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INFLUENCE OF USING DIETARY ANTIOXIDANT ON PRODUCTIVE AND REPRODUCTIVE PERFORMANCE OF MANDARA STRAINS UNDER SUMMER CONDITIONS

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ABSTRACT:

The present study aimed to investigate the influence of the dietary antioxidant addition to the basal diet on productive and reproductive performance of Mandara strains under summer conditions. A total number of 147 laying hens and 21 cocks at 24 weeks of age was allocated to 7 treatment groups (21 hens + 3 cocks / each treatment) until 36 weeks of age. The birds within each treatment were fed as follows:T1: Basal diet only without supplementation (served as a control), T2: Basal diet and 100 mg Vitamin C /kg feed, T3: Basal diet + 100 mg Vitamin E /kg feed, T4: Basal diet + 100 mg Vitamin C + E /kg feed, T5: Basal diet + 200 ml rosemary oil /kg feed, T6: Basal diet + 200 ml oregano oil /kg feed and T7: Basal diet + 100 ml rosemary and oregano oils as medicinal plants /kg feed, respectively.

***The obtained results showed that**, the major percentage components of rosemary and oregano oils by Gas chromatography-mass spectrometry (GC—MS) analysis were 1.8-cineole (44.33), α-Pinene (16.38) and Carvacrol (65.41) and p-cymene (14.58), respectively. Under the high ambient temperatures dietary oregano oil, Vitamin C, rosemary oil and an equal mixture of Vitamin C + E added to the basal basal diet were significantly (P≤0.05) increased the percentage of egg production and egg mass of Mandara layers. However, rosemary oil and an equal mixture of Vitamin C + E added to the diet were significantly (P≤0.05) heavier of egg weight than non-supplemented groups during the whole experimental period. Combinations of rosemary and oregano oils, combination of Vitamin C and E, rosemary oil, oregano oil, Vitamin C, Vitamin E alone in the diet treated groups were significantly (P≤0.05) enhancement of fertile eggs (%), hatchability/total eggs (%), sperm motility and sperm-cell concentration (X 10^9/ml) and decreased yolk cholesterol, serum*

cholesterol and glucose concentration, dead spermatozoa (%), sperm abnormalities (%) and acrosomal damage than in those controls treated group. Vitamin C addition alone or their combination with Vitamin E were significantly (P≤0.05) decreased body temperature and respiration rate of Mandara layers under Egyptian hot summer conditions. Blood pH was significant (P<0.05) decreased for layers fed Vitamin C, Vitamin E alone or their combination treated groups than in those controls. On the other hand, during the days post immunization control group created the lowest antibody levels, whereas individual antioxidant dietary supplementation alone or their mixtures had higher Sheep red blood cells (SRBC's) antibody levels when contrasted with the control group. However, blood serum activities of aspartate-aminotransferase (AST), alanine-aminotransferase (ALT) and malondialdehde (MDA) concentrations were significantly (P≤ 0.01) decreased, while total antioxidant capacity (TAOC) was significantly (P≤ 0.01) increased in all treated groups as compared with the control treated group.

In conclusion, *Vitamins C, E, rosemary oil, oregano oil alone or their combination showed better reduced the negative effects of heat stress and beneficial effect on maintaining immunity and antioxidant status under summer conditions.*

Key Words: Mandara strain, Vitamins C & E rosemary & oregano oils, productive & reproductive performance, summer conditions.

INTRODUCTION

Heat stress can influence on the reproductive function in poultry, under summer conditions. In females, heat stress can upset the normal status of reproductive hormones (Elnagar *et a*l., 2010). Çiftçi *et al.*, (2005) revealed that, presentation of laying hens to high ambient temperatures caused a reduction in reproductive activities and egg quality. Additionally, semen, ejaculate volume, sperm concentration, number of live sperm cells and motility were decreased of cocks to heat stress (Ilorin *et a*l., 2012). Heat stress causes oxidative damage, expanding mortality and reducing productivity in laying hens (Felver- Gant *et al.,* 2014). Different natural agents are used to minimize the harmful effects of heat stress and antioxidant on productive and reproductive performance of laying hen and

chicks. Such as, Vitamins E and Vitamin C (Çiftçi *et al.*, 2005) and medicinal plants such as oregano (Lambert *et al.*, 2001) and rosemary (Çiftçi *et al.*, 2013).

Rosemary (*Rosmarinus officinalis L*), utilized as a part of customary people pharmaceutical for the treatment of hyperglycaemia, is broadly acknowledged as one of the therapeutic herb with the most antioxidant activity (Tülay *et al.*, 2008). Addition Rosemary essential oil was imperative for medical use and its powerful antibacterial, cytotoxic, anti mutagenic, antioxidant, antiphlogistic and chemo preventive properties (Celiktas *et al.*, 2007). A study has exhibited the helpful impacts of rosemary essential oil as a natural antioxidant that counteracted shading decay, as well as, lipid oxidation (Hussain *et al.*, 2010). Çiftçi *et al.*, (2013) showed that rosemary oil supplemented with diet of Japanese quail under heat stressed condition; especially at a level of 250 ppm level supplemented diet had positive effects on performance and blood glucose level.

On the other hand, Oregano (*Origanum vulgare*) belongs to the family Lamiaceae. Real segments of oregano are carvacrol and thymol that constitute about 78 to 82% of the total oil (Lambert *et al.*, 2001). He *et al.* (2017) showed that the laying rate, egg weight, feed conversion ratio and the activities of amylase and trypsin of Hy-Line layers were significantly ($P < 0.01$) improved by a diet supplemented with 100 mg/kg oregano essential oils. Roofchaee *et al.* (2011) demonstrated that 600 mg/kg of oregano essential oil applied growth promoting impacts and furthermore showed strong antibacterial effects against cecal *E. coli* in grower diets of broiler.

Therefore, the present study aimed to the effect of dietary antioxidant supplementation alone or their combination, of Vitamin C, Vitamin E, Vitamin C + Vitamin E, rosemary oil, oregano oil, and rosemary + oregano oil on the productive and reproductive performance of Mandara laying hens, as well as, some blood parameters under Egyptian hot summer condition.

MATERIALS AND METHODS

The experimental work was carried out at the Inshas Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, Giza, Egypt, during the period from June, to Augusts, 2016.

A total number of 147 laying hens and 21 cocks of Mandara local strain at 24 weeks of age were utilized in a completely randomized design and randomly assigned into 7 treatment groups (21 hens + 3 cocks / each treatment). Laying hens of each group were nearly equal of average body

weight $\left(\overline{X} \pm SE\right)$. Each group was sub-divided into three replicates (7 hens and 1 cock). A corn-soybean meal basal experimental layer diet (16.03 % CP and 2770 kcal ME/kg diet) was formulated to cover all recommended nutrient requirements according to Feed. Composition Tables for Animal And Poultry Feedstuffs used in Egypt (2001) as shown in Table (1). The birds within each treatment were fed as follows: T1: Basal diet only without supplementation (served as a control) contained 10 mg Vitamin E/kg diet, T2: Basal diet + 100 mg Vitamin C (L- ascorbic acid, Roche, Egypt) /kg feed, T3: Basal diet+ 100 mg Vitamin E (as α- dl tocopherol acetate, Roche, Egypt)/kg feed, T4: Basal diet + 100 mg Vitamin C + E /kg feed, T5: Basal diet + 200 ml rosemary oil/kg feed. T6: Basal diet + 200 ml oregano oil/kg feed and T7: Basal diet + 100 ml rosemary and oregano oils as medicinal plants /kg feed, respectively.

The average minimum and maximum ambient temperatures during summer season ranged between 26.37 and 34.98 ^{0}C, relative humidity from 34.28 to 76.52% and temperature-humidity index (THI) from 23.94 to 33.47 % under Inshas, Sharkia Province, Egypt as shown in Table 2. THI was estimated according to the formula by Marai *et al.* (2000) as follows:

THI= db ^{o}C-{(0.31-0.31 RH) (db ^{o}C -14.4)},

Where db ^{o}C = bulb temperature in Celsius and RH= RH%/100. The values obtained indicate the following: <22.2 = absence of heat stress; 22.2 to <23.3 = moderate heat stress: 23.3 to <25.6 = severe heat stress and 25.6 and more = extreme severe heat stress.

All birds were housed individually in one cage. Each cage was provided with an individual feeder and automatic pipette drinkers. Birds were fed *ad-libitum* and fresh water was available at all the time during the experimental period. The photoperiod during the experimental period was fixed at 16h. Vitamin C, Vitamin E, rosemary and oregano oil were purchased from a local commercial market (Haraz Company for Medicinal plants), Cairo, Egypt. Essential oils were dissolved in vegetable oil (considered 100% pure) and then gently mixed with the standard diets. The diets were prepared freshly each 3 days at different of the experimental period.

Gas chromatography-mass spectrometry (GC—MS) analysis:

Rosemary and oregano basic oil parts were examined according to (AOAC, 1990) by GC/MS at the National Research Center, Dokki, Cairo, Egypt utilizing a Varian 3400 GC. furnished with a DB-5 combined silica hairlike segment (30 m X 0.25 mm; i.d. 0.25 µm film thickness). The multi-

Table (1). Ingredient and chemical compositions (%) of the experimental diet for laying hens

Ingredients	%
Yellow corn	66.00
Soybean meal (44% CP)	19.05
Corn gluten meal (60% CP)	2.59
Wheat bran	2.50
Dicalcium phosphate	1.50
Limestone	7.60
Salt	0.30
Vitamin & Min. Premix*	0.30
DL-Methionine	0.16
Total	**100**
Calculated analysis: **	
Crude protein (CP), %	16.03
ME; kcal/kg	2770
Ether extract	2.86
Crude fiber	3.09
Calcium	3.29
Av. Phosphorus	0.39
Lysine	0.73
Methionine	0.44
Methionine + cystine	0.74

* Vitamins and mineral premix provides per 3kg: Vitamin A 12000 IU; Vitamin D3 2000 IU; Vitamin E. 10mg; Vitamin k3 2mg; VitaminB1 1mg; Vitamin B24mg; Vitamin B6 1.5 mg; Pantothenic acid 10mg; VitaminB12 0.01mg; Folic acid 1mg; Niacin 20mg; Biotin 0.05mg; Choline chloride (50% choline) 500 mg; Zn 55mg; Fe 30mg; I 1mg; Se 0.1mg; Mn 55mg; ethoxyquin 3000 mg.

** Calculated analysis according to Feed Composition Tables for animal and poultry feedstuffs used in Egypt (2001).

step temperature program was increased from 60°C (held for 3 min) to 260°C (held for l0 min) at a rate of 5°C min^{-1}. The carrier gas was helium at a flow rate of l ml min^{-1} and the example, measure was 1 µl (injector temperature was 250°C). The mass spectrometer was a Varian-Finnigan SSQ 7000 working with an ionization voltage of 70 eV. Sweep time and mass range were 5 s and 40-400 m/z, as shown in Table 3.

Table (2): Means of air temperature, relative humidity (RH) and temperature-humidity index (THI) during summer season according to Egyptian Meteorological Authority

Summer months	Air temperature (°C)		Relative humidity (%)		Temperature-humidity index (THI)	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
June	26.17±0.54	38.7±0.57	17.87±1.28	75±3.36	23.17	36.82
July	26.52±0.29	32.94±0.1	42.59±0.99	77.13±1.21	24.36	31.63
August	26.42±0.38	33.3±0.16	42.39±1.04	77.42±1.59	24.27	31.98
Averages	**26.37±0.41**	**34.98±0.27**	**34.28±1.1**	**76.52±2.05**	**23.94**	**33.47**

Table (3). Chemical analysis of rosemary and volatile oil active component

Compound	Concentration (%)	
	Rosemary oil	Oregano oil
α-Pinene	16.38	2.24
β-Pinene	5.93	0.63
Camphene	9.81	0.59
Borneol	1.94	0.87
1,8-Cineole	44.33	--
p-Cymene	0.57	14.58
Camphor	9.10	--
Terpinolene	0.21	2.96
Thymol	--	3.85
Carvacrol	--	65.41
Carene	4.98	0.77
Caryophyllene	1.96	3.22
Limonene	2.48	1.43
Bornyl Acetate	0.75	1.39
Total	98.44	97.94
Others	1.56	2.06

Productive performance:

The body weight changes of laying hens calculated by the difference between finally and initial weight, while the egg number and egg weight were recorded daily and feed intake was calculated weekly. The egg production rate was calculated during the experimental period. Where: Egg production rate = Egg number / hen/ x 100. Egg mass was calculated by

multiplying egg number by average egg weight. Feed conversion (g feed/g egg) was also calculated. At 36 weeks of age, about 36 eggs from each treatment were collected and incubated. After hatching, the chicks were counted and non-hatched eggs were broken to determine the percentages of fertility and hatchability. Fertility was calculated as the percentage of fertile eggs from the total number of set eggs, while the hatchability was expressed as the chicks hatched from fertile eggs and from total eggs.

Physiological parameters:

The rectal temperature (RT) of three birds randomly selected out of each replicate was measured with a digital thermometer (0.1^{o}C accuracy) inserted into the rectum (colon) of the birds for one minute according to Yahav and McMurtry (2001). Respiratory rate (RR) of the birds was taken as the number of breaths per minute. Data on RT and RR were collected two consecutive days in every week.

Immunocompetence and Immunological analysis:

Sheep red blood cells (SRBC) were used as test antigens to quantitatively analyze specific antibody response as a measure of humeral immunocompetence. At 35 weeks of age, three birds in each treatment were injected intravenously with 0.5 ml of 10% SRBC suspension prepared in 0.9% sterile saline. At 3, 6 and 9 days, post immunization, blood samples was collected to determine the primary antibody response. Antibody levels were quantities using a micro titration hem agglutination technique (Van der Zijpp and Leenstra, 1980). Phagocytic activity (PA) of polymorph nuclear cells using Candida albicans and phagocytic index (PI) was performed according to the method described by Kawahara *et al.* (1991). Phagocytic activity (PA) = Percentage of phagocytic cells containing yeast cells.

$$\text{Phagocytic activity (PA)} = \frac{\text{Number of yeast cells phagocytized}}{\text{Number of phagocytic cells}}$$

Blood samples:

Blood samples were collected at the end of the experiment and were classified into three parts. The first part was collected in heparin (20 IU/ml) for evaluating the phagocytic activity of heterophils. The second part immediately after blood sample collection blood pH was determined using a digital electric pH meter (JENCO model No. 608 U.S.A). The third part of serum samples were taken from 3 hens within each treatment and placed in centrifuged tubes, left to clot, then centrifuged for separation of serum.

Serum samples were stored at -20°C until analysis of serum biochemical. Total cholesterol and lipid concentrations were colorimetrically determined in egg yolk, while total protein, albumin, total cholesterol, glucose, serum enzyme activities of aspartate-aminotransferase (AST) and alanine-aminotransferase (ALT), total antioxidant capacity (TAOC) and malondialdehde (MDA) were determined in blood serum using commercial kits (Bio-Diagonosis Co., Cairo, Egypt), following the same steps as described by the manufacturers.

Semen quality:

Semen was evaluated immediately after collection of cocks and artificially inseminated to hens (cock/7 hens) two times per week. Semen tests were exclusively gathered toward the end of test period of the massage method from all cocks. Immediately after semen collection, semen, ejaculate volume was measured using graduate collecting tubes and hydrogen-ion concentration (pH) was measured by Universal Indicator Paper and Standard Commercial Stain. A drop of semen with the aid of a micro-pipette was placed on a pre warmed microscope slide, which was then covered with a glass cover slip and examined at a magnification of ×400. Several fields were examined and an estimate to the nearest 10% of the motile sperm was made. Motility of semen samples was expressed as the percentage of motile spermatozoa having moderate to rapid progressive movement and cells that are motile under their own power (Ommati *et al.,* 2013). At least 10 microscopic fields were examined for each semen sample. Eosin-Nigrosine stain was used to determine the percent of morphologically sperm abnormalities and dead spermatozoa. For sperm cell concentration (X 10^9/ml) a droplet of diluted semen (1:200 in distilled water) semen was tenderly put on both councils of a Neubauer hemocytometer and the number of spermatozoa was determined microscopically (Ommati *et al.,* 2013). Acrosomal damage of spermatozoa was determined according to Waston (1975).

The economic efficiency (EEF):

The economic efficiency (EEF) of the experimental treatments was estimated depending up on feeding cost and price of egg produced.

Statistical analysis:

Data were analyzed by the least square analysis of variance according to Snedecor and Chochran (1982) using the General Linear Model Procedure (SAS, 2004) at the 5% level of significance as the following model:

$$Y_{ij} = \mu + N_i + e_{ij}$$

Where: Y_{ij} = Any observation, μ = Overall mean, N_i = Effect of treatment (i = 1….7)., e_{ij} = Experimental random error.

All percentages, data were transferred to percentage angle using arcsine equation before subject to statistical analysis. Significant differences among means were tested using Duncan Multiple New Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Chemical composition of rosemary and oregano essential oils:

Results presented in Table (3) showed that the major percentage component of rosemary oil by GC/MS analysis was 1.8-cineole (44.33). Other components such as α-Pinene (16.38), Camphene (9.81), Camphor (9.10), β-Pinene (5.93), Carene (4.98), Limonene (2.48), Caryophyllene (1.96), Borneol (1.94) and p-cymene (0.57), terpinolene (0.21), Bornyl Acetate (0.75) were also found at low amounts. These results are in agreement with those reported by Daferera *et al.* (2000) who showed that the chemical composition of rosemary essential oil was also characterized by the predominant presence of 1,8-cineole, which accounted for 88.9% of the total essential oil. Moreover, Pintore *et* al. (2002) reported that the major constituents of rosemary essential oils, collected from Morocco, Tunisia, Turkey, Greece, Yugoslavia, Italy, and France, were 1,8-cineole, which accounted for more than 40% of the essential oil. Mathlouthi *et al. (*2012) found that the essential oil isolated from rosemary was characterized by its increased content of 1,8-cineole (49.99%). However, the major percentage component of Oregano oil was Carvacrol (65.41). Other components such as pcymene (14.58), Thymol (3.85), Caryophyllene (3.22), terpinolene (2.96), α-Pinene (2.24), Limonene (1.43), Bornyl Acetate (1.39) and Borneol (0.87), Carene (0.77), β-Pinene (0.63), Camphene (0. 59) were also found in low amounts. These results are in agreement with those reported by Oussalah *et al.* (2006), who studied 3 oregano varieties: the first (*Origanum heracleoticum*) from France, rich in carvacrol (54%); the second (*Origanum compactum*) from Morocco, rich both in carvacrol (22%) and γ-terpinene (23%); and the third (*Origanum majorana*) from Egypt, rich in terpinene-4-ol (26%) and γ-terpinene (12%). Mathlouthi *et al. (*2012) and He *et al*. (2017) found that the essential oil isolated from oregano was characterized by its increased content of carvacrol (69.55%) and (73.06), respectively.

Productive performance:

Productive performance as affected by individual antioxidant dietary supplementation or their mixtures (Vitamin C, Vitamin E alone or their

combination, rosemary oil, oregano oil alone or their combination) in Mandara layers during the different experimental periods are shown in Table 4.

Antioxidant like Vitamins C and E did not improve body weight changes when incorporated in layer diets, similar to that reported by Jang *et al.* (2014) who found that dietary supplementation of Vitamin C or E did not influence on body weight, feed intake and feed conversion when birds are presented to high ambient temperature under summer conditions. The present investigation indicated additionally that the antioxidant dietary oregano oil (carvacrol, thymol and terpinolene) supplementation improved body weight changes in Mandara layers. Another study showed improvements in body weight at 21and 42 days when the chickens were fed a thymol plus carvacrol mixture, in combination (Hashemipour *et al.*, 2016). The main active components of oregano essential oil are thymol and carvacrol, which can improve feed digestibility, due to positive effects on nutrient digestibility (Jamroz *et al.*, 2005). These findings may be due to the essential oil of oregano more active biologically when incorporated in layer diets. In addition, Roofchaee *et al.* (2011) stated that dietary oregano essential oils showed significantly increased body weight of broiler chickens. The enhancements of growth performance could be in completely clarified by the increase in the apparent digestibility of dietary protein and the practical digestive capacity in general, which increase the intestinal availability of nutrients for retention and thus lead creatures to become speedier (Windisch *et al.*, 2008).

Data presented in Table 4 revealed that dietary antioxidant was insignificantly effected on feed intake of Mandara layers during all the experimental periods. Whereas, feed conversion (except at 28-32 and 32-36 weeks of age), percentage egg production (except at 32-36 weeks of age), egg weight (except at 28-32 and 32-36 weeks of age), and egg mass were significantly (($P \leq 0.05$ and 0.01) influenced by dietary antioxidant.

The obtained results demonstrated that the feed conversion ratio was enhanced by addition of Vitamin C alone or an equal mixture of Vitamin E, rosemary oil alone or oregano oil alone as compared with control treated from 24-36 weeks of age. These results are supported by the findings of Khan and Sardar (2005) who revealed that Vitamin C supplementation was enhanced feed conversion ratio in commercial white Leghorn chicken exposed to heat stress. The impacts of Vitamin C on feed conversion may be due to that Vitamin C helps to control the increase in body temperature and plasma corticosterone concentration. Çiftçi *et al.* (2005) detailed that dietary supplementation with 125 mg Vitamin E in addition to 200 mg Vitamin C/kg

in laying hen's diet presented to chronic stress (35°C) was significantly enhanced feed efficiency. Ahmed *et al*. (2008) found that FCR, was enhanced (P≤0.01) in hens presented in 34.5°C (28- 42°C) supplemented with Vitamin C at either 1000 or 1200 ppm/ L water as contrasted with the control birds. Bölükbaş *et al.* (2008) found that feed conversion ratios were significantly (P≤0.05) enhanced by supplementation of the essential oils (thyme, sage and rosemary oil). Radwan *et al.* (2008) noticed also that dietary rosemary enhanced FCR, while the FI was not measurably influenced. Dietary feeding of essential oil extracted from medicinal plants increased the secretion of digestive enzymes, so enhanced nutrient digestibility and improved the performance of broilers (Jang *et al.*, 2014).

In the whole experimental period, dietary oregano oil, Vitamin C, rosemary oil and an equal mixture of Vitamin C + E were significantly (P≤0.05 and P≤0.01) increased percentage egg production and egg mass of Mandara layers. However, rosemary oil and an equal mixture of Vitamin C + E were significantly (P≤0.05 and P≤0.01) increased of egg weight than non-supplemented groups. These results are in agreement with the findings of Ahmed *et al*. (2008), who detailed that egg production, egg weight and egg mass were enhanced (P<0.01) in hens presented at 34.5°C (28-42°C) when supplemented with Vitamin C at either 1000 or 1200 ppm/ L water when compared with the control birds. The beneficial impact of Vitamin C might be ascribed to in initiating thyroid gland, which affected the feed intake (El-Fiky, 1998). The addition of, Vitamin C scavenges free oxygen radicals which are vital, however, to prevent stress caused by oxidation of cell membrane in the digestive system and restore efficient feed utilization (Jaffy, 1984).

On the other hand, Abd El-Maksoud (2006) found that Vitamin E supplementation increased egg production (%) by reducing the adverse effects of high ambient temperature on the laying hens during summer months. Vitamin E was significantly enhanced egg production, this might be due to enhancing the synthesis of egg yolk forerunners (vitellogenine and very low density lipoprotein) in the liver through its activity as an antioxidant, which protect the liver from lipid peroxidation and damage to the cell membrane. Çiftçi *et al.* (2005) demonstrated that dietary supplementation with 125 mg Vitamin E added to 200 mg Vitamin C/kg in laying hen's diet presented with chronic stress (35°C) increased significantly

egg production. Radwan *et al.* (2008) noticed that dietary rosemary was increased egg production, and egg mass. The same later author reported that the use of 1.0% oregano in the diet was increased egg production and egg weight of hens. He *et al*. (2017) indicated that oregano essential oil enhanced average egg weight and feed efficiency on laying hens. These results the oregano essential oils may enhance the intestinal health and digestibility of the feeds and thus improve the performance of laying hens (He *et al*., 2017).

Reproductive performance:

Fertility (%), hatchability traits (%) and some constituents of the egg yolk extract as affected by individual antioxidant dietary supplementation or their mixtures (Vitamin C, Vitamin E alone or their combination, rosemary oil, oregano oil alone or their combination) in Mandara layers during the different experimental periods are shown in Table 5.

Combinations of rosemary and oregano oils, combination of Vitamin C and E, rosemary oil, oregano oil, Vitamin C, Vitamin E alone in the diet treated groups were significantly ($P \leq 0.05$ and 0.01) enhancement of fertile eggs (%) and hatchability/total eggs (%) and decreased yolk cholesterol than in those controls. At the same time, hatchability/fertility eggs (%) and total lipids were insignificantly effects with individual antioxidant dietary supplementation or their mixtures. Rosemary oil, oregano oil alone or their combination may have lowered effect on yolk cholesterol and total lipid, this may lead to produce enriched eggs that are healthier for human consumption and useful for those suffering from heart diseases. Egg yolk cholesterol and total lipid reduction did not have any negative effect on egg production rate. The increases of fertile eggs and hatchability/total eggs (%) in antioxidant treated groups could be due to the significantly increase of sperm motility, sperm-cell concentration and decreased in dead spermatozoa (%), sperm abnormalities (%) and acrosomal damage (%) compared with those in the control group. These antioxidants may block the production of ROS or counteract oxygen toxicity. Such increase may depend on egg shell thickness improvement in most treated groups compared with the control group. These results are in agreement with the findings of Radwan *et al.* (2008) who showed that the addition of 1% oregano to the hens' diets was significantly increased the percentages of fertility in comparison to hens fed control diets by 8.23. This

improvement can be explained as a result of oregano, which have antioxidant activities; decreased malondialdehyde formation in egg yolk and improved the semen characteristics consequently. Fertility rate might be increased by proactive qualities of plant oils containing some action items (Hashemi and Davoodi, 2012) and it could be associated with the vitamins and mineral contents of the extract (Kara, 2009). Soliman *et al.* (2016) found that fertility and hatchability percentages were significantly ($P\leq 0.05$) higher in oregano groups contrasted with the control group. On the other hand, Kontecka *et al.* (2001) detailed that Vitamin C was accounted to have a positive impact on the fertility and hatchability in Pekin ducks. Fitri *et al.* (2012) shown that increased levels of dietary Vitamin E were increased hatchability and fertility. Ipek and Dikmen (2014) recommends that a dietary combination of Vitamin E (240 mg) and Vitamin C (240 mg) supplementation may influence positively sexual hatching characteristics of quails reared under heat stress. Mohiti-Asli *et al*. (2010) found that egg yolk cholesterol concentrations decreased linearly by dietary Vitamin E and C supplementation ($P\leq 0.01$).

Physiological parameters:

Heat stress was increased in respiration rate and cloacal temperature recorded , similar to that da Silva *et al.* (2007) who demonstrated that under high temperatures, broilers exhibited a significantly increased in respiration rate and cloacal temperature.

Vitamin C, alone or with Vitamin E were significantly ($P\leq 0.05$) decreased body temperature and respiration rate of Mandara layers under Egyptian hot summer conditions. However, it has not affected with rosemary oil, oregano oil alone or their combination (Table 6). These results are in agreement with the findings of Kassim and Norziha (1995) who found that layer and broiler fed diets containing ascorbic acid in the tropics were less stressed due to having diminished body temperature and respiratory rates. Likewise, Kalamah *et al*. (2002) reported that, Vitamin C supplementation at a level of 250 mg/kg diet were reduced body temperatures and respiration rate of Norfa chickens exposed to hot temperature (37, 41 and 45ºC). Attia *et al.* (2015) demonstrated that Vitamin C, Cr or Se alone or their combination with enhanced body temperature, respiratory and blood pH of Dokki-4 laying hens strain under hot summer.

Blood pH and antibody titre against SRBC's:

Blood pH and antibody titre against SRBC as affected by individual antioxidant dietary supplementation or their mixtures (Vitamin C, Vitamin E alone or their combination, rosemary oil, oregano oil alone or their combination) in Mandara layers at the end of experimental period is shown in Table 7.

The blood pH increase in broiler exposed to heat stress and blood HCO3- concentrations decrease (Imik *et al.*, 2013). Blood, pH, was significantly ($P<0.05$) decreased for layers fed Vitamin C, Vitamin E alone or their combination, of treated groups than in those controls or with rosemary oil, oregano oil alone. These results partially agree with Attia *et al.* (2015) demonstrated that blood pH was lower in Cr, Se or Vitamin C alone or their combination in the treated groups and higher in the control group.

On the other hand, during the days post immunization control group created the lowest antibody levels, whereas individual antioxidant dietary supplementation or their mixtures (Vitamin C, Vitamin E alone or their combination, rosemary oil, oregano oil alone or their combination) had higher SRBC's antibody levels when contrasted with the control group. Higher antibody titre against SRBC at days post immunization in alone or in combination in treated groups may clarify the advantages of antioxidant dietary supplementation on humoral immune response, especially under heat stress. These results are in agreement with those of Hashemi and Davoodi (2012) reported that chicks were presented to high ambient temperature the diminishment of specific immune response in chicken. Boa-Amponsem *et al.* (2000) found an increased response of antibody titer against SRBC's in light to low (10 mg/kg) and high (300 mg/kg) doses of Vitamin E in broiler chicks measured at two different intervals. Mohiti-Asli *et al*. (2010) found that Vitamin E (α-tocopherol acetate) resulted in higher antibody titers for laying hens. Another impact of immune potentiating of Vitamin E might be clarified by its part in down regulating prostaglandin production by antagonizing the lipid peroxidation of arachidonic acid and limiting the entry of precursor into the prostaglandin cascade (Gore and Qureshi, 1997). Panda *et al.* (2008) expressed that the two Vitamins E and C altogether enhanced the antibody responses to inoculated sheep red blood cells (SRBC's). In our results, the increase in SRBC's in the rosemary oil and oregano oil groups may indicate a

more intense immune response to vaccine antigen in the treated subjects. Hashemipour *et al.* (2013) found an improved immune response in

broilers fed with a diet supplemented with thymol and carvacrol, characterized by an improvement of touchiness reaction and an increase of total IgG and IgG anti-sheep red blood cells with decreased heterophil to lymphocyte ratio.

Serum biochemistry parameters:

Serum components as affected by individual antioxidant dietary supplementation or their mixtures (Vitamin C, Vitamin E alone or their combination, rosemary oil, oregano oil alone or their combination) in Mandara layers during hot summer condition at the end of experimental period is shown in Table 8.

Serum total protein was significantly ($P<0.05$) increased for layers fed Vitamin C alone in the treated group than in those controls or with other treated groups. While, combination of Vitamin C and E and combinations of rosemary treated groups were significantly ($P\leq 0.01$) decreased serum cholesterol and glucose concentrations as compared with control treated group. Also, serum AST, ALT and MDA concentrations significantly ($P\leq 0.01$) decreased while TAOC significantly ($P\leq 0.01$) increased in all treated groups as compared with control treated group. These results are in agreement with those obtained by Hassan *et al.* (2013) demonstrate that dietary supplemental Vitamin C alone or their combination with Vitamin E were significantly increased concentrations of plasma protein and reduced the levels of plasma cholesterol contrasted with the control group reared under high environmental temperature. However, dietary supplementation with Vitamin E was an insignificant impact on plasma levels of total protein. Panda et *al.* (2008) noted that Vitamins E and C, as well as interactions between them, significantly affected the exercises of glutathione reductase and MDA. The same authors found that a combination of Vitamin E at 125 IU/kg with Vitamin C at 200 mg/kg diet had an additive effect on lessening the action of oxidative enzyme MDA and increasing the activity of antioxidant enzyme glutathione reductase (GSHR). Separately or as a combination supplemental Vitamin C and Cr resulted in a decrease in MDA concentration (Tawfeek *et al.,* 2014). Jang *et al.* (2014) revealed that total antioxidant status in serum of birds fed Vitamin C supplemented diet was significantly ($P<0.05$) higher with than that in birds a basal diet. MDA in serum and liver resulted in a significant ($P<0.05$) decrease in response to dietary Vitamin C or E supplementation.

Bölükbaşi *et al.* (2008) revealed that rosemary supplementation to laying hen diets were significantly depressed serum triglyceride and total cholesterol levels. The reduction of cholesterol in our results might be due to the impact of rosemary oil and oregano oil mixes display in these herbs on lipid digestion. In broiler, Osman *et al.* (2010) noticed that rosemary supplementation at levels of 0.5 and 1 g/kg diet was not significantly influence on serum concentration of protein, albumin, creatinine or cholesterol. Polat *et al.* (2011) expressed that the lowest AST concentration ($P< 0.001$) was resolved in broiler chickens treated with rosemary (5.7, 8.6 or 11.5 g/kg). Mansoub and Myandoab (2011) in their investigation found that including of oregano oil in broiler diet decreased cholesterol content of broiler. Abd El-Latif *et al.* (2013) exhibited that 100 or 200 mg rosemary oil /kg was not indicated significantly changes in serum levels of total protein, while AST concentration was significantly ($P\leq 0.01$) decreased. ÇİFTÇİ *et al.* (2013) watched that birds kept in heat stress conditions had a more prominent glucose level than hens kept in thermo neutral condition and dietary rosemary oil had a positive effect on blood glucose level. This finding shows that the rosemary oil might be producing its hypoglycemic action by a component free from insulin secretion by the inhibition of endogenous glucose production or the inhibition of intestinal glucose absorption (Eddouks *et al.,* 2003). Changes in these chemicals could be attributed to the nearness of phenolic mixes in the rosemary plant. Hashemipour *et al.* (2013) noticed that the admission of herbs or their contents (thymol and carvacrol) resulted in an increase in serum antioxidant enzyme activities, for example, SOD and glutathione peroxidase and a decrease in the MDA concentration. It appears that rosemary supplementation of layer diets was effective in enhancing the antioxidant ability of birds. Rosemary is a rich source of beneficial phenolic mixes, carnosol, carnosic and rosmarinic acids, and successful mixes having strong antioxidant, anti-cancer and anti-inflammatory activities (Celiktas *et al.,* 2007).

Semen physical characteristics:

Semen physical characteristics as affected by individual antioxidant dietary supplementation or their mixtures (Vitamin C, Vitamin E alone or their combination, rosemary oil, oregano oil alone or their combination) in Mandara cockerels during hot summer condition at the end of experimental period are shown in Table 9.

The percentage of sperm motility and sperm-cell concentration (x 10^9/ml) were ($P \leq 0.05$ and 0.01) significantly higher, while the percentage of dead spermatozoa, sperm abnormalities and acrosomal damage were significantly

(P≤0.05 and 0.01) improved as affected in the treated cocks compared with the control. These results are in agreement with those of McDaniel *et al.* (2004) who expressed that semen ejaculate volume, sperm-cell concentration, percentage of live spermatozoa cells and percentage of sperm motility were decreased in the males were exposed to heat stress. In males, heat stress initiates testicular damage by hoisting testicular lipid peroxidation because of increases in ROS, which negatively affect the weight of testes, semen, ejaculate volume, sperm-cell concentration, percentage of live and normal sperm count, spermatogonia, spermatocytes, spermatids, the positive dead sperm count and motility (Türk *et al.*, 2016). However, Radwan *et al.* (2008) found that expansion of 0.5 or 1.0 oregano to El-Salaam cock's diets increased semen ejaculate volume, sperm motility (%) and live sperm (%), and decreased dead spermatozoa (%) and abnormal spermatozoa (%) compared to the control group. Soliman *et al.* (2016) found that spermatozoa motility (%), live sperm (%), sperm-cell concentration were significantly (P≤0.05) increased for oregano treated groups contrasted with the control group. While, abnormal spermatozoa (%) was significantly (P≤0.05) decreased in the fed cocks on 0.6 g oregano /kg diet compared with other groups. Monsi and Onitchi (1991) supplemented the feed of heat-stressed broiler breeders with 0, 125, 250 or 500 ppm of vitamin C and who found that semen volume, total sperm, and motile sperm per ejaculate were significantly increased due to the addition of vitamin C.

Economical efficiency (EEf):

Data shown in Table (10) clear that Mandara layers as affected by Vitamin C fed diet recorded the highest net revenue and best economic efficiency followed by those fed oregano oil diet, however control group had the lowest net revenue and economic efficiency.

Conclusively, the addition of Vitamin C, E, rosemary oil, oregano oil and their combination of the diet of Mandara laying hens and cocks showed better productive and reproductive performance, especially egg production, semen quality, elevated the immunity as well as reduced rectal temperature and respiration, and best economic efficiency under summer season conditions.

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تأثير مضادات الأكسدة الغذائية على الأداء الإنتاجي والتناسلي لسلالة المندرة تحت الإجهاد الحراري

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صممت التجربة لدراسة تأثير اضافة مضادات الأكسدة الغذائية على الأداء الإنتاجي والتناسلي لسلالة المندره تحت الإجهاد الحراري). وأجريت هذه الدراسة علي عدد 147 دجاجة بياضة و 21 ديك قسمت عشوائيا إلي 7 مجموعات (كل مجموعة 21 دجاجة و3 ديوك) في اقفاص فردية حتى 36 أسابيع من العمر . وقد تم تغذيه الطيور داخل كل معاملة علي النحو التالي: T1 عليقه أساسية (كنترول بدون اى إضافة) . T2: عليقه أساسية مضاف إليها 100 مليجرام فيتامين ج / كجم عليقه . T3: عليقه أساسية مضاف إليها 100 مليجرام فيتامين هـ / كجم عليقه .T4 عليقه أساسية مضاف إليها 100 مليجرام / كجم عليقه من كل من فيتامين ج و فيتامين هـ. T5: عليقه أساسية مضاف إليها 200 مليجرام من زيت الروزماري / كجم عليقه . T6: عليقه أساسية مضاف إليها 200 مليجرام من زيت الأوريجانو / كجم عليقه . T7: عليقه أساسية مضاف إليها 100 مليجرام / كجم عليقه من كل من زيت الروزماري وزيت الأوريجانو.

وأظهرت النتائج ان معظم النسبة المئوية لزيت زيت الروزماري و زيت الأوريجانو من خلال تحليل GC/MS كانت 1.8-cineole (44.33)، α-Pinene (16.38) و Carvacrol (65.41)، p-cymene (14.58) علي الترتيب. وتحت

درجات محيطة عالية الإضافة الغذائية بزيت الأوريجانو، فيتامين ج، زيت الروزماري ، وخليط متساو من فيتامين ج و فيتامين ه ـ تزيد معنويا انتاج البيض وكتلة البيض لدجاج المندرة. ومع ذلك زاد معنويا وزن البيض في الفترة التجريبية بأكملها عند اضافة زيت الروزماري وخليط متساو من فيتامين ج و فيتامين هـ عن مجموعه المقارنة (الكنترول). ووجد ان خليط زيت الروزماري و زيت الأوريجانو وخليط فيتامين ج و فيتامين هـ، زيت الروزماري، زيت الأوريجانو، فيتامين ج ، فيتامين هـ كلا منفردا في المجاميع المعاملة يحسن معنويا نسبة معدل الخصوبة ونسبة الفقس /للمجموع الكلي للبيض وحركة الحيوانات المنوية وتركيزها وانخفاض كولسترول البيض و كولسترول وجلوكوز السيرم والنسبة المئوية لكل من الحيوانات المنوية الميتة والشاذة وتشوهات رأس الحيوان المنوي عن مجموعة المقارنة. وإضافة فيتامين ج منفردا او مع فيتامين هـ ينقص معنويا درجة حرارة الجسم ومعدل التنفس لدجاج المندرة البياض تحت ظروف الصيف المصرية. وانخفاض معنويا حموضة الدم للدجاج البياض المعامل والمغذي بفيتامين ج ، فيتامين هـ منفردا او مجتمعا عند المقارنة مع بمجموعة الكنترول.

ومن ناحية أخرى كانت مجموعه المقارنة خلال الايام اللاحقة للتحصين في ادني مستوي للأجسام المضادة في حين اضافة المواد المضادة للأكسدة سواء كانت منفردة او مجتمعة في اعلي مستوي للاستجابة المناعية لكرات الدم الحمراء للأغنام عند المقارنة مع مجموعه المقارنة.

ومع ذلك انخفض معنويا تركيزات سيرم إنزيمات الكبد AST, ALT ومضاد الأكسدة MDA في حين زاد منعويا مضاد الأكسدة TAOC لكل معاملات الأكسدة مقارنة مع مجموعه المقارنة.

التوصية: فيتامين ج، فيتامين هـ، زيت الروزماري، زيت الأوريجانو منفردا او معا يخفض خفض الآثار السلبية للإجهاد الحرارة الحراري وتأثيرهم مفيد على الحفاظ على المناعة ومضادات الأكسدة تحت ظروف الصيف