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Effect of Natural Antioxidant on Oxidative Stability of Eggs and Productive and Reproductive Performance of Laying Hens

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Abstract: The main target of this study was to determine the effect of dietary natural antioxidants: Thyme, rosemary, oregano and curcuma longa on production and reproduction performance of laying local hens and study the oxidative stability of yolk shell egg storage at room temperature (16°C±2). A total number of 360 hens and 36 cocks from El-Salaam strain of 28 weeks old were divided into 12 groups with 3 replicate each (10 hens +1 cock). Birds were fed on the experimental diets, using two levels (0.5 and 1.0%) from four types of herbs (oregano, thyme, rosemary, curcuma longa) as natural antioxidants, in comparison to two levels of vitamin E (100 and 200) mg/Kg diet, in addition to two diets, one using vitamins and minerals premix with vitamin E (control) and the other without vitamin E (Negative-control).The main results obtained from this study can be summarized as follows: Addition of herbs as natural antioxidants during the laying period can improve the production performance especially at 1.0% thyme, rosemary, oregano or 0.5% curcuma longa increased egg production, egg mass and improved feed conversion. Addition of 1.0% curcuma longa increased numerically values of shell weight and egg shape index. This treatment increased percentage of yolk weight and improved yolk colour by 10.87% and 15.62%, respectively compared to control group. Addition 1.0% oregano gave the highest values of all nutrient digestibility coefficients; except ether extract digestibility that was the lowest value compared to control diet, however it was statistically insignificant. Statistically, the highest values ($P<0.05$) of antibody titter against sheep red blood cells were recorded for hens fed 1.0% thyme or rosemary. Thyme or rosemary at 1.0% significantly decreased plasma total lipid, in comparison to the control by 17.15 and 27.15%, respectively while total cholesterol and LDL- cholesterol decreased insignificantly. Addition of thyme, rosemary or curcuma longa at 1.0% significantly decreased yolk total lipid, in comparison to the control group by 12.14, 13.19 and 13.95%, respectively. Addition of 1% oregano or rosemary or 0.5 and 1.0% curcuma longa during laying period significantly decreased malonaldehyede formation in egg yolk and had positive effect on oxidative stability of shell eggs storage at room temperature(16ºC±2). Addition of 1.0% oregano, rosemary or 0.5% curcuma longa significantly increased the percentages of fertility. While 1.0% thyme or 0.5-1.0% curcuma longa significantly increased the percentages of hatchability of fresh eggs. It could be concluded from this study that herbs could be used as natural antioxidants during laying period. Oregano, rosemary or thyme at 1.0% or curcuma longa at 0.5% can improve productive performance, fertility and hatchability and had positive effect on oxidative stability of shell eggs during storage.

Key words: Natural antioxidants, herbs, oxidative stability, productive performance, fertility and hatchability

Introduction

Lipid oxidation during food processing and storage is of major importance. As the polyunsaturated lipids oxidize, they form hydro peroxides, which are susceptible to further oxidation or decomposition to secondary reaction products such as short-chain aldehydes, ketones and other oxygenated compounds that may adversely affect on the overall quality of foods, including flavour, taste, nutritional value and production of toxic compounds (Vercellotti *et al*., 1992). The oxidative stability of shell eggs in storage has not been a major problem normally. Eggs seemed to have built-in antioxidant characteristics that maintain the flavour in extended storage. Constituents such as position appear to be very effective in preventing oxidation of yolk lipids. Recently, however, dietary modified eggs have been produced and there is extensive interest in their sales potential. Such eggs are highly unsaturated lipids and therefore, they may be more prone to oxidation during storage, particularly, at low pH as in yolk containing acidic foodstuff preparations. Eggs contain oxidation products that may lower their nutritive value (Marshall *et al*., 1994). Therefore, as the emerging egg technology produces more dietary modified eggs, there may be an increasing interest in the oxidative deterioration of marketed eggs. A major preventive measure of lipid oxidation is the use of natural and synthetic antioxidants that function either by scavenging chain-carrying peroxyl radicals or by diminishing the formation of initiating lipid radicals (Yamamoto and Niki, 1990). Synthetic antioxidants such as butylated hydroxy toluene and butylated hydroxy anisole are currently approved to control lipid oxidation

in foods, but recently cancer restrict their use (Imaida *et al.*, 1983; Okada *et al.*, 1990). Therefore, we need to create research for alternative antioxidants. Dietary supplementation with alpha tocopherol has been demonstrated to beneficially affect enhancement of lipid stability in foods from animal origin, such as poultry meat (Ajuyah *et al.*, 1993) and eggs (Cherian *et al.*, 1996; Galobart *et al.*, 1999). Natural antioxidants such as herbs are used as a substitute for synthetic antioxidants to stabilize fat containing foodstuffs. Rosemary leaves has essential oils 1.50% (Radwan, 2003). Rosemary extracts have a wide range of different phenolic compounds with biological activities; Carnosic acid is the most active antioxidant present in rosemary (Cuvelier *et al.*,1996; Richheimer *et al.*, 1996; Offord *et al.*, 1997) with an antioxidant activity three times higher than carnosol and seven time higher than butylated hydroxy toluene and butylated hydroxy anisole (Richheimer *et al* .,1996).

Eugenol, carvacrol and thymol are the major components present in clove, oregano and thyme essential oils, respectively and butylated hydroxy anisole have similar molar equivalent antioxidant capacities (Dorman *et al.*, 2000).

The essential oil of thyme ranged between 1.10-1.40%; the main active components of the essential oil were thymol (40-60%) and carvacrol (1-5%), Radwan (2003). Studies on the stabilizing activity of the essential oil of thyme in lipid systems; Botsoglou *et al.* (1997) found that hens fed thyme have a lower concentration of malondialdehyde yolk and indicated that possible transfer of the antioxidant constituents of thyme into the hen through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into the yolk. Carvacrol and particularly thymol are the phenolic components in thyme which are primarily responsible for its antioxidant activity (Farag *et al.*, 1989; Deighton *et al.*, 1993).

Botsoglou *et al.* (2002) reported that, iron-induced lipid oxidation showed that as oregano oil increased in the diet, malondialdehyde values decreased in tissue samples and so might be promising as dietary supplements. The rhizome of Curcuma Longa L. (family: Zingiberaceae) named turmeric is a perennial herb. Curcuma Longa L. has received attention as a component of designer foods for its cancer-preventing ability (Kelloff *et al.*, 1996). Curcumin a major component in turmeric has a potent antioxidant activity (Ruby *et al.*, 1995; Sreejayan, 1994).

Chick embryos may be subjected to stress caused by excessive production of heat during the later period of egg incubation (Tullett, 1990). The development of the chick embryo is associated with the accumulation of highly polyunsaturated fatty acids within the lipids of several embryonic tissues (Noble and Speake, 1997; Speake *et al.*, 1998). This makes embryonic tissues highly susceptible to lipid peroxidation and free radicals through hatching period (Surai, 1999a). Tissue - specific features in the susceptibility to lipid peroxidation were found with the brain being the most susceptible to lipid peroxidation at day 25 and in day-old poults of Turkey (Surai *et al.*, 1999) and at day 15 of incubation period of chicken eggs (Noble *et al.*, 1993). Therefore, the integrated antioxidant systems in the chicken tissues are responsible for protection of polyunsaturated fatty acids, protein and DNA from damaging effect of free radicals and toxic products of their metabolism (Surai *et al.*, 2003). In such condition, oxidative stress may be a problem during the last days of prenatal and 1st days of postnatal chick life. This lead to decrease hatchability and increase mortality post hatch and consequently economic impact on the poultry industry. These necessitate the development of effective antioxidant capacities in the tissues to prevent lipid peroxidation. The antioxidant system of the embryo and newly hatched chick is based on antioxidant enzymes such as superoxide dismutase, glutathione reoxidase and catalase (Surai, 1999a, b) and vitamin E (Surai, 1999b), carotenoids (Surai *et al.*, 2001a, b) ascorbic acid (Surai *et al.*,1996) and reduced glutathione (Surai, 1999b). The antioxidant system of the developing chick could be enhanced through of the maternal diet. The objective of this research was to determine the effect of supplemented natural antioxidants; Thyme, Rosemary, Oregano and Curcuma longa on production and reproduction performance of laying local hens and study the effect on yolk oxidative stability of shell eggs during storage.

Materials and Methods

The present study was carried out at Sakha Animal Research Station, Animal Production Research Institute, Ministry of Agriculture, Egypt. The chemical analyses were carried out at Laboratories of the Animal Production Research Institute, Ministry of Agriculture, Egypt. A total number of 360 Al-Salaam hens and 36 Al-Salaam cocks, 28 weeks of age were randomly distributed into 12 groups with 3 replicates each (10 hens+1 cock). Birds fed on the experimental diets, used two levels (0.5 and 1.0%) from four types of herbs (oregano, thyme, rosemary, curcuma longa) as natural antioxidants, in comparison to two levels of vitamin E (100 and 200) mg/Kg diet, in addition to control diets (using premix vitamins and minerals with vitamin E (control) and without vitamin E (Negative-control). Herbs were purchased from local market in Cairo. The experimental period lasted 90 days, from December 2005 to February 2006.The experimental diets were formulated on the basis of a basal diet (Table 1) and to be isonitrogenous (16% CP) and isocaloric (2700 Kcal ME/Kg diet) and to satisfy the nutrient requirements

Table 1: Composition of the experimental laying hen basal diet and calculated analysis.

Ingredients	Quantity %
Yellow corn	63.14
Soybean meal (44%)	27.10
Di Calcium phosphate	1.50
Limestone	7.60
Vitamins and Minerals mixture*	0.30
NaCl	0.30
Dl-Methionine	0.06
Total	100.00
Calculated analysis	
Crude Protein %	16.82
Metabolism Energy(kcal/kg diet)	2721.70
Ether extract %	2.81
Available phosphorus %	0.41
calcium %	3.27
Lysine %	0.95
Methionine %	0.36
Methionine + Cystine %	0.64

Each 3 kg of Vitamins and Minerals mixture* contains: Vit. A 10000,000 IU; Vit. D_3 2000,000 IU ; Vit. E 10,000 mg ; Vit. k_3 1000 mg ; Vit. B_1 1000 mg; Vit. B_2 5000 mg ; Vit. B_6 1500 mg ; Vit. B_{12} 10 mg; Pantothenic acid 10,000 mg; Niacin 30,000 mg; Folic acid 1000 mg; Biotin 50 mg; Choline 300,000 mg; Manganese 60,000 mg; Zinc 50,000 mg; Copper 10,000 mg; Iron 30,000mg; Iodine 1000 mg; Selenium 100 mg; Cobalt 100 mg ; $CaCO_3$ to 3,000 gm.

according to Agriculture Ministry Decree (1996). The birds were reared under the same managerial conditions in open-sided house on floor. Photoperiod was 17 hours daily. Feed and water were offered *ad libitum* during the experimental period. Feed intake, egg production (%) and egg weight were recorded. Feed Conversion was determined as follow: Feed Conversion = [Feed Intake / Egg Mass] / day.

Thirty representative eggs from each treatment (10 from each replicate) were collected to determine egg quality. Shape index and yolk index were determined according to Romanoff and Romanoff (1949) as follows:

Yolk index (%) = (height / diameter) x 100.

Shape index (%) = [width / length] x 100

Egg shell thickness, including shell membranes, was measured using a micrometer at the equator. Haugh unit score was applied from a special chart using egg weight and albumin height which was measured by using a micrometer according to Haugh (1937), Kotaiah and Mohapatra (1974) and Eisen *et al.* (1962). The egg yolk visual colour score was determined by matching the yolk with one of the 15 bands of the "1961, Roche Improved Yolk Color Fan".

At the end of the experimental period three egg yolk samples from each treatment were separated from the broken eggs, calculated and extracted according to Folch *et al.* (1957). Total lipids, total cholesterol, Low Density Lipoprotein - cholesterol (LDL - cholesterol), High Density Lipoprotein-cholesterol (HDL - cholesterol) were determined in both of blood plasma and egg yolk, while AST, ALT, total protein, albumin and globulin were determined in blood plasma using commercial kits, following the same steps as described by manufactures. Total of 1080 eggs (30 eggs x 12 treatments x 3 period) were collected during the last days of the feeding trial, 360 eggs freshly were directly incubated. The remaining eggs were stored at room temperature (16°C±2), 360 eggs were incubated after 15 days and other 360 eggs after 30 days of storage and chick hatching weight were recorded. Nine eggs from each dietary treatment collected during the last days of the feeding trial were used to investigate the effect of treatments on yolk oxidation of shell eggs in storage. At the start of this investigation, three egg yolk samples from each treatment were separated from the broken fresh eggs were directly stored in deep freezer at -20°C. The remaining eggs were stored at room temperature (16°C±2) and three egg yolk samples from each treatment were separated from the broken eggs stored after 15 days and the remaining eggs were broken after 30 days of storage. All samples stored in deep freezer at -20ºC were analyzed for malonaldehyde. Malonaldehyde (MDA) reacts with Thiobar Bituric Acid (TBA) in an acid medium and the produced coloured TBA-complex that could be measured calorimetrically according to the method of Uchiyama and Mihara (1978). Semen samples were collected between 10 and 11 AM at 40 weeks of age (at the end of the experiment, after 90 days) from three cocks of each group by abdominal massage technique according to the method of Burrows and Quinn (1937). Semen ejaculate volume was measured by tuberculin syringe graduated to nearest 0.01 ml percentage of sperm forward motion. Sperm abnormalities were determined according to Vontienhoven and Steel (1957).

Digestion coefficients of Dry Matter (DM), Organic Matter (OM), Crude Protein (CP %), Crude Fibre (CF %), Ether Extract (EE %) and Nitrogen Free Extract (NFE %) were determined at the end of the study using 3 hens from each group. Faecal nitrogen content was determined according to the method outlined by Jakobsen *et al.* (1960), while the urinary organic matter fraction was calculated according to Abou-Raya and Galal (1971). Proximate analyses of feed and excreta were carried out following A.O.A.C. (1990).

At the end of the experimental period, three birds were chosen randomly from each treatment for slaughter test and carcass weights were determined and presented as a percentage of live body weight. Physical characteristics of meat tissues determined included: pH value of meat tissues and drip were measured by pH - meter with glass electrode (Aitken *et al.*, 1962). The water holding capacity (WHC) was measured by method described by Volovinskaia and Kelman (1962). Meat of birds were analyzed for moisture, crude protein, ether extract and ash content. At the 40 weeks of age, nine

Table 2: Effect of experimental treatments on productive performance parameters

Treatments	Egg Production (%)	Egg weight (g)	Egg mass (g/hen/90days)	Feed intake (g/hen/day)	Feed conver sion (FI/EM)/day	Body weight gain (g)
N-control	52.00^{c}	49.08^{c}	2296.85^{e}	95.41	3.74^{a}	180.00^{c}
Control	53.85^{bc}	49.71^{b}	2409.32^{d}	96.69	3.61^{bc}	200.00^{bc}
100 mg vit. E	54.04^{bc}	49.79^{ab}	2421.38^{d}	97.84	3.64^{b}	215.00^{ab}
200 mg vit. E	54.22^{bc}	49.89^{ab}	2434.8^{cd}	97.17	3.59^{bc}	213.33^{ab}
0.5% Thyme	54.85^{ab}	49.76^{ab}	2456.36^{bcd}	96.9	3.55^{cd}	203.33^{b}
1.0% Thyme	57.59^{a}	49.96^{ab}	2589.25^{a}	99.97	3.47^{de}	220.00^{ab}
0.5 % Oregano	54.29^{bc}	49.74^{b}	2430.25^{cd}	98.10	3.63^{b}	205.00^{b}
1.0% Oregano	57.11^{a}	49.93^{ab}	2566.62^{ab}	97.12	3.41^{e}	231.67^{a}
0.5% Rosemary	55.18^{ab}	49.77^{ab}	2471.45^{bcd}	99.09	3.61^{bc}	203.33^{b}
1.0% Rosemary	56.33^{ab}	50.08^{a}	2539.23^{abc}	97.95	3.47^{de}	230.00^{a}
0.5% Curcuma L.	57.18^{a}	49.91^{ab}	2568.78^{ab}	98.57	3.45^{e}	216.67^{ab}
1.0 % Curcuma L.	56.63^{ab}	50.07^{a}	2551.94^{ab}	97.64	3.44^{e}	223.33^{ab}
S E M	±0.342	±0.048	±16.380	±0.405	±0.017	±14.530
P value	00.002	00.0001	00.0001	NS	0.0001	000.002

a,b,...=Means in the same column with different superscripts, differ significantly ($p<0.05$). NS = Not Significant ($p > 0.05$). SEM = Standard Error of Means. N-control = Negative control diet with Vitamins and Minerals mixture free vit.E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit. E

birds from each treatment (3 hens / replicate) were injected intra-muscular with 0.5 ml of 10% suspension packed sheep red blood cells (SRBC's). Pre-injection antibody titters were zero. Blood samples were collected at 7 days post immunizing with SRBC's. Antibody titter against SRBC's was determined using the micrometer procedure described by Vanderzjpp and Leenstra (1980).

The statistical analysis of data was computed using the General Linear Models (G LM) procedure (SAS, 2000). The significant differences among treatments means were separated by Duncan's Multiple Range test (Duncan, 1955).

Results and Discussion

Production Performance: Effects of dietary natural antioxidant supplementation to layer diets on production performance are summarized in Table 2. The data showed that there is significant effect of natural antioxidant on production performance .However, insignificant effect was only observed on feed intake. Hens fed 0 mg Vit. E /Kg diet (Negative-control) recorded significantly lower Egg Weight (EW), Egg Mass (EM) and better Feed Conversion (FC) values, but percentage of Egg Production (EP), Feed Intake (FI) and Body Weight Gain (BWG) values were insignificantly lower compared with those receiving 10 mg Vit. E /Kg diet (control). The results suggest that hens fed on corn-soybean meal diet without supplemental vit. E might receive the minimum requirement for pullets regardless of their performance. The addition of 100 or 200 mg/Kg of vit. E to control diet numerically increased EW, EM, EP, FI and BWG and improved FC. The results are agreement with Bartov *et al.* (1991) who found that dietary concentration of vit. E (125 mg/Kg diet) fed from one to 32 weeks of age did not affect pullet growth rate, age at first egg or average EW. Lin *et al.* (2004) reported that the addition of 80 mg/Kg of vit. E gave the best performance in EP, EM and FC. However, Sunder *et al.* (1999) reported that supplementation of vit. E (0 to 20000mg/Kg) did not significantly influence pullet health or performance. In this respect, Mezes and Hidas (1992) indicated that during eggshell formation, excess amounts of vit. E (100 and 200mg/bird) inhibited prostaglandins biosynthesis. Prostaglandins may regulate ovulation and are correlated with reproduction. However, diets supplemented with 125 mg/Kg or more of vit. E significantly increased EP of hens after heat stress (Bollengier - Lee *et al.*, 1999). Data in Table 2 indicated that supplementation of herbs improved production performance compared with control group. The addition of 0.5% thyme, oregano or rosemary leaves to laying hens diets numerically increased EW, EM, BWG and EP and improved FC. However, this effect increased significantly by increasing the level to 1.0% compared with control. Addition of rosemary to diet had no significant effect on EP. The beneficial effect of labiatae family (thyme, rosemary and oregano) may be due to the phenolic compounds which considerably exhibit antimicrobial and antifungal activity (Sivropoulou *et al.*, 1996; Adam *et al.*, 1998; Dorman and Deans 2000; Giannenas *et al.*, 2003; Arcila-Lozano *et al.*, 2004 and Bozin *et al.*, 2006). This activity may be due to thymol and carvacrol which are present in the essential oil of thyme and oregano (Basilico and Basilico, 1999). These findings agree with those obtained by Radwan (2003) who reported that, the use of 0.5% rosemary or thyme leaves in diets increased body weight and body weight gain and improved feed conversion of chicks. This improvement could be attributed to its content of essential oil that have active components which have possess antimicrobial, antifungal and antioxidant activities; and accordingly could improve the bird utilization of dietary nutrients. In addition, Abdel - Latif *et*

Table 3 : Effect of experimental treatments on external and internal egg quality

	External egg quality			Internal Egg quality				
Treatments	Shell weight (%)	Shell Thickness (mm)	Egg shape index (%)	Albumen weight (%)	Yolk weight (%)	Yolk colour score	Yolk index (%)	Hough unit
N-control	10.31	0.349	73.86	52.03^{a}	37.67^{c}	6.30	43.33^{d}	72.22
Control	10.27	0.350	73.71	52.02^{a}	37.71^{c}	6.40	42.35^{d}	75.73
100 mg vit. E	10.23	0.347	74.28	51.50^{ab}	38.26^{bc}	6.80	46.05^{c}	74.07
200 mg vit. E	10.48	0.352	74.26	51.24^{ab}	38.27^{bc}	7.10	50.36^{ab}	74.27
0.5% Thyme	10.70	0.352	74.91	49.68^{ab}	39.61^{abc}	6.90	49.82^{b}	73.53
1.0% Thyme	10.61	0.354	74.72	49.06^{bc}	40.32^{ab}	7.00	51.84^{ab}	75.27
0.5 % Oregano	10.82	0.357	74.56	50.14^{ab}	39.04^{bc}	6.70	51.64^{ab}	72.86
1.0% Oregano	10.72	0.354	75.16	50.04^{ab}	39.24^{bc}	6.80	52.19^{a}	74.07
0.5% Rosemary	10.70	0.356	74.77	50.58^{ab}	38.72^{bc}	7.00	51.19^{ab}	74.67
1.0% Rosemary	10.66	0.358	75.04	49.31^{bc}	40.03^{abc}	7.00	50.25^{ab}	76.80
0.5% Curcuma L.	10.67	0.358	74.34	50.27^{ab}	39.06^{bc}	7.00	51.98^{ab}	74.66
1.0 % Curcuma L.	11.19	0.355	75.58	47.00^{c}	41.81^{a}	7.40	50.85^{ab}	74.20
S E M	±0.085	±0.001	±0.202	±0.255	±0.235	±0.067	±0.3610	±0.349
P value	NS	NS	NS	0.001	0.011	NS	0.0001	NS

a,b,...= Means in the same column with different superscripts, differ significantly ($p < 0.05$). NS = Not Significant ($p > 0.05$); SEM = Standard Error of Means. N-control = Negative control diet with Vitamins and Minerals mixture free vit E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit.E.

al. (2002) attributed the improvement in growth and feed conversion of chicks fed thyme leaves to the enhancement in thyroid activity and the biological role of such medicinal plant in the metabolic functions and biosynthesis of hormones. Ali *et al.* (2007) found that addition of 0.25% thyme leaves to laying hens, significantly decreased BWG; numerically increased egg number and improved feed conversion compared to hens fed basal diet. Moreno *et al.* (2006) reported that, carnosic acid and rosmarinic acid may be the main bioactive antimicrobial compounds present in rosemary. Dietary feeding of essential oil extracted from herbs improved the secretion of digestive enzymes, so improved the digestibility of the feeds and improved the performance for broiler (Hernandez *et al.*, 2004 and Jang *et al.*, 2004). Radwan and Abdel - Khalek (2007) suggested that the herb mixture of equal parts of sage, oregano and sweet basal at 0.5% supplementation level increased both of villi height, crypt depth and absorption area and improved growth and health of rabbits grown under high ambient temperature conditions. The addition of 0.5 or 1.0% tumeric (Curcuma longa) significantly increased EW, EM, EP and improved FC values. However, these levels numerically increased BWG and FI compared to hens fed basal diet. These results agree with Al-Sultan (2003) who found that the use of turmeric as feed additive at level 0.5% enhance the overall performance of broiler chickens. The increased body weight gain is due to the antioxidant activity of turmeric (Osawa *et al.*, 1995); that stimulate protein synthesis by bird enzymatic system. Iqbal *et al.* (2003) reported that the dietary supplementation of curcumin (a major component of turmeric) to male ddY mice for 30 days (2.0%, w/v), significantly increased the activity of antioxidant and phase II-metabolizing enzymes in liver and kidney.

In general, the results indicated that the addition of 1.0% thyme, rosemary, or oregano or 0.5% tumaric to laying hens diets can give the best performance in the term of Egg production, Egg mass and Feed Conversion (feed intake/egg mass).

Egg Quality

External Egg Quality: External egg quality was not influenced significantly by any of the experimental treatments (Table 3). However, the addition of 200 mg vit. E /Kg diet or 0.5-1.0% thyme, rosemary, oregano or curcuma Longa to hen's diets numerically increased the percentage of egg shape index and shell weight and shell thickness. These results agree with Ali *et al.* (2007) who found that addition of thyme increased insignificantly the percentage of egg shape index and shell weight and shell thickness compared to hens fed control diet. The highest numerical value observed in shell weight and egg shape index were for 1.0% curcuma Longa (11.19 and 75.58% vs.10.27 and 73.71% for the control, respectively). Increased shell weight and shell thickness by addition of Curcuma Longa may be attributed to curcumin and turmeric acid; the main components in Curcuma Longa which have antioxidant activities (Ramirez-Tortosa *et al.*, 1999). Curcuma Longa may improve the small environment in uterus (Site of calcium deposition) and consequently increase shell weight and shell thickness more than the other treatments.

Internal egg quality: There were significant differences between treatments in percentages of both albumen and yolk weight and percentage of yolk index. Whereas, there were insignificant effect in yolk colour and Haugh

Table 4: Effect of experimental treatments on nutrient digestibility

Treatments	D.M (%)	O.M (%)	C.P (%)	C.F (%)	E.E (%)	N.F.E (%)
N-control	77.17	76.30	79.40	22.50	73.33	73.87
Control	77.63	77.12	79.57	22.73	76.17	73.97
100 mg vit. E	77.23	77.43	82.50	22.77	76.80	74.90
200 mg vit. E	77.43	76.83	82.60	23.27	77.23	75.17
0.5% Thyme	78.33	77.27	82.27	22.77	77.63	75.27
1.0% Thyme	78.03	76.67	81.83	23.67	76.73	74.00
0.5 % Oregano	78.00	77.67	81.03	22.20	77.10	73.53
1.0% Oregano	77.57	77.00	82.97	24.03	75.07	76.83
0.5% Rosemary	78.77	77.23	82.30	23.57	77.57	74.83
1.0% Rosemary	78.67	77.17	82.10	22.73	77.50	76.20
0.5% Curcuma L.	78.03	78.33	82.63	22.70	77.20	75.87
1.0 % Curcuma L.	78.40	78.80	81.87	23.47	78.07	75.83
S E M	± 0.147	±0.174	±0.371	±0.26	±0.311	±0.356
P value	NS	NS	NS	NS	NS	NS

SEM = Standard Error of Means; NS = Not Significant (p > 0.05). N-control = Negative control diet with Vitamins and Minerals mixture free vit. E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit. E.

unit (Table 3). The addition of vit. E (100 or 200 mg/Kg diet) or herbs (0.5%) in laying hens diets, insignificantly decreased albumen weight % with increased yolk weight %. While, addition of 1.0% thyme, rosemary or curcuma longa show significant effect. These results disagree with (Osawa *et al.*, 1995) who reported that curcuma stimulate protein synthesis by bird enzymatic system. Also, yolk index percentages were significantly higher for all treatments as compared with the N-control (without vit. E) or control groups. Yolk colour was not affected by adding vit. E or herbs in laying hens diet. However, addition of 1.0% curcuma longa increased numerically yolk colour than the control group (7.40 vs. 6.40).In general, curcuma at level 1.0% increased percentage of yolk weight and improved yolk colour by 10.87% and 15.62%, respectively compared to control group. In this respect, Ramirez-Tortosa *et al.* (1999) reported that Curcumin; a yellow pigment obtained from rhizomes of curcuma longa is commonly used as a spice and food colouring.

Nutrient Digestibility: The effect of treatments on the nutrient digestibility coefficients are summarized in Table 4. The results showed that the addition of (100 or 200 mg/Kg) vit. E or (0.5 or 1.0%) herbs to hen's diets numerically increased all nutrient digestibility coefficients. Addition 1.0% oregano gave the highest values of all nutrient digestibility coefficients, except that ether extract digestibility was the lowest value compared to control diet. The decrease of ether extract digestibility may be due to the effect of essential oil components present in oregano on lipid metabolism. Improvement of their nutrient digestibility coefficients may be due to that essential oils from plant extracts stimulates secretion of digestive enzymes in chickens (Williams and Losa, 2001). The phenolic compounds carvacrol and thymol present in the essential oil from Oregano has a good antioxidant capacity and also antimicrobial activity against pathogenic microorganisms like Salmonella typhimurium, *Escherichia coli,* Staphylococcus aureus and Staphylococcus epidermidis. These are all characteristics of interest for the food industry because they may enhance the safety and stability of food (Arcila-Lozano *et al.*, 2004). Hernandez *et al.* (2004) reported that, supplementation of 200 ppm essential oil extract from oregano, cinnamon and pepper and 5000ppm labiatae extract from sage, thyme and rosemary improved the digestibility of the feeds for broilers. Plant extracts contain different molecules that have intrinsic bio-activities on animal physiology and metabolism. They suggested that the plant extracts could control and limit the growth and colonization of numerous pathogenic and non-pathogenic species of bacteria in the gut. Thymol and carvacrol disrupt the membrane integrity, which further affects pH homeostasis and equilibrium of inorganic ions (Lambert *et al.*, 2001). Thymol is currently used to inhibit oral bacteria (Twetman and Peterson, 1997).

Carcass Characteristics: Table 5 shows that the percentage of the dressing, liver, heart, gizzard and spleen were not significantly affected by the experimental treatments. However, it was observed that values of spleen weight % increased numerically. Hens fed 0.5% oregano, 0.5 or 1.0% curcuma Longa increased spleen weight % in comparison to the control by 11.46, 19.10 or 14.65%, respectively. The increase of spleen weight % may be due to immunostimulate activity of curcumin which is the active compound in curcuma longa (Antony *et al.*, 1999).The results were supported by Al-Sultan (2003) who reported that, higher spleen weight index was observed in birds received feed contained 1.0% tumeric. While Ali *et al.*, (2007) reported that hens fed thyme or anise had no significant effect on carcass parameters and internal organ.

Meat Quality and Chemical Analysis of Meat: The effect of treatments on the meat quality is summarized in

Table 5: Effect of experimental treatments on carcass characteristics

Treatments	Live body weight (g)	Dressing (%)	Liver (%)	Heart (%)	Spleen (%)	Gizzard (%)
N-control	1826	67.87	2.04	0.518	0.146	1.848
Control	1850	68.63	2.05	0.512	0.157	1.865
100 mg vit. E	1826	69.71	2.23	0.575	0.167	1.995
200 mg vit. E	1973	69.59	2.29	0.509	0.166	1.771
0.5% Thyme	1906	68.10	2.11	0.566	0.169	1.864
1.0% Thyme	1960	69.63	2.09	0.557	0.161	1.631
0.5% Oregano	2003	69.55	2.44	0.534	0.175	1.852
1.0% Oregano	2023	68.17	2.22	0.557	0.165	1.529
0.5% Rosemary	1940	69.37	2.44	0.554	0.170	1.785
1.0% Rosemary	1913	70.18	2.31	0.544	0.164	1.807
0.5% Curcuma L.	1883	69.94	2.27	0.553	0.187	1.757
1.0% Curcuma L.	1956	68.37	2.18	0.547	0.180	1.959
S E M	±25.860	±0.381	±0.047	±0.011	±0.006	±0.059
P value	NS	NS	NS	NS	NS	NS

SEM=Standard Error of Means; NS = Not Significant (p>0.05). N-control = Negative control diet with Vitamins and Minerals mixture free vit.E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit.E.

Table 6. Neither vit. E nor herbs (thyme, rosemary, oregano and curcuma Longa) had significant effect on meat quality. The results were supported by Al-Sultan (2003) who indicated that results of organo-leptic test revealed that curcuma Longa did not induce any abnormal flavour, color or smell. There were insignificant differences between treatments in values of meat chemical analysis (Table 6). The protein percent of meat (breast and thigh muscles) were nearly the same in all treatments. While, fat percent decrease at 1.0% thyme, oregano, rosemary or curcuma longa in comparison to the control by 9.15, 8.60, 13.55, 10.80%, respectively. The lower fat percentage may be due to the effect of essential oil compounds presents in herbs on lipid metabolism. The results agreed with Al-Sultan (2003) who reported that protein percent of breast and thigh muscles of birds were nearly the same in all treatments. The lower fat percentage was recorded in carcasses of birds fed on 1.0% dietary curcuma longa followed by 0.5%.

Biochemical Parameters

Immune Response: Data of antibody titter against sheep red blood cells (SRBC's) expressing immune response of birds are present in Table 7. There was gradual increases in values of antibody titter by increasing levels of vit. E or herbs (thyme, rosemary, oregano and curcuma longa) in the diets. Regarding addition of Vit. E in the hen's diets. Data showed that hens received 200 mg/Kg of vit. E had significantly higher antibody titter than N-control or control groups. However, N-control and control group (0 to 10 mg Vit. E /Kg, respectively) or 10 to 100 mg Vit. E /Kg increased antibody titter more than high levels (100 to 200 mg Vit. E /Kg).The percentage of increase were 14.13, 10.50 and 9.33%, respectively. These results were supported by Leshchinsky and Klasing (2001) who indicated that moderate 25 to 50 IU/Kg levels of vit. E supplementation were most immunomodulatory and high levels (100 to 200 IU/Kg) were less effective. Vitamin E at high concentrations can serve as a per oxidant (Thomas and Stocker, 2000) and may be the highest levels (100 to 200 IU/Kg) increase free radical formation in cytosolic pools that influence leukocyte responses. Gonzalez-Vega-Aguire *et al.* (1995) demonstrated that the combination of supplementation of 200 ppm of vitamin C and 75000 IU/ton of vitamin E improved antibody levels of broiler chickens against *Brucella abortus* and Newcastle modified live and dead virus vaccine. Vitamin E stimulated the mitogenic responses of lymphocytes to *Salmonella typhosa* (Corwin and Shloss, 1980 and Puthpongsiriporn *et al.*, 2001) and that vitamin E positively affected maturation of T cells. Immunoregulatory mechanism of vit. E is the modulation of arachidonic acid metabolism via cyclooxygenase and lipoxygenase pathways (Blumberg, 1994) which lead to the synthesis of prostaglandins and leukotrienes, respectively. In addition, Corwin and Shloss (1980) suggested that Vitamin E could enhance lymphocyte activity by protecting lymphocytes from lipid oxidation by its antioxidant activity. Regarding, addition of herbs in the hen's diets, antibody titter was increased in all treatments compared to control group. The highest values (P<0.05) were for hens fed 1.0% thyme or rosemary followed by hens received 0.5% rosemary or 1.0% oregano and this may be due to the antioxidant activity of essential oil components. The results agreed with Ibrahim *et al.* (2000) found significant increase in levels of red blood cells count, hemoglobin and the packed cell volume for rabbits fed diets with 0.5% thyme. Radwan (2003) found increase in antibody titter values for broiler fed diets content 0.5, 1.0 or 2.0% thyme leaves; and reported that the improvement in antibody titter values may be due to the high level of iron in thyme leaves (743 PPm) which may affect the transport of oxygen needed for hemoglobin synthesis in blood. In addition, the effective component of thyme such as

Table 6: Effect of experimental treatments on meat quality and chemical analysis of meat

	Meat quality				Chemical analysis of meat(breast and thigh)			
Treatments	pH value	Colour score	Tenderness (cm^2)	W. H.C* (cm^2)	Dray matter (%)	Crude protein (%)	Fat (%)	Ash (%)
N-control	6.64	0.269	2.896	5.85	25.73	18.60	5.58	1.53
Control	6.70	0.279	2.993	5.92	25.88	18.84	5.46	1.54
100 mg vit. E	6.68	0.250	2.910	5.85	26.05	19.17	5.34	1.54
200 mg vit. E	6.64	0.246	2.827	5.88	26.08	19.12	5.36	1.58
0.5% Thyme	6.70	0.278	2.967	5.96	25.83	18.72	5.74	1.44
1.0% Thyme	6.57	0.218	2.950	5.98	25.97	19.43	4.96	1.53
0.5% Oregano	6.62	0.269	2.883	5.96	26.39	19.07	5.81	1.48
1.0% Oregano	6.68	0.215	2.903	5.96	25.77	19.31	4.99	1.43
0.5% Rosemary	6.66	0.246	3.013	5.83	25.83	18.79	5.56	1.45
1.0% Rosemary	6.63	0.205	2.856	5.93	25.52	19.22	4.72	1.56
0.5% Curcuma L.	6.58	0.248	2.940	5.89	26.53	19.37	5.89	1.56
1.0% Curcuma L.	6.65	0.226	2.930	5.81	25.52	19.15	4.87	1.47
S E M	±0.012	±0.006	±0.026	±0.031	±0.093	±0.093	±0.093	±0.015
P value	NS	NS	NS	NS	NS	NS	NS	NS

SEM = Standard Error of Means; NS = Not Significant ($p > 0.05$); W.H.C* = water holding capacity. N-control = Negative control diet with Vitamins and Minerals mixture free vit.E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit. E.

thymol and carvacrol may stimulate the immune response as well as the growth and metabolic changes in broilers. On the other hand, the use of rosemary might not have a generalized immunoenhancing effect.

Al-Sultan (2003) reported that the higher levels of tumeric inclusion (0.5 and 1.0%) increased both erythrocytic and total leukocytic count in broiler. These results may be due to immunostimulate activity of curcumin which is the active compound in curcuma longa (Antony *et al.,* 1999).

Blood Constituents: Results of blood constituents as affected by different treatments are shown in Table 7. Either vit. E nor herbs had any significant effect on blood constituents, except total lipid which decreased significantly. Thyme or Rosemary at 1.0% significantly decreased total lipid, in comparison to the control by 17.15 and 27.15%, respectively. While total cholesterol and LDL - cholesterol decreased insignificantly compared to control group. Curcuma longa or oregano at 1.0% insignificantly decreased these parameters. It could be concluded from the study that vit. E or herbs had no adverse effects on liver functions (AST and ALT) or blood constituents. The decrease of total lipid and cholesterol may be due to the effect of essential oil compounds present in these herbs on lipid metabolism. These results agree to a large extent with those obtained by Ali *et al.,* (2007) who found that addition of thyme to hen's diets significantly decreased plasma LDL, HDL, total cholesterol, triglyceride and total lipid. Radwan (2003) reported that the decrease of total lipid and cholesterol may be attributed to the lowering effect of thymol and carvacrol on HMG-COA that is needed for cholesterol synthesis in liver. Lee *et al.* (2003) found that dietary carvacrol significantly lowered plasma triglyceride and phospholipids by 12 and 7%, respectively and indicating that dietary carvacrol, but not thymol may have more impact on lipogenesis than on cholesterol biosynthesis. But, Case *et al.* (1995) reported that the feeding of thymol at a dietary concentration of 150 ppm to leghorn chickens for 21 days reduced serum cholesterol by 9%.While; Kermanshahi and Riasi (2006) reported that turmeric had a profound positive effect on lowering blood triglyceride, total cholesterol and LDL - cholesterol. Turmeric also improved HDL- cholesterol and might be used as an ingredient in laying hen diets for manipulating egg composition.

Some Constituents of Egg Yolk Extract: The data in Table 8 indicated that there were insignificant differences between treatments in yolk LDL- cholesterol, HDL- cholesterol and total cholesterol, while there was significant difference in total lipid, as well as their trend on plasma. Egg yolk content of total lipid, LDL-cholesterol, HDL- cholesterol and total cholesterol were not affected significantly by supplementation of vit. E as compared with the N-control (without vit. E) or control (with 10 mg vit. E/ Kg) groups. There was significant effect of herbs in total lipid. The hens fed 1.0% thyme, rosemary or Curcuma longa significantly decreased yolk total lipid, while the other treatments decreased it insignificantly as compared with the N-control or control groups. The hens fed 0.5% or 1.0% curcuma longa or 1.0% rosemary insignificantly recorded the lowest values of yolk LDL - cholesterol and total cholesterol. Rosemary at 1.0% or Curcuma longa at 0.5 or 1.0% decreased both of total lipid by 13.2, 8.98, 13.95%, total cholesterol by 6.2, 6.09, 5.67% and LDL - cholesterol by 8.2, 14.5 and 15.6% in comparison to the control ,respectively. The decrease of total lipid and cholesterol in plasma was found to be correlated to decreasing these parameters in yolk egg. These finding may be due to the effect of essential oil components present in herbs on lipid metabolism. Close relationship between

Table 7: Effect of experimental treatments on antibody titter and some blood constituents

Treatments	Anti-body titter	A.S.T (U/L)	A.L.T (U/L)	T. Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	T. Lipid (g/dl)	Cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)
N-control	4.67^{d}	32.32	23.24	6.57	4.23	2.34	1.367^{a}	170.97	72.32	55.80
Control	5.33^{cd}	32.58	23.55	6.36	3.94	2.42	1.370^{a}	171.00	72.35	55.78
100 mg vit. E	5.89^{bc}	31.70	23.74	6.66	4.35	2.31	1.365^{a}	170.83	71.60	56.37
200 mg vit. E	6.44^{ab}	32.31	24.05	6.84	3.89	2.95	1.357^{a}	170.05	70.26	56.92
0.5% Thyme	6.22^{abc}	31.22	23.80	6.02	2.86	3.16	1.262^{ab}	169.79	70.02	56.90
1.0% Thyme	6.89^{a}	33.23	24.72	6.65	3.18	3.47	1.135^{bc}	167.19	67.85	56.47
0.5% Oregano	5.78^{bc}	32.49	23.46	6.42	3.52	2.90	1.167^{abc}	170.36	71.54	55.93
1.0% Oregano	6.33^{ab}	33.91	23.87	6.72	3.54	3.18	1.189^{abc}	168.23	69.76	55.61
0.5% Rosemary	6.33^{ab}	32.35	24.18	6.19	3.37	2.82	1.188^{abc}	168.49	69.08	56.57
1.0% Rosemary	6.89^{a}	33.11	24.74	6.52	3.68	2.83	0.998^{c}	165.62	67.38	55.39
0.5% Curcuma L.	6.22^{abc}	32.08	23.65	7.09	3.80	3.29	1.274^{ab}	168.75	70.34	55.55
1.0% Curcuma L.	6.11^{abc}	32.89	24.88	6.95	4.39	2.56	1.246^{ab}	165.10	68.34	53.90
S E M	±0.099	±0.388	±0.659	±0.110	±0.115	±0.112	±0.023	±1.70	±1.236	±0.999
P value	0.0001	NS	NS	NS	NS	NS	0.007	NS	NS	NS

a, b,...=Means in the same column with different superscripts, differ significantly (p<0.05); NS = Not Significant (p>0.05). SEM=Standard Error of Means; N-control = Negative control diet with Vitamins and Minerals mixture free vit.E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit.E.

Table 8: Effect of experimental treatments on some constituents of egg yolk extract

Treatments	Total lipid (mg/g)	Total cholesterol (mg/g)	LDL-cholesterol (mg/g)	HDL-cholesterol (mg/g)
N-control	282.82^{a}	16.580	9.890	5.920
Control	282.13^{a}	16.570	9.900	5.900
100 mg vit. E	280.03^{a}	16.390	9.650	5.950
200 mg vit. E	273.91^{ab}	16.350	9.590	5.990
0.5% Thyme	260.31^{abc}	16.240	9.520	5.960
1.0% Thyme	247.88^{bc}	16.290	9.570	5.960
0.5% Oregano	271.37^{ab}	16.140	9.330	6.060
1.0% Oregano	268.75^{abc}	15.910	9.250	5.900
0.5% Rosemary	259.85^{abc}	15.780	9.360	5.900
1.0% Rosemary	244.91^{c}	15.540	9.090	5.930
0.5% Curcuma L.	256.78^{abc}	15.560	8.460	5.680
1.0% Curcuma L.	242.77^{c}	15.630	8.360	5.850
S E M	±3.000	±0.121	±0.121	±0.065
P value	0.007	NS	NS	NS

a,b,...=Means in the same column with different superscripts, differ significantly (p<0.05), NS = Not Significant (p>0.05). SEM=Standard Error of Means. N-control = Negative control diet with Vitamins and Minerals mixture free vit. E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit.E.

some of the components of the diet and egg composition was found by (Sim, 1999). The pure components of essential oils inhibit hepatic 3-hydroxy - 3-methylglutaryl coenzyme A (HMG-COA) reductive activity (Crowell, 1999) which is a key regulatory enzyme in cholesterol synthesis and consequently the hypocholeterolemic effect (Lee *et al.*, 2004). These results agree with those found by Ali *et al.* (2007) who found that addition of 0.25% thyme to laying hens diets significantly decreased the yolk total lipid, LDL, HDL and total cholesterol compared with control group. From These results, it could be concluded that rosemary or Curcuma longa may have lowering effect on total lipid, total cholesterol and LDL-cholesterol without effect on HDL - cholesterol. This may lead to produce enriched eggs that are healthier for human consumption and seful for those suffering from heart diseases.

Lipid Oxidation: The effect of dietary treatments on lipid yolk oxidation during storage of shell eggs stored in room temperature (16°C±2), is shown in Table 9. The extent of lipid oxidation, measured by malonaldehyede (MDA) content. Showed significant differences (P<0.01) between the experimental treatments but there was no significant difference as an effect of storage time. Supplemental vitamin E during the laying period had insignificant influence on MDA formation. There was a gradual decrease by increasing vitamin E level in the diet. These results is in accordance with those found by Galobart *et al.* (2001a, b). They did not find any change in TBA-reactive substances (malonaldehyede) values in eggs not enriched with n - 3 polyunsaturated fatty acids from hens fed on diets contain of alpha tocopherol acetate. Franchini *et al.* (2002) evaluated the effect of vitamin E (100 and 200 mg/Kg) on the content of vitamin E in the yolk and on the lipid stability of fresh and stored eggs(30, 60 and 90 days at (4°C) or stored 28 days at room temperature (25°C) in Hy-Line Brown hens and found that the yolk content of vitamin E depended on the level of dietary addition and decreased after 90 days of storage at (4°C) or after 28 days at (25°C). During storage, malonaldehyede (MDA) were almost stable in all treated group, whereas in the long-term (90 days) the oxidative products of control eggs significantly increased. The oxidation products increased in all groups after 4-weeks storage at room temperature and reduced the content of vitamin E presumably consumed in oxidative processes, these results could confirm our results. Botsoglou *et al.* (2005) found lower value of malonaldehyede in eggs from the hens fed alpha tocopherol as compared to control. Dietary supplementation with alpha tocopherol has been demonstrated to beneficially affect enhancement of lipid stability in foods from animal origin, such as poultry meat (Ajuyah *et al.*, 1993) and eggs (Cherian *et al.*, 1996; Galobart *et al.*, 1999). Yolk content of vitamin E increased by increasing level of dietary addition, this increase was quite stable during long- term storage in

Table 9: Effect of treatments and storage time at room temperature on yolk egg content of malonaldehyde(mg/100g)

Treatment	Fresh	Stored 15 days	Stored 30 days	Over all of treatments
N-control	2.940^{a}	3.130^{a}	3.353^{a}	3.141^{a}
Control	2.830^{ab}	3.010^{ab}	3.156^{ab}	2.999^{ab}
100 mg vit. E	2.763^{ab}	2.870^{abc}	2.980^{abc}	2.871^{abc}
200 mg vit. E	2.730^{ab}	2.973^{ab}	2.750^{abcd}	2.818^{bcd}
0.5% Thyme	2.763^{ab}	2.830^{abcd}	2.956^{abc}	2.850^{bcd}
1.0% Thyme	2.600^{abcd}	2.647^{abcd}	2.700^{bcd}	2.649^{cde}
0.5% Oregano	2.650^{abc}	2.687^{abcd}	2.736^{bcd}	2.691^{cd}
1.0% Oregano	2.250^{d}	2.280^{d}	2.313^{d}	2.281^{g}
0.5% Rosemary	2.540^{bcd}	2.576^{bcd}	2.590^{bcd}	2.569^{def}
1.0% Rosemary	2.280^{cd}	2.333^{cd}	2.380^{cd}	2.331^{fg}
0.5% Curcuma L.	2.340^{cd}	2.390^{cd}	2.423^{cd}	2.384^{efg}
1.0% Curcuma L.	2.300^{cd}	2.340^{cd}	2.366^{cd}	2.336^{fg}
S E M	±0.047	±0.061	±0.070	±0.035
S.O.V	---------- P value ----------			
Treatments	0.001	0.009	0.008	0.0001
Storage time		NS		
Treatments x Storage time		NS		

a,b,...=Means in the same column with different superscripts, differ significantly ($p<0.05$). NS = Not Significant ($p>0.05$); SEM=Standard Error of Means. N-control = Negative control diet with Vitamins and Minerals mixture free vit. E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit E. S.O.V. = Source of variation

refrigerated eggs. These results support the hypothesis that since the shell egg is a closed system, little exposed to oxygen and oxidant agents, thereby little involved in oxidative reactions, vitamin E is not consumed in preventing oxidative processes (Franchini *et al.*, 2002).

Regarding the effect of herbs on malonaldehyede formation, there were significant effects among treatments. The addition of 1% oregano or rosemary or 0.5-1.0%curcuma to hens' diets significantly decreased MDA values in comparison to control diet. While, the other treatments decreased these values insignificantly compared to diets with vitamin E at 100 or 200 mg/Kg. Malonaldehyede values increased in all treatments with increasing storage time without significant difference. The results agreed with Botsoglou *et al.*, 1997. They evaluated the effect of dietary thyme on the oxidative stability of shell eggs over a 60-day refrigerated storage and found that hens fed 3.0% thyme show lower concentration of malonaldehyede (MDA) in yolk, but did not change with storage time. Although yolk MDA cannot be produced during storage of shell eggs, it is present in the yolk of fresh eggs. The malonaldehyede found in the yolk of fresh eggs might be due to either the consumption or subsequent deposition of MDA that was already present in the diets or the in vivo production of MDA by the hens fed the diets. Generally, the over all effect of treatments suggested that hens receiving 1% oregano or rosemary or 0.5-1.0% curcuma Longa significantly decreased MDA values compared to diets with supplemented vitamin E at the experimental levels. It could be concluded that, supplemental herbs during laying period had positive effect on oxidative stability of shell eggs stored in room temperature ($16^{o}C\pm2$). These finding can be explained on the basis that oregano has essential oils, 1.25% (Radwan and Abdel - Khalek 2007) and the major components in oregano essential oil are carvacrol and thymol that constitute about 78-82% of the total oil(Adam *et al.*, 1998), that have antioxidant activity. Botsoglou *et al.* (2002) reported that, iron-induced lipid oxidation showed that as oregano oil increased in the diet, MDA values decreased in meat tissue samples of broilers and suggesting that the lower MDA values are probably due to antioxidant compounds which enter the circulatory system that are distributed and retained in tissues. With increased oregano essential oil in rabbit diets, MDA values decreased in both raw and thermally treated muscles during refrigerated storage (Botsoglou *et al.*, 2004). Oregano essential oil exerted a significant antioxidant effect at the level of 200 mg/Kg, but inferior effect in lipid oxidation was obtained compared with 200 mg/Kg of alpha tocopherol. Carnosic acid is the most active antioxidant present in rosemary (Cuvelier *et al.*, 1996; Richheimer *et al.*, 1996; Offord *et al.*, 1997). Supplementing hen diets with 500 or 1000 mg of Carnosic acid /Kg diet, established that the transfer rate of Carnosic acid from feed to eggs was of 0.0025% (Krause and Ternes, 2000). Turmeric has essential oils, 2.4-4% (Srimal, 1997). Curcumin a major component in turmeric has a potent antioxidant activity (Ruby *et al.*,1995 and Sreejayan, 1994); Curcumin has a unique conjugated structure including two methoxylated phenols and an enol form of ß-diketone and the structure shows a typical radical trapping ability as a chain -breaking antioxidant. Masuda *et al.* (2001) Stated that, an antioxidant mechanism of curcumin in polyunsaturated lipids was proposed, which consisted of an oxidative coupling reaction at the 3-position of the curcumin with the lipid and a subsequent intramolecular Diels - Alder reaction. Reddy and Lokesh (1994) suggested that dietary turmeric lower lipid peroxidation by enhancing the activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) .While (Miquel *et al.*, 2006) reported that curcuma longa may help to prevent antioxidant deficiency with resulting protection of mitochondria against premature oxidative damage with loss of ATP synthesis and specialized cellular functions .

Extracts of the labiatae family (rosemary, thyme and oregano) offer potential to increase the oxidative stability of chicken meat and eggs (Economou *et al.*, 1991; Schwartz *et al.*, 1996; Botsoglou *et al.*, 1997; Lopez-Bote *et al.*,1998). Other studies show that, apart from carvacrol and thymol, other phenolic (caffeic acid, p-cymene-2, 3-diol and several biphenolic and flavonoid) compounds also exhibit anti-oxidative activity which, for some of them, is higher than that of alpha tocopherol (Schulz and Herrmann, 1980; Miura and Nakatani, 1989a, b). The high antioxidant activity of thymol is due

Table 10: Effect of experimental treatments on semen quality

Treatments	Volume Ejaculate (ml)	Motility (%)	Live sperm (%)	Dead sperm (%)	Abnormal sperm (%)
N-control	0.300	75.00	66.00^{d}	34.00^{a}	4.33
Control	0.313	73.33	70.33^{d}	29.67^{a}	3.00
100 mg vit. E	0.330	75.00	80.33^{c}	19.66^{b}	4.00
200 mg vit. E	0.340	80.00	82.00^{abc}	18.00^{bcd}	3.00
0.5% Thyme	0.336	76.67	81.00^{bc}	19.00^{bc}	2.66
1.0% Thyme	0.340	80.00	87.00^{ab}	13.00^{cd}	2.66
0.5% Oregano	0.330	78.33	81.33^{abc}	18.66^{bcd}	3.00
1.0% Oregano	0.346	81.66	84.66^{abc}	15.33^{bcd}	2.00
0.5% Rosemary	0.340	80.00	84.00^{abc}	16.00^{bcd}	3.00
1.0% Rosemary	0.340	78.33	86.67^{ab}	13.33^{cd}	2.66
0.5% Curcuma L.	0.326	80.00	87.33^{a}	12.66^{d}	3.33
1.0% Curcuma L.	0.336	80.00	86.33^{abc}	13.66^{bcd}	2.33
S E M	±0.003	±1.063	±1.176	±1.1760	±0.187
P value	NS	NS	0.0001	00.0001	NS

a,b,...=Means in the same column with different superscripts, differ significantly ($p<0.05$). NS = Not Significant ($p>0.05$). SEM = Standard Error of Mean. N-control = Negative control diet with Vitamins and Minerals mixture free vit.E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit.E.

to the presence of phenolic OH groups which act as hydrogen donors to the peroxy radicals produced during the first step in lipid oxidation, thus retarding the hydroxyl peroxide formation (Farag *et al*., 1989). Therefore, malonaldehyde is not produced significantly during storage of shell eggs and reduced oxidation of yolk in herbs-treated than vitamin E. As a result of herbs treatments decreased total lipid in plasma (Table 7) and egg yolk (Table 8), may be due to decreasing the oxidation products transferred into the yolk and decreased substrate that produce free radical, consequently malondialdehyde decreased in eggs. In this respect, Galobart *et al*. (2001a) found that eggs produced from the carnosic acid - hen diets (from dietary rosemary extract) showed a delay in the iron-induced lipid oxidation and indicated that carnosic acid may effectively act as an antioxidant in eggs when high enough doses are used in the diets. Ali *et al*. (2007) reported that the thyme treatment increased antioxidant capacity and decreased total lipid and LDL in plasma. This may be due to antioxidant compounds present in thyme transferred to eggs and deposited into yolk (Krause and Ternes, 1999). In this respect Botsoglou *et al*. (1997) indicated that possible transfer of the antioxidant constituents of thyme into the hen through feeding might inhibit the chain reaction involved in oxidation of the consumed lipids, thus decreasing the oxidation products transferred into the yolk.

Reproductive Performance

Semen quality: The effect of the experimental treatments on semen characteristics (ejaculate volume, motility, live sperms, dead sperms and the abnormal sperms) are present in Table 10. There were insignificant differences between treatments in ejaculate volume, motility and the abnormal sperms. While there were significant differences in live and dead sperms%. Ejaculate volume and motility % were increased numerically in control group than N- control group. This effect increased numerically by increasing vit. E level up to 200 mg/Kg by 8.63 and 9.10% for ejaculate volume and motility respectively, compared to control group. This results may be due to addition of vit. E. Also, the values of ejaculate volume and motility % increased numerically in herbs treatments (0.326-0.346 vs.0.313ml and 76.67-81.66 vs.73.33%, for the control group, respectively) without significant differences between the treatments. The highest values were recorded with 1.0% oregano in diets. Abnormal sperm% was not influenced significantly ($P > 0.05$) by any of the treatments (Table 10). However, the addition 0.5-1.0% thyme or 1.0% oregano, rosemary or curcuma decreased numerically abnormal sperm% and the lowest value at 1.0% oregano (2.00 vs.3.00 % for the control). Live sperms% increased with decreased dead sperms%, significantly by adding vit. E and there was more increase by increasing level. The addition of 200 mg/Kg in diet increased live sperms% by (16.59%) while decreased dead sperms% by (39.33%) compared with control group. This results may be due to addition of vit. E; which could have antioxidant activities. Also, the addition of 0.5-1.0% herbs increased live sperms% by (15.17-24.17%) and decreased dead sperms% by (35.96-57.33%) in comparison to the control group. The highest value of live sperms% and the lowest value of dead sperms % were recorded with 0.5% curcuma longa in diets. It could be concluded that, 0.5% curcuma longa or 1.0% thyme or rosemary improved significantly the semen characteristics than 100 mg vit. E /Kg. But, 0.5-1.0% herbs (thyme, oregano, rosemary or curcuma longa) improved insignificantly in comparison to 200 mg vit. E /Kg. The results could be due to herbs, which have been classified as antioxidants (Economou *et al*., 1991; Schwartz *et al*., 1996; Lopez-Bote *et al*., 1998; Botsoglou *et al*., 1997). The importance of the antioxidants in this regard was explained by Kelso *et al*. (1996) and Aitken (1994) who reported that avian spermatozoa are characterized by the presence of high concentrations of polyunsaturated fatty acids within the phospholipids. The presence of such polyunsaturated fatty acids requires an efficient antioxidant system to protect sperm membranes against peroxidative damage. Also, Pappas *et al*. (2006) indicated that the phospholipids of avian spermatozoa are characterized by high proportions of arachidonic and docosatetraenoic fatty acid, which are very susceptible to oxidation.

Fertility: Table 11 shows percentages of fertility as affected by the experimental treatments and storage time of fertile eggs (fresh, after 15 and 30 days) in room temperature (16^{o}C±2).Significant differences were found between treatments. On the other hand, no significant effect was observed with storage time in percentages of

Table 11: Effect of experimental treatments and storage time of fertile eggs on (%) fertility

Treatments	Fresh	Stored 15 days	Stored 30 days	Over all of treatment
N-control	71.67^{c}	77.00^{b}	73.33^{b}	74.00^{c}
Control	81.00^{b}	81.67^{ab}	84.33^{a}	82.33^{b}
100 mg vit. E	85.67^{ab}	85.67^{a}	85.00^{a}	85.44^{a}
200 mg vit. E	86.67^{ab}	87.67^{a}	86.33^{a}	86.89^{a}
0.5% Thyme	86.00^{ab}	85.33^{a}	86.33^{a}	85.89^{a}
1.0% Thyme	86.67^{ab}	86.67^{a}	89.00^{a}	87.44^{a}
0.5 % Oregano	86.67^{ab}	86.67^{a}	87.67^{a}	87.00^{a}
1.0% Oregano	87.67^{a}	88.00^{a}	88.66^{a}	88.11^{a}
0.5% Rosemary	86.67^{ab}	85.67^{a}	87.67^{a}	86.67^{a}
1.0% Rosemary	87.67^{a}	86.33^{a}	88.00^{a}	87.33^{a}
0.5% Curcuma L.	88.33^{a}	86.00^{a}	88.33^{a}	87.56^{a}
1.0 % Curcuma L.	86.67^{ab}	87.67^{a}	88.67^{a}	87.67^{a}
S E M	±0.863	±0.697	±0.812	±0.455
Source of variation	----------P value----------			
Treatments	0.0001	0.038	0.0001	0.0001
Storage time		NS		
Treatments x Storage time		NS		

a,b,...=Means in the same column with different superscripts, differ significantly ($p<0.05$). NS = Not Significant ($p>0.05$). SEM=Standard Error of Means. N-control = Negative control diet with Vitamins and Minerals mixture free vit.E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit.E.

fertility. Supplemental vitamin E at level 200 mg /Kg to control diet during the laying period had significantly greater fertility than those fertile eggs fresh without vit. E or stored (15 and 30 days). These results indicate that supplemental vitamin E had a significant positive effect on improving fertility. The percentages of fertility increased by increasing Vit. E level; hens fed 200 mg/Kg increased fertility by 7.0, 7.35 and 2.37% for fertile eggs fresh or stored for 15 and 30 days, respectively as compared with those fed control diet. It could be concluded that, supplemental vitamin E had a significant positive effect on improving fertility and the fertile eggs could be stored up to 30 days without negative effect on fertility. These results agree with those found by Lin *et al.* (2004) who found that addition of 80 mg /Kg vitamin E during the laying period increased fertility by 7.7% as compared with those fed 0 mg /Kg.

Regarding addition of herbs to hens' diets, there was significant effect of herbs on fertility for fertile eggs fresh (Table 11). The addition of 1% oregano, rosemary or 0.5% curcuma longa to hens' diets significantly increased the percentages of fertility in comparison to hens fed control diets by 8.23, 8.23 or 9.05%, respectively. While, the other treatments increased fertility insignificantly as compared to hens fed control diets or control diets with vitamin E 100 mg /Kg. The addition of vitamin E or herbs increased fertility insignificantly of fertile eggs stored (15 and 30 days) compared to hens fed control diets. Regarding, fertile eggs stored 15 days; the highest values of fertility were for hens fed 1.0% oregano or curcuma longa which increased fertility by 7.75 and 7.35%, respectively. Hens fed 1% rosemary or 0.5% curcuma longa showed an increase by 5.7 and 5.3%, respectively. Fertile eggs stored 30 days showed that; hens fed 1% oregano, rosemary, 1.0 or 0.5% curcuma Longa increased fertility by 5.13, 4.35, 5.15 and 4.74%, respectively. Generally, the over all of treatments showed that the addition of herbs or vitamin E to hens' diets significantly increased the percentages of fertility in comparison to hens fed control diets. The highest percentages of fertility were for hens receiving 1% oregano, rosemary or thyme or 0.5-1.0% curcuma longa. It could be concluded that, the use herbs or vitamin E could improve fertility without significant change with storage time. Storing fertile eggs up to 15 days show the best fertility. This improvement can be explained as a result of herbs or vitamin E which have antioxidant activities; decreased malondialdehyde formation in egg yolk (Table 9) and improved the semen characteristics (Table 10) consequently, improved fertility.

Hatchability and Body weight of Hatched Chicks: The data in Table 12 indicated that there were significant differences between treatments, in percentages of hatchability, through different storage periods, but did not change significantly with storage time as well as their trend on fertility (Table 11). Supplemental vitamin E to diets had a significant influence on hatchability. The hens receiving vitamin E at 10 (control) to 200 mg /Kg had significantly greater hatchability than those receiving 0 mg /Kg (N-control) for fertile fresh eggs or stored for 15 and 30 days. The percentages of hatchability increased by increasing vit. E level, hens fed 200 mg/Kg increased hatchability by 11.62, 11.06 and 1.30% for fertile fresh eggs or stored for 15 and 30 days, respectively as compared with those fed control diets. It could be concluded that, supplemental vitamin E had a significant positive effect on improving hatchability and fertile eggs could be stored up to 15 days without deleterious negative effect on hatchability. These results agree with those found by Lin *et al.* (2004) who found that hens fed 80 mg /Kg vit. E increased percentages of hatchability by 13.4% as compared with those fed 0 mg /Kg. Regarding addition of herbs in laying diets, there was significant effect on hatchability for fertile fresh eggs and stored for 15 days; while insignificant effect was obtained for fertile eggs stored for 30 days. The addition of 1% thyme or 0.5-1.0% curcuma longa to hens diets significantly increase the percentages of hatchability in comparison to hens fed control diets by 18.60, 14.87 and 13.48%, respectively for fertile fresh eggs. While, in fertile eggs stored for 15 days increased hatchability by 12.44, 10.60 and 11.99%, respectively. The other treatments increased values insignificantly compared to hens fed control diets or 100 mg vit. E /Kg (fertile fresh eggs). Regarding fertile eggs stored for 30 days, hens fed 1% thyme or 0.5 - 1.0% curcuma longa recorded higher percentages of hatchability by 5.22, 4.77 and 6.08%, respectively in comparison to hens fed control diets.

Table 12: Effect of experimental treatments and storage time of fertile eggs on hatchability and chicks weight at hatch

	Hatchability (%)				Body weight of chicks at hatch (g)			
Treatments	Fresh	Stored 15 days	Stored 30 days	Over all of treatments	Fresh	Stored 15 days	Stored 30 days	Over all of treatments
N-control	56.67^{c}	63.33^{c}	63.33^{b}	61.11^{d}	34.97	35.00	35.00	34.99
Control	71.67^{b}	72.33^{b}	76.67^{a}	73.56^{c}	35.10	35.13	35.23	35.16
100 mg vit. E	76.67^{ab}	79.33^{a}	76.00^{a}	77.33^{bc}	35.27	35.20	35.27	35.24
200 mg vit. E	80.00^{ab}	80.33^{a}	77.67^{a}	79.33^{ab}	35.30	35.47	35.53	35.10
0.5% Thyme	80.00^{ab}	79.33^{a}	78.00^{a}	79.11^{ab}	35.30	35.27	35.37	35.31
1.0% Thyme	85.00^{a}	81.33^{a}	80.67^{a}	82.33^{a}	35.37	35.20	35.57	35.38
0.5 % Oregano	77.67^{ab}	77.33^{ab}	80.33^{a}	78.44^{ab}	35.20	35.20	35.30	35.23
1.0% Oregano	81.00^{ab}	81.67^{a}	80.66^{a}	81.11^{ab}	35.33	34.97	35.43	35.24
0.5% Rosemary	78.33^{ab}	81.67^{a}	82.33^{a}	80.78^{ab}	35.23	35.27	35.40	35.30
1.0% Rosemary	80.33^{ab}	83.00^{a}	80.66^{a}	81.33^{ab}	35.10	35.60	35.50	35.40
0.5% Curcuma L.	82.33^{a}	80.00^{a}	80.33^{a}	80.89^{ab}	35.50	35.20	35.33	35.34
1.0 % Curcuma L	81.33^{a}	81.00^{a}	81.33^{a}	81.22^{ab}	35.23	35.33	35.33	35.30
S E M	±1.374	±1.027	±1.005	±0.657	±0.059	±0.089	±0.070	±0.043
Source of variation	P value				P value			
Treatments	0.0001	0.0001	0.001	0.0001	NS	NS	NS	NS
Storage time		NS				NS		
Treatments x Storage time		NS				NS		

a,b,...= Means in the same column with different superscripts, differ significantly ($p < 0.05$). NS = Not Significant ($p > 0.05$). SEM = Standard Error of Means; N-control = Negative control diet with Vitamins and Minerals mixture free vit.E. Control = Control diet with Vitamins and Minerals mixture contain requirement of vit.E.

Ali *et al.* (2007) reported that the addition of 0.25% thyme to laying hens diets significantly increased the percentages of fertility and hatchability of eggs compared to laying hens fed control diets by 1.77 and 4.96%, respectively. Thyme decreased total lipid, LDL and increasing antioxidant capacity in plasma and consequently decreased the sources of free radicals passing to egg. Generally, the over all of treatments suggested that the addition of herbs or 200 mg vit. E /Kg to hens' diets significantly increased the percentages of hatchability in comparison to hens fed control diet. The highest percentage of hatchability was obtained by hens receiving 1% thyme followed by 1.0% rosemary, oregano and 0.5-1.0% curcuma longa. It could be concluded that, these treatments or 200 mg vit. E /Kg had positive effect on hatchability and fertile eggs could be stored without negative effect on fertility and hatchability up to 15 days. These could be explained as a result of antioxidant activity (Farag *et al.*, 1989; Deighton *et al.*, 1993; Ajuyah *et al.*, 1993; Cherian *et al.*, 1996; Galobart *et al.*, 1999). In addition to that, such treatments decreased malonaldehyde in yolk (Table 9). In this respect, Speake *et al.* (1998) reported the fact that chick embryo development is associated with an accumulation of polyunsaturated fatty acids in tissue lipids. This making them susceptible to lipid peroxidation (Surai, 1999a). Antioxidant compounds of herbs such thymol present in thyme and carnosic acid present in rosemary are transferred to eggs and deposited into yolk (Botsoglou *et al.*, 1997; Krause and Ternes, 1999; Galobart *et al.*, 2001a) and increase the adaptation mechanism to deal with over production of free radicals; consequently increase hatchability.

Body weight of hatched chick was not influenced significantly by any of the experimental treatments through differenced storage periods. Also; storage time had no significant effect (Table 12). The values of hatched chick weights for the over all treatments were 35.23 - 35.43 vs.35.15g for the control without significant differences between the treatments.

Conclusion:

- Addition of 1.0% (thyme, rosemary or oregano) or 0.5% Curcuma longa as natural antioxidants to laying hens diets increased egg mass, egg production and improved feed conversion.
- Addition of 1.0% (thyme or rosemary) or 0.5% Curcuma longa decreased total lipid; total cholesterol and LDL- cholesterol in blood and egg yolk.
- Addition of 1.0% (oregano or rosemary) or 0.5% curcuma longa during laying period decreased malonaldehyede formation in egg yolk and had positive effect on oxidative stability of shell eggs storage.
- Addition of 1.0 % (oregano, thyme, rosemary) or 0.5% curcuma longa improved fertility and hatchability.
- Further studies must be carried out to study the possibility of using herbs as natural antioxidants in commercial broiler breeder's diets.

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