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Using Natural Antioxidants with or Without Sulphate to Improve Productive and Reproductive Performance of Two Local Strains at Late Egg Production Period

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Abstract: This study examines the hypothesis that natural antioxidants can improve the performance of local laying hens in late egg production period (48-60 weeks) especially in presence of sulphate ion. A total number of 288 hens plus 36 cocks 48 weeks old from Inshas and Dokki 4 strains (144 hens and 18 cocks from each strain) were equally divided into 12 groups with 3 replicates (8 hens + 1 cocks each) and housed in wire cages. The experimental hens fed from 48 to 60 week of age the control diet without or with 0.25% *Cuminum cyminum* L (CC), 0.5% Anhydrous Sodium Sulphate (SS), SS + CC, 20 mg commercial Canthaxanthin/kg diet (CAN) and CAN + SS. Compared to control diet, addition of CC, SS, CC + SS, CAN or CAN + SS increased egg number and egg yolk color score. All feed additives used in this study increased calcium, phosphorus, total protein, albumin, globulin and total antioxidants capacity in plasma while decreased LDL, HDL, total cholesterol and total lipids in both plasma and egg yolk. Feed additives increased T_3 and estrogen hormones compared to control diet. Feed additives tended to improve semen quality parameters, fertility and hatchability of total and fertile eggs. The combination of CAN and SS was the most successful additive under the condition of this study. Further studies are needed to elucidate the mechanism of protecting hen hormones from free radical attack at the late egg production period.

Key words: Natural antioxidants, laying hens, egg production

INTRODUCTION

Breeding hens are continuously exposed to oxidative stress over their lifetimes and the cumulative stress may cause diseases (Allen *et al*., 1998; Enkvetchakul *et al*., 1993). The current theory suggests that aging is largely a consequence of the synergistic relationship between free radical damage and the formation of advanced Maillard products (Droge, 2002) and the oxidative stress plays an unique role in age-related hyperthermic injury (Zhang *et al*., 2004). So, an effective antioxidant system for breeding pullets becomes very important. On the other hand, embryo and chicks exposed to an oxidative stress during the hatching period were expected to react with a compensatory induction of endogenous antioxidants (Lin *et al*., 2005). *Cuminum cyminum* L (CC) is an annual plant of the Umbelliferae family. This plant, considered one of the important spices in the world, is native to Egypt. It is used as a condiment and as an ingredient in many foods industries. Shaath and Azzo (1993) reported that Cumin aldehyde was found as the main component in cumin seed oil. Birjees Bukhari *et al*. (2009) suggested CC to be a potent source of antioxidants. Falany (1991) showed that sulfation has evolved as a key step in xenobiotic metabolism. Ali *et al*. (2010) found with growing chicks that the *Cuminum cyminum* L plus sulphate seemed to be the best additive under the heat stress condition. Also, they showed that diets supplemented with sulphate to some extent increased the activity of natural antioxidants.

Carotenoids have been shown to be one of the crucial importance during embryo development, specially during the period of intense growth which associated with increasing oxidative stress (Surai *et al*., 1999a). Carotenoids are used in physiological processes as antioxidants, but also have a protective and recycling role for other antioxidants like vitamins E and A (Surai and Speake, 1998) and they have the ability to modulate antioxidant enzyme function (Surai *et al*., 2001). Inclusion of a carotenoid mixture in the laying hen diets was associated with increased lutein, citranaxanthin, canthaxanthin and carotenoic acid accumulation in the egg yolk (Surai and Speake, 1998) and hen tissues (Surai *et al*., 1999b). Eggs of hens have high level of carotenoids are characterized by higher hatchability in comparison to eggs with low carotenoid levels (Inborr, 1996; Kemp *et al*., 2001). In the same connection, Koutsos *et al*. (2006) indicated that chicks hatched from hens fed carotenoid-deplete diet had greater systemic inflammatory responses than did chicks hatched from hens fed 40 mg lutein/kg diet. Grashorn and Steinberg (2002) found that the deposition rate of canthaxanthin of roughly 40% of dietary intake in yolks with strict linearity. The factors affecting absorption of carotenoids are fat content in feed (Han *et al*., 1987; Jayarajan *et al*., 1980),

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vitamins in feed (Surai and Sparks, 2001), breed of chickens (Jensen *et al*., 1998) and gender (Hinton *et al*., 1973; Twining *et al*., 1971). Polarity of carotenoids also affected absorption and accumulation in chickens. While vast majority of the 732 recorded naturally occurring carotenoids are hydrophobic (Britton *et al*., 2004); water dispersibility has been reported for carotenoid sulfates (Liaaen-Jensen, 1996; Oliveiros *et al*., 1994).

The reproductive performance decreases gradually in hens during aging, with a decrease in the length of clutch and an increase in the interval between ovulations. This already occurs at the end of the first year of laying (Williams and Sharp, 1978) and is closely connected with endocrine changes, primarily with variations in circulating levels of gonadotropins and sex steroids (Burger *et al*., 2002). Therefore, this study examines the hypothesis that natural antioxidants can improve the laying hen productive and reproductive performance in late egg production period (48-60 week) and cocks semen quality especially in presence of sulphate ion in their diets.

MATERIALS AND METHODS

This study was carried out at Sakha Animal Production Research Station, Animal Production Research Institute, Agricultural Research Center, Egypt during the period from April to June. A total number of 288 hens plus 72 cocks from Inshas and Dokki4 developed strains (48 weeks old) were used in this study. Birds of each strain were equally divided into six experimental groups of three replicates each (8 hens + one cock each) and housed in cage (100 cm long x 100 cm wide x 90 cm height). The other 36 cocks (18 cocks from each strain) were also divided into 12 groups of 3 cocks each fed experimental diets and housed separately for semen quality evaluation.

The minimum and maximum ambient temperatures were 26±1 and 32.2±1°C with 76±2.5% relative humidity. Six experimental diets were prepared from a hen control diet (Table 1). The experiment had a 2 x 6 factorial arrangement (two strains and six dietary treatments).

The experimental diets were offered for the two strains (144 hens + 18 cocks from each strain) from 48-60 weeks of age as follows:

1) The basal diet without any supplements and served as a control diet.
2) Control diet +0.25% *Cuminum cyminum* L (CC).
3) Control diet +0.50 % Anhydrous Sodium Sulphate (SS).
4) Control diet +0.25% CC + 0.50 % SS.
5) Control diet + 20 mg commercial Canthaxanthin (contains 10% canthaxanthin, CAN)/kg diet.
6) Control diet + 20 mg CAN/kg diet + 0.5% SS.

Anhydrous Sodium Sulphate was supplied by the Egyptian Salt and Mineral Company. The *Cuminum cyminum* L (CC) was purchased from local market in Cairo while canthaxanthin was provided by BASF Germany.

Table 1: Composition and calculated analysis of the control diet

Ingredients	%
Yellow corn	63.50
Soybean meal (44%)	24.57
Wheat bran	2.00
Lime stone	7.77
Di-calcium phosphate	1.50
NaCl	0.30
Vitamin and Min. Mix*	0.30
DL-methionine	0.06
Total	100.00
Calculated analysis**	
CP %	16.00
ME kcal/kg	2703.30
Crude fiber %	3.47
Crude fat %	2.86
Calcium %	3.32
Available phosphorus %	0.406
Lysine %	0.88
Methionine %	0.35
Methionine + Cysteine %	0.62
Sodium %	0.13

*Vitamin and mineral mix contain per 3 kg vit A 15 000 000, vit D3 3 300 000 IU, vit E 80 000mg, Vit K3 4000 mg, vit B1 2200 mg, vit B2 12 000 mg, vit B6 5500 mg, vit B12 20 mg, pantothenic acid 20 000 mg, Niacin 40 000 mg, Biotin 300 mg, Folic acid 1500 mg, Choline chloride 1400 gm, Selenium 300 mg, Copper 10000 mg, Iron 60 000 mg, Manganese 100 000 mg, Zinc 80 000 mg, Iodine 2000 mg, Cobalt 100 mg and $CaCO_3$ to 3000 g

**According to Egyptian Feed Composition Tables for Animal and Poultry Feedstuffs (2001)

Data collected included weight gain, egg weight and feed intake (g/hen/day). Egg mass was calculated (egg number x egg weight) and feed conversion (g feed/g egg). Also, some reproductive performance (semen characteristics, fertility % and hatchability %) were measured.

Total number of 240 eggs (20 eggs from each group) were collected twice (at the end of 54 and 60 weeks of age) to determine the egg quality traits.

At the end of 52 and 56 weeks of age, the other 36 cocks (three cocks from each treatment within each strain) were used to determine sperm concentration, mass motility, sperm abnormality and dead sperms were measured according to Kamar (1959 and 1960).

At the end of the experimental period (60 weeks of age), three hens from each treatment within each strain were randomly selected, weighed, slaughtered and sacrificed to obtain relative some organs weight. Blood samples were collected from slaughtered hens into heparinized test tubes, centrifuged at 4000 rpm for 15 min. Plasma was separated and stored in deep freezer at -20°C until assayed, Aspartate Transaminase (AST) and Alanine Transaminase (ALT) enzyme, Calcium, Phosphorus, Low Density Lipoprotein (LDL), total cholesterol and total lipids were determined by colorimetric methods using

commercial kits. Total antioxidant capacity in plasma was determined using commercial kit produced by Biodiagnostic Company. Hormones of T_3, T_4 and estrogen were determined by Radioimmunoassay (RIA). After measuring the egg quality, three yolk samples from each treatment were separated from the broken eggs and extracted to determine yolk cholesterol, LDL and total lipids according to Folch *et al.* (1957). High Density Lipoprotein (HDL) concentration of each assayed sample (plasma or yolk) was calculated by subtracting the LDL value from its total cholesterol. After 4 weeks from beginning of the experiment, a total number of 5760 eggs (20 eggs from each replicate/week until end of experiment) were incubated to evaluate the fertility and hatchability percentage.

The data collected were subjected to two-way analysis of variance to clear the main effects (treatments, strains and their interaction). To obtain the differences among specific all 12 groups (six dietary treatment with two strains), data were analyzed as one-way analysis of variance. The statistical analysis was computed using the General Linear Models (GLM) procedure and the significant differences among treatments means were separated by Duncan's Multiple Range test as described in the SAS (SAS, 1990).

RESULTS

Hens performance: Results in Table 2 showed that there was a significant effect of both dietary treatment and strain on egg number and egg weight while there was a significant effect of only dietary treatment on egg mass. In both strains, all feed additives used in this study improved egg number and egg mass reached to highest value with CAN+SS. Conversely, hens of control diet recorded the highest value of egg weight while the lowest value was recorded by those fed CAN+SS. The same manner, feed conversion was significantly improved by all the dietary treatments comparing with control group. However, the averages of EN, EM, FI and FC were almost similar for hens which received the CC+SS and CAN treatments. On the other hand, no significant alterations were recorded in hens weight gain due to using any of the dietary treatments.

Egg quality: The data in Table 3 showed that strain had a significant effect on egg length, shape index, albumin weight percentage, yolk weight percentage and shell weight percentage while the dietary treatment affect only the yolk color score. All feed additive used in this study increased yolk color score and hens fed CAN+SS recorded the highest value.

Table 2: Effect of treatment, strain and their interaction on productive performance of two local hens

Main effect		Egg number (egg/all period)	Egg weight (g)	Egg mass (g)	Feed intake g/hen/day	Feed conversion g feed/g egg	Body gain (g)
Treatment effect							
	Control	29.69^{e}	51.86^{a}	1538^{e}	116.79^{a}	6.84^{d}	346.87
	CC	48.39^{c}	51.20^{cd}	2478^{c}	115.88^{ab}	4.21^{c}	361.87
	SS	53.27^{b}	51.13^{cd}	2720^{b}	115.05^{bc}	3.81^{b}	323.95
	CC+SS	46.50^{d}	51.48^{b}	2393^{d}	115.33^{abc}	4.34^{c}	283.12
	CAN	46.02^{d}	51.29^{bc}	2359^{d}	114.97^{bc}	4.38^{c}	315.83
	CAN+SS	59.27^{a}	51.02^{d}	3019^{a}	114.26^{c}	3.41^{a}	295.62
Strain effect							
	Inshas	46.08^{b}	52.84^{a}	2433	116.71^{a}	4.55	334.65
	Dokki4	48.91^{a}	49.80^{b}	2433	114.05^{b}	4.44	307.77
Source of variation							
Treatment		0.0001	0.0001	0.0001	0.01	0.0001	NS
Strain		0.0001	0.0001	NS	0.0001	NS	NS
Treatment* strain		0.0001	0.009	0.0002	NS	NS	NS
Significant levels derived from one-way ANOVA							
Inshas	Control	28.42^{g}	53.31^{a}	1515^{g}	118.67^{a}	7.06^{f}	367.08
	CC	48.45^{cd}	52.62^{bc}	2550d^{e}	116.72^{abc}	4.12^{cd}	384.58
	SS	50.45^{c}	52.62^{bc}	2655^{d}	115.61^{bcd}	3.91^{bc}	337.08
	CC+SS	46.00^{de}	53.26^{a}	2451^{ef}	117.18^{ab}	4.31^{cd}	295.41
	CAN	45.33^{e}	52.83^{b}	2395^{f}	116.12^{bc}	4.36^{d}	344.58
	CAN+SS	56.04^{b}	52.43^{c}	2938^{b}	115.97^{bcd}	3.55^{ab}	279.16
Dokki4	Control	30.97^{f}	50.42^{d}	1561^{g}	114.91^{cde}	6.62^{e}	326.66
	CC	48.33^{cd}	49.78^{e}	2405^{f}	115.05^{bcde}	4.30^{cd}	339.16
	SS	56.08^{b}	49.65^{e}	2784^{c}	114.48^{cdef}	3.70^{b}	310.83
	CC+SS	47.02^{de}	49.67^{e}	2335^{f}	113.48^{ef}	4.37^{d}	270.83
	CAN	46.72^{de}	49.73^{e}	2323^{f}	113.83^{def}	4.41^{d}	287.08
	CAN+SS	62.50^{a}	49.60^{e}	3099^{a}	112.54^{f}	3.26^{a}	312.08
Pooled SEM		0.36	0.05	18.24	0.24	0.18	8.27
p-value		0.0001	0.0001	0.0001	0.0001	0.0001	NS

a,b,..etc.: Means within the same column with different superscripts are significantly different ($p<0.05$)

Table 3 Effect of treatment, strain and their interaction on egg quality parameters

Main effect		Egg length (mm)	Shape index %	Albumin weight (%)	Yolk weight (%)	Shell weight (%)	Shell thickness (Mm)	Yolk colour
Treatment effect								
	Control	53.97	74.70	57.04	33.26	9.69	39.02	6.16^{c}
	CC	53.37	74.96	56.56	33.38	10.04	40.05	7.50^{b}
	SS	53.17	75.70	56.80	33.04	10.15	40.21	7.66^{b}
	CC+SS	53.68	75.31	56.60	33.11	10.28	40.63	7.50^{b}
	CAN	53.80	75.87	56.41	33.50	10.08	39.71	7.65^{b}
	CAN+SS	53.21	76.03	57.36	32.78	09.85	39.44	8.50^{a}
Strain effect								
	Inshas	54.77^{a}	73.69^{b}	57.41^{a}	32.70^{b}	9.88^{b}	39.74	7.54
	Dokki4	52.32^{b}	77.19^{a}	56.21^{b}	33.62^{a}	10.15^{a}	39.96	7.43
Source of variation								
Treatment		NS	NS	NS	NS	NS	NS	0.0001
Strain		0.0001	0.0001	0.0002	0.001	0.01	NS	NS
Treatment* strain		NS	0.03	NS	NS	NS	NS	NS
Significant levels derived from one-way ANOVA								
Inshas	Control	55.12^{a}	73.40^{efg}	57.69^{ab}	32.93	9.36^{bc}	39.00	6.20^{cd}
	CC	54.05^{abc}	73.78^{defg}	57.26^{abc}	32.92	9.81^{abc}	40.35	7.30^{bc}
	SS	54.90^{ab}	72.52^{fg}	57.52^{abc}	32.61	9.87^{abc}	40.15	7.60^{ab}
	CC+SS	55.25^{a}	72.16^{g}	57.35^{abc}	32.49	10.16^{ab}	40.95	7.60^{ab}
	CAN	55.39^{a}	74.50^{cdefg}	56.49^{abc}	33.52	9.99^{abc}	39.34	7.62^{ab}
	CAN+SS	54.00^{abc}	75.43^{bcdef}	58.25^{a}	31.76	9.98^{abc}	38.68	8.66^{a}
Dokki4	Control	53.00^{bcd}	75.79^{abcde}	56.49^{abc}	33.53	9.96^{abc}	39.05	6.00^{d}
	CC	52.58^{cd}	76.35^{abcde}	55.75^{c}	33.92	10.31^{ab}	39.70	7.60^{ab}
	SS	51.52^{d}	78.72^{a}	56.10^{bc}	33.46	10.43^{a}	40.28	7.50^{ab}
	CC+SS	52.19^{cd}	78.31^{ab}	55.88^{bc}	33.71	10.39^{ab}	40.33	7.30^{bc}
	CAN	52.21^{cd}	77.24^{abc}	56.33^{bc}	33.48	10.17^{ab}	40.08	7.60^{ab}
	CAN+SS	52.52^{cd}	76.56^{abcd}	56.58^{abc}	33.67	9.73^{bc}	40.12	8.30^{ab}
Pooled SEM		0.19	0.3	0.16	0.14	0.05	0.18	0.14
p-value		0.0001	0.0001	0.03	NS	0.01	NS	0.001

a,b,..etc.: Means within the same column with different superscripts are significantly different ($p<0.05$)

Carcass characteristics: The data in Table 4 showed that there was no significant effect due to either strain or dietary treatment on carcass percentage, heart and liver while there was a significant effect due to strain on gizzard percentage. Compared to control diet, most of carcass traits studied of the both strains were not significantly affected by experimental treatments, except for CAN+SS treatment which increased heart percentage of Inshas strain. Meanwhile, abdominal fat percentage was decreased significantly by using CC treatment in Inshas hens, while in Dokki4 hens it was reduced by adding CAN in their diets.

Plasma parameters: Dietary treatment significant affect plasma phosphorus and calcium levels (Table 5). All feed additives used in this study significantly increased level of phosphorus and calcium compared to control diets. Also, the hens fed control diet recorded significantly the lowest values of either total protein or albumin. Although the feed additives increased the plasma globulin level compared to control diet, there were insignificant effect of dietary treatment on this parameter. It seemed that the Dokki 4 strain has a higher plasma globulin level compared to Inshas strain. The data in Table 6 showed that there was a significant effect due to dietary treatment on cholesterol, LDL, HDL and total lipids. All feed additives decreased previous parameters compared to control diet. Also, a significant effect of both dietary treatment and strain was observed on total plasma antioxidants capacity levels and all feed additives increased its levels compared to control diets.

Plasma T_3, T_4 and estrogen hormones: The effect of dietary treatment and strain and their interaction on plasma thyroid hormones are shown in Table 7. There was a significant effect of dietary treatment on plasma T_3, T_4 hormones and T_3/T_4 ratio. All feed additives used in this study increased T_3 hormone especially CAN+SS which recorded the highest values of T_3 and T_4 hormones. The same trend was observed with estrogen hormone. All feed additives increased plasma estrogen compared to control diet and significant interaction between treatment and strain was also observed for plasma estrogen.

Yolk chemical parameters: There was a significant effect of dietary treatment on all yolk parameters in this study (Table 8). There was a significant effect of strain on only LDL and total lipids while there was a significant interaction between treatment and strain on total lipids. All feed additives used in this study decreased all yolk parameters. The hens fed CAN+SS in both strains

Table 4: Effect of treatment, strain and their interaction on carcass characteristics

Main effect		Carcass	Heart	Liver	Gizzard	AF	P	Pancreas	Spleen
		(%)							
Treatment effect									
	Control	68.56	0.49	2.53	1.65	4.48^{ab}	0.35	0.21	0.14
	CC	67.22	0.50	2.32	1.39	3.42^{c}	0.34	0.15	0.13
	SS	66.79	0.47	2.38	1.75	4.37^{ab}	0.36	0.18	0.15
	CC+SS	78.09	0.48	2.39	1.53	4.55^{a}	0.42	0.24	0.13
	CAN	70.30	0.48	2.21	1.77	3.15^{c}	0.48	0.26	0.15
	CAN+SS	75.61	0.60	2.39	1.87	3.61^{bc}	0.52	0.19	0.16
Strain effect									
	Inshas	72.98	0.52	2.18	1.88^{a}	3.39^{b}	0.44	0.23^{a}	0.17^{a}
	Dokki4	69.22	0.49	2.56	1.44^{b}	4.47^{a}	0.38	0.18^{b}	0.12^{b}
Source of variation									
Treatment		NS	NS	NS	NS	0.007	NS	NS	NS
Strain		NS	NS	NS	0.006	0.0002	NS	0.03	0.005
Treatment* strain		NS	0.02	NS	NS	0.01	NS	NS	NS
Significant levels derived from one-way ANOVA									
Inshas	Control	68.03	0.42	2.44	1.73	3.73^{bcd}	0.36	0.18^{bc}	0.15
	CC	64.62	0.49	1.93	1.49	2.12^{e}	0.40	0.16^{c}	0.16
	SS	69.23	0.54	2.31	2.16	3.67^{bcd}	0.36	0.22^{abc}	0.17
	CC+SS	79.09	0.50	2.14	1.60	4.20^{abc}	0.39	0.30^{ab}	0.14
	CAN	74.12	0.48	2.09	2.02	3.57^{bcd}	0.47	0.32^{a}	0.18
	CAN+SS	82.77	0.72	2.15	2.27	3.27^{cde}	0.69	0.20^{abc}	0.20
Dokki4	Control	69.10	0.57	2.62	1.57	5.23^{a}	0.35	0.24^{abc}	0.12
	CC	69.83	0.52	2.71	1.30	4.72^{ab}	0.28	0.13^{c}	0.09
	SS	64.34	0.39	2.44	1.34	4.84^{ab}	0.37	0.15^{c}	0.12
	CC+SS	77.09	0.47	2.65	1.45	5.07^{a}	0.46	0.19^{bc}	0.12
	CAN	66.47	0.49	2.33	1.53	2.72^{de}	0.48	0.20^{abc}	0.12
	CAN+SS	68.46	0.48	2.63	1.47	3.95^{abcd}	0.34	0.18^{c}	0.12
Pooled SEM		01.37	0.01	0.09	0.07	0.20	0.02	0.01	0.008
p-value		NS	NS	NS	NS	0.0006	NS	0.03	NS

a,b,..etc.: Means within the same column with different superscripts are significantly different ($p<0.05$). AF = Abdominal Fat; P = Proventriculus

Table 5: Effect of treatment, strain and their interaction on some plasma parameters of experimental hens at 60 weeks of age

Main effect		ALT U/L	AST U/L	Phos. mg/dl	Calcium mg/dl	TP mg/dl	Albumin mg/dl	Globulin mg/dl
Treatment effect								
	Control	25.66	63.33	3.94^{b}	11.57^{c}	4.70^{c}	2.66^{b}	2.05
	CC	27.00	65.33	4.93^{a}	12.57^{ab}	5.67^{ab}	3.63^{a}	2.21
	SS	25.66	68.66	4.90^{a}	12.95^{a}	6.00^{a}	3.63^{a}	2.38
	CC+SS	26.33	66.00	4.43^{ab}	12.07^{bc}	5.47^{ab}	3.10^{ab}	2.36
	CAN	26.66	64.66	4.76^{a}	12.56^{ab}	5.54^{ab}	3.20^{ab}	2.34
	CAN+SS	26.33	68.00	4.97^{a}	12.23^{b}	5.00^{bc}	2.83^{b}	2.16
Strain effect								
	Inshas	26.22	65.66	4.53	12.37	5.36	3.18	2.17^{b}
	Dokki4	26.33	66.33	4.78	12.27	5.44	3.17	2.32^{a}
Source of variation								
Treatment		NS	NS	0.0041	0.0017	0.01	0.005	NS
Strain		NS	NS	NS	NS	NS	NS	0.03
Treatment* strain		NS	NS	NS	NS	NS	NS	NS
Significant levels derived from one-way ANOVA								
Inshas	Control	25.33	58.66	3.87^{d}	11.68^{c}	4.51^{d}	2.57^{c}	1.95^{c}
	CC	26.66	65.33	4.49^{abcd}	12.93^{ab}	6.12^{a}	3.85^{a}	2.26^{abc}
	SS	25.33	70.00	5.00^{a}	13.00^{a}	5.91^{ab}	3.57^{ab}	2.33^{abc}
	CC+SS	26.66	66.66	4.10^{bcd}	11.99^{bc}	5.48^{abcd}	3.15^{abc}	2.33^{abc}
	CAN	26.66	65.33	4.74^{abc}	12.35^{abc}	5.40^{abcd}	3.28^{abc}	2.11^{bc}
	CAN+SS	26.66	68.00	4.99^{a}	12.30^{abc}	4.73^{cd}	2.65^{c}	2.07^{bc}
Dokki4	Control	26.00	68.00	4.02^{cd}	11.46^{c}	4.89^{bcd}	2.74^{bc}	2.15^{bc}
	CC	27.33	65.33	5.36^{a}	12.21^{abc}	5.23^{abcd}	3.40^{abc}	2.16^{bc}
	SS	26.00	67.33	4.80^{abc}	12.90^{ab}	6.09^{a}	3.69^{a}	2.43^{ab}
	CC+SS	26.00	65.33	4.76^{abc}	12.14^{abc}	5.45^{abcd}	3.06^{abc}	2.39^{ab}
	CAN	26.66	64.00	4.77^{abc}	12.78^{ab}	5.69^{abc}	3.12^{abc}	2.56^{a}
	CAN+SS	26.00	68.00	4.94^{ab}	12.17^{abc}	5.28^{abcd}	3.01^{abc}	2.26^{abc}
Pooled SEM		00.29	01.01	0.09	00.03	0.11	0.09	0.10
p-value		NS	NS	0.01	0.01	0.04	0.04	0.04

a,b,..etc.: Means within the same column with different superscripts are significantly different ($p<0.05$). Phos. = Phosphorus; TP = Total Protein

Table 6: Effect of treatment, strain and their interaction on total cholesterol, LDL, HDL, total lipids and total antioxidants capacity in plasma of experimental hens at 60 weeks of age

Main effect		Cholesterol mg/dl	LDL mg/dl	HDL mg/dl	Total lipids g/dl	Total antioxidants capacity mmol/L
Treatment effect						
	Control	136.49^{a}	86.07^{a}	50.42^{a}	16.74^{a}	0.36^{c}
	CC	113.83^{b}	71.60^{b}	42.23^{b}	14.53^{b}	0.47^{b}
	SS	116.96^{b}	73.35^{b}	43.61^{b}	14.50^{b}	0.47^{b}
	CC+SS	123.83^{ab}	78.16^{ab}	45.67^{ab}	15.62^{b}	0.51^{ab}
	CAN	120.98^{b}	75.79^{b}	45.19^{ab}	14.55^{b}	0.56^{a}
	CAN+SS	118.71^{b}	75.28^{b}	42.65^{b}	14.95^{b}	0.58^{a}
Strain effect						
	Inshas	122.37	77.05	45.05	15.19	0.45^{b}
	Dokki4	121.23	76.36	44.87	15.10	0.53^{a}
Source of variation						
Treatment		0.03	0.03	0.03	0.0007	0.0001
Strain		NS	NS	NS	NS	0.0009
Treatment* strain		NS	NS	NS	NS	NS
Significant levels derived from one-way ANOVA						
Inshas	Control	134.63	84.72	49.91	16.49^{a}	0.34^{d}
	CC	111.97	70.86	41.11	14.34^{b}	0.45^{cd}
	SS	119.10	74.23	44.86	14.39^{b}	0.38^{d}
	CC+SS	130.80	82.38	48.42	15.54^{ab}	0.45^{cd}
	CAN	120.71	76.05	44.65	14.73^{b}	0.50^{abc}
	CAN+SS	117.00	74.09	41.34	15.67^{ab}	0.59^{ab}
Dokki4	Control	138.35	87.43	50.92	16.99^{a}	0.37^{d}
	CC	115.69	72.34	43.35	14.73^{b}	0.49^{bc}
	SS	114.82	72.47	42.35	14.62^{b}	0.56^{abc}
	CC+SS	116.86	73.93	42.93	15.70^{ab}	0.57^{ab}
	CAN	121.25	75.52	45.72	14.36^{b}	0.62^{a}
	CAN+SS	120.43	76.47	43.96	14.23^{b}	0.57^{ab}
Pooled SEM		02.09	01.33	00.79	00.19	0.01
p-value		NS	NS	NS	0.005	0.0001

a,b,..etc.: Means within the same column with different superscripts are significantly different (p<0.05)

Table 7: Effect of treatment, strain and their interaction on T_3, T_4, T_3/T_4 and estrogen hormone in plasma of laying hens at 60 weeks of age

Main effect		T_3 ng/dl	T_4 ng/dl	T_3/T_4	Estrogen Pg/ml
Treatment effect					
	Control	3.67^{b}	13.57^{b}	0.27^{b}	200.82^{d}
	CC	4.27^{b}	13.54^{b}	0.31^{ab}	297.00^{c}
	SS	3.97^{b}	13.40^{b}	0.29^{ab}	303.04^{c}
	CC+SS	5.01^{a}	14.11^{ab}	0.35^{a}	253.32^{c}
	CAN	3.91^{b}	13.75^{ab}	0.28^{b}	430.19^{b}
	CAN+SS	5.15^{a}	14.57^{a}	0.35^{a}	486.30^{a}
Strain effect					
	Inshas	4.40	13.86	0.31	344.06
	Dokki4	4.26	13.79	0.30	320.94
Source of variation					
Treatment		0.001	0.04	0.01	0.0001
Strain		NS	NS	NS	NS
Treatment* strain		NS	NS	NS	0.0098
Significant levels derived from one-way ANOVA					
Inshas	Control	3.72^{e}	13.69	0.27	185.51^{f}
	CC	4.42^{abcde}	13.61	0.32	296.77^{d}
	SS	4.04^{abcde}	13.41	0.30	390.15^{c}
	CC+SS	5.07^{abc}	14.10	0.35	232.14^{def}
	CAN	3.97^{bcde}	13.72	0.29	413.00^{bc}
	CAN+SS	5.18^{a}	14.64	0.35	493.53^{a}
Dokki4	Control	3.62^{e}	13.45	0.26	211.03^{ef}
	CC	4.12^{abcde}	13.47	0.30	297.22^{d}
	SS	3.91^{cde}	13.40	0.29	244.97^{def}
	CC+SS	4.95^{abcd}	14.12	0.35	274.50^{de}
	CAN	3.86^{de}	13.79	0.28	455.99^{abc}
	CAN+SS	5.12^{ab}	14.51	0.35	479.06^{ab}
Pooled SEM		0.12	00.11	0.008	020.28
p-value		0.01	NS	NS	0.0001

a,b,..etc.: Means within the same column with different superscripts are significantly different (p<0.05)

Table 8: Effect of treatment, strain and their interaction on cholesterol, LDL, HDL and total lipids of eggs yolk

Main effect		Cholesterol mg/g	LDL mg/g	HDL mg/g	Total lipids mg/g
Treatment effect					
	Control	16.98^{a}	12.17^{a}	4.81^{a}	330.03^{a}
	CC	15.49^{b}	11.04^{b}	4.45^{ab}	286.53^{b}
	SS	15.01^{bc}	10.69^{bc}	4.32^{bc}	289.76^{b}
	CC+SS	14.66^{bc}	10.76^{bc}	3.89^{c}	296.53^{b}
	CAN	14.43^{bc}	10.32^{bc}	4.11^{bc}	293.00^{b}
	CAN+SS	13.69^{c}	09.67^{c}	4.02^{bc}	270.75^{c}
Strain effect					
	Inshas	14.70	10.40^{b}	4.30	290.50^{b}
	Dokki4	15.38	11.15^{a}	4.23	298.36^{a}
Source of variation					
Treatment		0.0006	0.003	0.002	0.0001
Strain		NS	0.023	NS	0.0300
Treatment* strain		NS	NS	NS	0.0015
Significant levels derived from one-way ANOVA					
Inshas	Control	16.96^{a}	12.17^{a}	4.79^{a}	332.12^{a}
	CC	15.38^{ab}	10.67^{abc}	4.71^{a}	268.63^{ef}
	SS	14.25^{bc}	9.88^{bc}	4.37^{ab}	286.86^{cde}
	CC+SS	14.62^{bc}	10.74^{abc}	3.88^{b}	307.94^{b}
	CAN	13.76^{bc}	9.84^{bc}	3.92^{b}	281.55^{def}
	CAN+SS	13.25^{c}	9.10^{c}	4.15^{ab}	265.91^{f}
Dokki4	Control	17.01^{a}	12.17^{a}	4.83^{a}	327.93^{a}
	CC	15.60^{ab}	11.42^{ab}	4.18^{ab}	304.44^{bc}
	SS	15.78^{ab}	11.50^{ab}	4.28^{ab}	292.66^{bcd}
	CC+SS	14.69^{bc}	10.79^{abc}	3.90^{b}	285.12^{cdef}
	CAN	15.10^{abc}	10.80^{abc}	4.30^{ab}	304.46^{bc}
	CAN+SS	14.13^{bc}	10.24^{bc}	3.89^{b}	275.58^{def}
Pooled SEM		00.24	00.19	0.07	003.77
p-value		0.004	00.01	0.01	0.0001

a,b,..etc.: Means within the same column with different superscripts are significantly different (p<0.05)

Table 9: Effect of treatment, strain and their interaction on semen characteristics of cocks

Main effect		Volume (ml)	Motility %	Concentration milion/ml	Abnormality %	Dead %
Treatment effect						
	Control	0.81^{bc}	85.62^{b}	282.43^{c}	23.00^{a}	15.43^{a}
	CC	0.85^{abc}	87.93^{ab}	290.37^{bc}	23.93^{a}	13.62^{b}
	SS	0.79^{c}	86.87^{b}	291.18^{b}	24.31^{a}	14.12^{ab}
	CC+SS	0.88^{ab}	90.81^{a}	300.56^{a}	20.68^{b}	11.87^{c}
	CAN	0.86^{abc}	89.06^{ab}	294.93^{ab}	20.50^{b}	14.25^{ab}
	CAN+SS	0.89^{a}	90.56^{a}	294.56^{ab}	19.25^{b}	11.43^{c}
Strain effect						
	Inshas	0.85	88.70	295.0^{a}	21.97	13.14
	Dokki4	0.84	88.25	289.6^{b}	21.91	13.77
Source of variation						
Treatment		0.02	0.01	0.001	0.0001	0.0001
Strain		NS	NS	0.02	NS	NS
Treatment* strain		NS	NS	NS	NS	NS
Significant levels derived from one-way ANOVA						
Inshas	Control	0.81	86.25	287.12^{bc}	23.25^{ab}	14.75^{ab}
	CC	0.86	88.50	292.00^{b}	23.37^{ab}	13.75^{abc}
	SS	0.83	86.50	286.75^{bc}	25.00^{a}	14.25^{abc}
	CC+SS	0.89	91.75	307.25^{a}	20.37^{bcd}	11.62^{cd}
	CAN	0.84	88.50	297.50^{ab}	21.62^{abcd}	13.87^{abc}
	CAN+SS	0.90	90.75	299.37^{ab}	18.25^{d}	10.62^{d}
Dokki4	Control	0.82	85.00	277.75^{c}	22.75^{abc}	16.12^{a}
	CC	0.85	87.37	288.75^{bc}	24.50^{a}	13.50^{abc}
	SS	0.75	87.25	295.62^{ab}	23.62^{ab}	14.00^{abc}
	CC+SS	0.86	89.87	293.87^{b}	21.00^{bcd}	12.12^{bcd}
	CAN	0.87	89.62	292.37^{b}	19.37^{cd}	14.62^{ab}
	CAN+SS	0.88	90.37	289.75^{bc}	20.25^{bcd}	12.25^{bcd}
Pooled SEM		0.009	0.48	1.31	0.36	0.26
p-value		NS	NS	0.0007	0.0002	0.0004

a,b,..etc.: Means within the same column with different superscripts are significantly different (p<0.05)

Table 10: Effect of treatment, strain and their interaction on some reproductive performance mean of 8 hatches

Main effect		Fertility %	Hatchability of fertile eggs %	Hatchability of total eggs %	Chick weight (g)
Treatment effect					
	Control	83.04^{c}	74.84^{e}	62.18^{e}	34.21
	CC	86.62^{b}	81.28^{cd}	70.44^{c}	34.05
	SS	89.43^{a}	86.99^{b}	77.80^{b}	34.01
	CC+SS	86.05^{b}	81.86^{c}	70.48^{c}	34.01
	CAN	85.31^{b}	79.62^{d}	68.04^{d}	34.10
	CAN+SS	89.90^{a}	89.68^{a}	81.04^{a}	33.39
Strain effect					
	Inshas	87.88^{a}	82.26	72.48^{a}	34.93^{a}
	Dokki4	85.55^{b}	82.47	70.80^{b}	33.00^{b}
Source of variation					
Treatment		0.0001	0.0001	0.0001	NS
Strain		0.0001	NS	0.0074	0.0001
Treatment* strain		NS	NS	NS	NS
Significant levels derived from one-way ANOVA					
Inshas	Control	82.96^{f}	73.98^{e}	61.38^{f}	35.31^{a}
	CC	85.40^{def}	81.60^{cd}	70.32^{de}	33.94^{bc}
	SS	90.11^{ab}	86.77^{b}	78.19^{bc}	34.78^{ab}
	CC+SS	87.72^{bcd}	82.43^{c}	72.36^{d}	34.81^{ab}
	CAN	86.33^{cde}	79.61^{d}	68.86^{e}	35.01^{a}
	CAN+SS	92.38^{a}	89.54^{a}	82.75^{a}	34.80^{ab}
Dokki4	Control	83.13^{f}	75.71^{e}	62.98^{f}	33.12^{cd}
	CC	85.47^{def}	81.33^{cd}	69.55^{de}	33.25^{cd}
	SS	88.75^{bc}	87.22^{ab}	77.41^{c}	33.24^{cd}
	CC+SS	84.38^{ef}	81.29^{cd}	68.61^{e}	33.22^{cd}
	CAN	84.30^{ef}	79.64^{d}	67.22^{e}	33.20^{cd}
	CAN+SS	89.91^{ab}	89.66^{a}	80.61^{ab}	32.85^{d}
Pooled SEM		0.24	0.33	0.41	0.05
p-value		0.0001	0.0001	0.0001	0.0001

a,b,..etc.: Means within the same column with different superscripts are significantly different ($p<0.05$)

recorded the lowest values of yolk cholesterol, LDL and total lipid. For example, the addition of CAN+SS to control diet decreased yolk total lipids of Inshas strain by 19.93%.

Reproductive performance

Semen characteristics: The data in Table 9 showed that there was a significant effect of dietary treatment on all semen parameters while the strain affect only concentration of sperm. In both strain, the cocks fed CAN+SS recorded the highest values of volume while, all additives improved motility of semen compared to control group.

Fertility and hatchability percentage: There was a significant effect of dietary treatment on fertility, hatchability of fertile and total eggs while strain affect fertility, hatchability of total eggs and chick weight (Table 10). All feed additives used in this study improved all hatch performance except chick weight especially CAN+SS which recorded the highest values of fertility, hatchability of fertile and total eggs. For example, hens of Inshas strain fed CAN+SS recorded value of hatchability of fertile eggs found to be higher by 21.03% compared to control diet.

DISCUSSION

This experiment was conducted during the period from April to June, which consider relatively higher in temperature using two strains of aged hens (48-60 weeks). Also, the hens reared in wire cages which are known that it increase the detrimental effect of heat stress in open system (Hooper *et al*., 1996). Heat stress is well known to reduce the reproductive performance of laying hens by interrupting egg production, which caused not only by a reduction in feed intake but also by a disruption of hormones responsible for ovulation and a decrease in responsiveness of granulose cells to luteinizing hormone (Donoghue *et al*., 1989; Novero *et al*., 1991).

Free radicals production by high temperature increase by increasing age of hen. In this respect, Ando *et al*. (1997) found that heat stress-inducible oxygen radical damage becomes more severe in aged rats. Therefore, the control diet recorded the worst values of egg production and feed conversion. The enhanced antioxidant ability and decreasing lipid peroxidation are considered a part of the anti-heat stress strategies in laying hens during summer time (Sahin *et al*., 2003; Lin *et al*., 2006). Addition of CC significantly increased egg production and improved feed conversion. These results

are partly supported by Ather (2000), who found with 48-wk-old broiler breeders given diets supplemented with a polyherbal additive that consisted of six herbs that hen-day egg production and fertility significantly improved for aged hens receiving the herbal additive supplementation in their diet during the 8-week trial period. One hypothesis to explain the increase egg number with CC supplementation may due to its CC estrogenic activity (Table 7). Malini and Vanithakumari (1987) reported that acetone extract of CC has estrogenic activity in immature ovariectomized rats.

It was surprise that addition of SS alone significantly increased egg production compared to control diet. The SS may play a role in sexual hormones protection from free radical attack since its addition to hen diets increased estrogen hormone compared to control diet (Table 7).

For example, in human, serum levels of estrone sulphate are as much as 10 times higher than those of unconjugated estrone and estradiol and the half life of estrone sulphate is much longer than the half-life of unconjugated estrogen (Bhattacharyya and Tobacman, 2007). Also, Rees *et al*. (2008) indicated that sulfation confers resistance to oxidation. The sulphate may conjugate with estradiol and protect it from free radical attack since steroidal estrogens have been reported to function as antioxidants and free radical scavengers (Wiseman and Halliwell, 1993). The reproductive performance in females is known to deteriorate with age and is closely connected with endocrine changes, primarily with variations in circulating levels of gonadotropins and sex steroids (Burger *et al*., 2002) thus any additive increase or protect sex steroids hormones will increase egg production. Sulphate may also plays a role in synthesis of steroid hormone since cholesterol sulfate consider a precursor for sulfated steroid hormones without desulfation (Moser *et al*., 1966). On the other hand, it is known that sulphate is used in tyrosine sulphation of hormones receptors. For example, tyrosine sulfation plays an important role in high-affinity binding of all three glycoprotein hormones Thyroid-stimulating Hormone Receptors (TSHR), Luteinizing Hormone Receptors (LHR) and the Follicle-Stimulating Hormone Receptors (FSHR) to their corresponding receptors (Stone *et al*., 2009). Sulphate may play role in protection tyrosine from free radical which is known the target site to free radical attack. In this respect, it has been reported that myeloperoxidase can use free tyrosine as a mediator of free radical damage and that such reactions may be involved in the development of atherosclerosis in human (Leeuwenburgh *et al*., 1997). However, Moudgal and Razdan (1985) reported that the sensitivity of the hen's follicle to Luteinizing Hormone (LH) as indicated by its ability to ovulate declines with age. The sulphate is not only plays a role in receptors of hormone, but also links to hormone structure. Green *et al*. (1984) proved that Sulfate is covalently links to the oligosaccharides on LH hormone and boiling LH had no major effect on incorporation of sulfate since the sulfotransferase (the enzyme which transfer sulphate to oligosaccharides) is not sensitive to heating denaturation. From nutritional view, sulphate may protect and increase circulating of vitamin D_3. Axelson (1985) showed that in man that 25-hydroxy vitamin D_3 3ß-sulphate is a major circulating form of vitamin D_3 in man. The estrogen and vitamin D_3 which may conjugate with sulphate (as we discussed before) are known as alleviator of heat stress. Hansen *et al*. (2004) found that exogenous estrogen, high levels of dietary vitamin D, or both, before a heat stress episode, are efficacious in alleviating at least some of the bad effects of heat stress. Also, vitamin E metabolites conjugate with sulphate (Leonard *et al*., 2005). A long with previously reports (Mustacich *et al*., 2010; Grammas *et al*., 2004) stated that cytoplamic sulfotransferase enzymes may catalyze sulfation of the intermediates to increase their solubility and allow excretion to prevent accumulation of alpha-tocopherol intermediates and these metabolites have been reported to have potential health benefits. From the previous discussion, we can indicate that sulphate has a positive effect on laying hens performance at late period of age. Further studies are needed to elucidate these mechanisms. The addition of CAN significantly increased egg production and this beneficial effect may due to its antioxidants capacity. Costantini (2008) showed that carotenoids may still be important antioxidants in cell membranes by protecting their phospholipids and they may also have a function in antioxidant defense by participating in the process of recycling vitamin E. Moreover, beta-carotenoids could be easily converts to vitamin A by avian species (Damron *et al*., 1984; Schaffer *et al*., 1988). It was observed that hens fed diet containing SS plus CC did not increase egg production comparing to those fed diet containing CC alone, while combination of CAN+SS had recorded the highest value of egg production. Concerning the present study, it is interesting to notice that hens fed CAN plus SS in their diets were significantly attained the highest egg production (number and mass) comparing with those fed CC+SS or CC alone. This may be attributes to the difference work mechanism of CAN+SS on egg ovulation. Whereas, SS may increase solubility and polarity of CAN since water dispersibility has been reported for carotenoid sulfates (Liaaen-Jensen, 1996; Oliveiros *et al*., 1994) and consequently reached to all bird tissues. Na *et al*. (2004) found that polar carotenoids were efficiently absorbed by chickens, especially into blood. Thus it causes an increase in pigmentation of muscle, skin and egg-yolk. CAN is lipid-soluble, hence, it can protect the lipid from free radical. At the same time, bird needs also water-soluble

antioxidants to protect other non lipid tissues. Thus, sulphate may help CAN to be more polar which aid to reach to other tissues. Surai (2002) showed that to achieve optimum protection from free radical, the tissues deploy an integrated antioxidant system that consists of a diverse array of lipid-soluble (e.g. vitamin E, carotenoids), water-soluble (e.g. ascorbic acid, glutathione) and enzymic (e.g. glutathione peroxidase, superoxide dismutase) components. The author showed that these various components act in synergy. Feed additives used in this study improved egg shell thickness indicating an improvement in calcium metabolism. However, Bar and Hurwitz (1987) showed that the ability of the old hen to adapt itself through vitamin D metabolism and its expression, to physiological or nutritional calcium deficiency, declines. In eggs of both strain, addition of CAN to control diet increased yolk score from 6.16 to 7.65 whereas the normal egg-yolk score was 6-7 (Roche, 1988). The increase in yolk color score of hens fed CAN+SS (8.50) compared to those fed CAN(7.65) alone meaning that SS increases the transfer of CAN from hen to egg yolk. Ali *et al*. (2007) indicated that SS may increases the transfer of thyme active ingredients from hen to egg. Here, in both strains of hens, all feed additives used increased plasma phosphorus and calcium compared to control diet. These results agree with those obtained by Ali *et al*. (2007) who found that addition of thyme (as a natural antioxidants) numerically increased plasma phosphorus and calcium.

The same findings found in total protein, albumin and globulin indicating that these feed additives have a beneficial effect on protein metabolism. The significant reduction of total plasma cholesterol, LDL, HDL and total lipid by dietary treatments agree with the results obtained from Ali *et al*. (2007) who found that addition of thyme (as a natural antioxidants) significantly decreased plasma total lipid. On the other hand, all feed additives used in this study increased plasma total antioxidants capacity which in harmony with the data of hen performance (Table 2). For example, hens of both strains fed CAN+SS recorded the highest values of total plasma antioxidants capacity (0.58) and egg number (59.27). In the present study, all groups had lower values of all yolk parameters studied compared to control diet and these results are supported by Hassan *et al*. (2007) who found that adding of 2% fenugreek (as natural additives) to Japanese quail diet significantly decreased yolk cholesterol, LDL, HDL and total lipids compared with control group. In the present study, the improvement in hatchability of fertile and total eggs (Table 8) was corresponding to the decreasing of egg yolk cholesterol, LDL, HDL and total lipids. In this respect, Yilmaz Dikmen and Sahan (2007) found that negative correlations between egg yolk cholesterol content and hatchability of fertile and total eggs. They suggested that modifying the diets of old age breeder flocks, to determine exactly how altering yolk cholesterol content affects hatchability and may lead to improvements in the hatchability and viability of chicks from old breeders. Keeping in mind that high ambient temperature reduces thyroid activity in poultry (Bowen and Washburn, 1985), all feed additives used in this study increased T_3 compared to control diet (Table 7). Sulphate in combination to either CC or CAN may play role in T_3 and T_4 metabolism since the major conjugation of T_3 is with sulfate (Sekura *et al*., 1981). Sulfation of thyroid hormone is catalysed by sulfotransferases, while desulfation is catalysed by arylsulfatases (Visser *et al*., 1990). Thyroid hormone synthesis was dependent on tyrosine sulfation and hormone synthesis decreased when tyrosine sulfation decreased (Nlend *et al*., 1999). Since thyroid hormones had LDL-antioxidant properties (Chomard *et al*., 1998), CAN or another natural antioxidants can protect it from free radical attack (saving effect). Hens fed CAN+SS treatment had recorded the highest plasma T_3 value meaning that CAN protected T_3 from free radical attack and SS increased T_3 metabolism and/or sulfation by SS protect T_3 from free radical attack. Similar to our results, vitamin C as an antioxidant induced elevations in thyroid hormones (T_3 and T_4) have been also reported in poultry maintained under heat-stress temperatures (Abdel-Wahap *et al*., 1975). As we discussed before, CAN or CC as a natural antioxidants can protect estrogen hormone from free radical attack and consequently increased its level in plasma (Table 7). On the other hand, SS increased estrogen level may due to another mechanism (conjugate with estrogen and protect it from free radical) thus the addition of both CAN and SS+CAN recorded the highest values (430.19 and 489.30). The significant beneficial effect of dietary treatments on semen characteristics (Table 9) meaning that these additives improved the male condition and consequently improved fertility percentage (Table 10). The beneficial effect of CC on semen parameters are agree with the results of Shanoon (2011) who found that administration of 5 or 10 kg/ton ginger (as a natural antioxidants) for twenty consecutive weeks significantly increased sperm motility and viability in both levels as compared to the control group. The good effect of dietary treatment on fertility and hatchability percentages of both fertile and total eggs (Table 10) could be explained on the base that these parameters affect by free radical production which removes by supplying these natural antioxidants. The beneficial effect of SS on fertility percentage can explained because of that SS increased T_3 and estrogen hormones which improved the internal reproductive condition in both hens and cocks. However, in human spermatozoa, it is known that cholesterol sulphate comprises up to 20% of the sperm head surface area (Lin *et al*., 1993). In this study, addition of CAN significantly improved the hatchability of fertile and all

eggs (Table 10) which in agreement with those obtained by Rosa *et al.* (2012) who observed an increase in hatchability of total and fertile eggs in breeders fed canthaxanthin in their diet. Liaurado *et al.* (1997) reported a 5.6% improvement in hatchability by adding 6 mg/kg canthaxanthin into broiler breeder diets. However, Surai *et al.* (2003) showed that including the carotenoid canthaxanthin in the maternal diet reduces the susceptibility of tissues of newly hatched chicks to lipid peroxidation. The increase in hatchability by feeding natural antioxidants was observed by several authors (Kemp *et al.*, 2001; Ali *et al.*, 2007). Ali *et al.* (2007) found that addition of thyme to hen diets significantly increased the hatchability of eggs compared to those fed control diets. It was observed in both strains that hen fed CAN+SS recorded the highest value of all hatch parameters except chick weight. In previous study, Ali *et al.* (2007) used SS to increase the response to natural antioxidants in hens at 32 weeks old, they found that combination of thyme and sulphate was the most successful additive for laying hens and showed that hen need sulphate ion to conjugate with natural antioxidants and transfer it to egg before oxidation in digestive tract. In this study, using older laying hen (48-60 weeks old) may the reason of increasing the response to SS alone. SS increased plasma T_3 and estrogen hormone as shown in Table 7, hence, it may increases the transfer of hormones to egg and consequently increased the hatchability. In this respect, McNabb (2007) showed that the inhibition of thyroid function in hens decreases their egg production and the hatchability of their eggs. Also, Freeman (1974) showed that T_3 is the "hatching" hormone. Christensen (1985) found that small amounts of exogenous T_4 introduced into turkey eggs prior to incubation can improve hatchability. Avian eggs contain several maternally produced steroid hormones (Schwabl, 1993) and increased its levels can increase embryonic and posthatching development rates within species (Schwabl, 1996). Steroidal estrogens have been reported to function as antioxidants and free radical scavengers under a variety of experimental conditions. For example, phenolic and catecholic estrogens prevent lipid peroxidation induced by diverse pro-oxidants in microsomes (Wiseman and Halliwell, 1993). Sulphate also increased estrogen hormone (Table 7) and may increase transfer steroid hormones to egg. For example, sulphation enhances elimination of steroids from adrenal gland (Miyazaki *et al.*, 1969). The lower response to addition of CC+SS (fertility and hatchability) compared to SS alone may be due to that cumin aldehyde (the main component active ingredient in CC) may not work synergy with SS. Conversely, CAN which works synergy with SS increased all parameters studied like egg production (Table 2), total plasma antioxidants capacity (Table 6), plasma hormones (Table 7) and hatchability (Table 10). Also, during embryo development, fat-soluble antioxidants are transferred from the yolk to embryonic tissues and particularly to the liver (Surai *et al.*, 1998; Surai and Speake, 1998), which will concentrate and redistribute them after hatching (Surai *et al.*, 2001). From previous discussion, we can expected that these additives may increase livability of chicks after hatching and during fatting period. Further studies are needed with different levels of natural antioxidants and SS in both broiler and laying breeder hens to determine the best level of these additives on reproductive performance and elucidate the mechanism of protecting the hormones from free radical attack at the late egg production period.

Conclusion: The Egyptian local hen strains in late egg production period under environmental ambient temperature decreased their egg production, fertility and hatchability. It could be concluded that the feed additives used in this study improved productive, physiological and reproductive performances of old laying hens as well as fertility of the old cocks. The superiority improvements in most studied parameters were achieved by adding canthaxanthin and sodium sulphate together. It is suggested that further studies in this area should be conducted to elucidate the mechanism of protecting the hormones from free radical attack at the late egg production period.

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