagerstow. Jaychandran, M.; Jayantht, B.; Sundaravadtvel, B. and Paneerselvam,C. (1996). Status of lipids, lipid peroxidation and antioxidant system with vitamin C supplementation during aging in rats. J.Nutr. Biochem. 7,270-275.
- Jensen, L.S.; Schumaier,G.W.; Funk, A.D.; Smith,T.C. and Falen,L. (1974). Effect of selenium and lipotropic factors on liver fat accumulation in laying hens. Poultry Sci. 53, 296-302
- Kaasgaard,S.G.; Holmer,G.; Hoy, C.-E.; Behrens, W.A. and Bear-Rogers, J.L. (1992). Effects of dietary linseed oil and marine oil on lipid peroxidation in monkey liver in vivo and in vitro. Lipids, 27, 740-745. Killingworth,L.M. et al. (1980). Protein Analysis, Diag Med. pp 47-58.

- Knight, J.A.; S.Anderson and J.M.Rawle (1972). Chemical basis of the sulfo phospho-vanillin reaction for estimating serum total lipids. Clin. Chem.18. No. (3).
- Lawrence, L.M.; Mathias, M.M.; Nockels, C.F. and Tengerdy, R.P. (11985). The effect of vitamin E on prostaglandin levels in the immune organs of chicks during the course of an E.coli infection. Nutr. Res. 5,497-509.
- Mezes, M. (1994). Effect of vitamin E treatment on early postnatal changes of vitamin E status of chickens. Acta Veterinaria Hungarica 42, pp.477-480.
- Mezes, M.; and Salyi,G. (1994). Effect of acute selenium toxicosis on the lipid peroxide status and the glutathione system of broiler chickens. Acta Veterinaria Hungarica 42, pp.459-463.

Nockels, CF. (1996). Antioxidants improve cattle immunity following stress. Animal Feed Science Technology. 62, 29-68.

- Paglia, D. E. and Valentine W.N. (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab.Clin. Med.70:158-169
- Ramnath, V.; P. S. Rekha and K. S. Sujatha (2007). Amelioration of Heat Stress Induced Disturbances of Antioxidant Defense System in Chicken by Brahma Rasayana. Oxford J. eCAM Advance Access published online on February 9, 2007.
- Rao, G., Xia, E., Nadakarukaren, M.J. and Richardson, A. (1990). Effect of dietary restriction on the age-dependent changes in the expression of antioxidant enzymes in rat liver. J. Nutr. 120, 607-637.
- SAS, 1998. SAS Procedure Guide. Version 6.12 Edition. SAS Institute INC., Cary, NC, USA.
- Sahin,K; N.Sahin; M.Onderci; S.Yarallioglu and O.Kucuk (2001) Protective role of supplemental vitamin E on lipid peroxidation, vitamin E,A and some mineral concentrations of broilers reared under heat stress. Vet. Med-Czech, 46,5,140-144

Sawada, M. and Crlson, J.C. (1987). Changes in superoxide radical and lipid peroxide formation in thee brain, heart and liver during the lifetime of the rat. Mech. Ageing Dev. 41, 125-137

Sinha, A. S. (1972). Colorimetric assay of catalase. Anal. Bioch. 47, 389-394.

- Squires, E.J.and Leeson, S. (1988). Aetiology of fatty liver syndrome inlaying hens. Bri. Vet.J. 144, 602-609.
- Squires, E.J.and Wu, J. (1992). Enhanced induction of hepatic lipid peroxidation by ferric nitrilotriacetate in chickens susceptible to fatty

liver rupture. *Bri. Poultry Sci.* 33, 329-337. Strove, E.A. and Makarova, V.G. (1989). Laboratory Manual in Biochemistry. 1<sup>st</sup> Ed. Mir. Publishers Moscow. pp. 251-253.

- Sugiyama, J., and Muramatsu,K. (1987). Effects of excess D- and L-methionine diets on growth and hepatic enzyme activities in rats. *Agric.Biol.Chem.* 51,3411-3413.
- Sugiyama, J., Kushima, Y. and Muramatsu,K. (1987). Effect of dietary glycine on methionine metabolism in rats fed high methionine diet. J.Nutr.SciVitaminol. 33,195-205.
- Uchiyama, M. and Mihara, M. (1978). Determination of malonal dehyde precursore in tissues by thiobarbitric acid test. Anal. Biochem., 86: 271-278.
- Yutaka, O.; Kasai, T. and Kiriyama, S. (1995). Vitamin E prevents the elevation of thiobabituric acid-ractive substances but not hemolytic anemia in rats fed excess methionine. *Nutr.Biochem.* 7:77-84.
- Yu, B. P. (1994). Cellular defenses against damage from reactive oxygen species. Physiol. Rev. 74: 139-162.

ت أثير فيت امين ه والثير وكسين على بعض المؤشرات الحيوية والفسيولوجية فىالدجاج البياض سلالة إيزا البنية

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صممت هذه التجربة لدراسة تأثير فيتامين هـ وهرمون الثيروكسين على القياسات البيوكيميائية والفسيولوجية في الدواجن البياض من سلالة (ISA Brown Strain) . تم استخدام مستويين من فيتامين هـ (250 ، 500 مجم) ومستوى واحد من هرمون الثيروكسين (0.25 مجم/كجم وجبة).

أظهرت النتائج زيادة في مستوى البروتينات من نوع بيتا جلوبيولين وألفا 2 ، كما حدث نقص معنوى في مستوى الكوليستيرول وفي نفس الوقت صاحبه زيادة في مستوى البروتين العالي الكثافة مع حدوث نقص في مستوى البروتين المنخفض الكثافة . كما أظهرت القياسات الدالة على حالة الأكسدة الليبيدية أن نسبة الأكسدة الليبيدية قد انخفضت وتبين ذلك من تناقص مستوى المالونالدهيد كما حدث زيادة في نشاط إنزيم الجلوتاثيون بير أكسيديز وفي إنزيم الكتاليز نتيجة المعاملات. ويلاحظ أن نشاط الجلوتاثيون بير اكسيديز زاد في وجود هرمون الثير وكسين وذلك يمكن أن يكون بسبب أن الهرمون أدى إلى زيادة الميتابوليزم ، وبالتالي زيادة الإحتياج إلى التخلص من نواتج الأكسدة مثل فوق أكسيد الهيدروجين الناتج في مسار التمثيل الغذائي ، مما أدى إلى زيادة نشاط الإنزيم . ويلاحظ تناقص درجة نشاط الإنزيم في وجود فيتامين هـ مع الهرمون حيث أن الجرعة (250مجم/كجم وجبة) أدت إلى خفض النشاط بدرجة أقل من الجرعة 500 مجم من الفيتامين أكما يلاحظ أن فيتامبن هـ الجرعة 250 ، 500 بدون الهرمون كانت في مدى قيم الكنترول . وتخلص الدراسة إلى أن تدعيم الوجبة بفيتامين هـ مع الهرمون يؤدى إلى خفض الإجهاد الناتج من زيادة الميتابوليزم .

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