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Strengthening the Antioxidant Status of Laying Hens Through Summer Season by Using Different Categories of Antioxidant Sources

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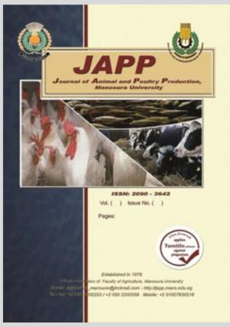

ABSTRACT

This study aimed to investigate the effect of natural antioxidants sources on laying performances, egg quality and economic efficiency compared with dietary vitamin E supplementation during summer season. Two hundred and seventy 20 week old Babcock Brown that reached 40% egg production were distributed randomly into 9 treatments. Each treatment contained 30 birds in 6 replicates. The first treatment performed control treatment and treatments 2, 3, 4 and 5 fed diets contain Vitamin E (E) 100 mg/ kg diet, Green Tea extract (G) 4ml/kg diet, Tomatoes Puree (T) 5g/kg and Canthaxanthin (C) 4mg/kg diet respectively. Treatments 6, 7 ,8 and 9 fed diets contain G+T, G+ C, T+C and G+T+C, respectively. The overall results showed that, significant enhancer effect of all experimental treatments on average feed conversion, egg numbers, weight, math and yolk color score more than control groups. The combination between more than antioxidant source resulted in better values of egg production, egg quality and yolk analysis, the highest combination were triple mixture G+T+C and double mixture T+C. The group fed T+C showed the highest values of albumen height, yolk color, and yolk index. All supplementation treatments significant decreased yolk triglycerides (TG) and significant increased yolk Total Antioxidant Capacity (TAOC) compared with control. Single, double and triple supplementation improved egg production, egg quality, TG, TAOC and economic efficiency. Also, T + C and G +T + C were the best among all parameters and could be used as VitE as natural antioxidant sources for laying hens through summer season.

Keywords: Hen, Green Tea, Tomatoes, Canthaxanthin, egg, production, quality..

INTRODUCTION

The optimal ambient temperature for laying hens is approximately 20°C to 25°C (Tumova and Gous, 2012). Therefore, When the temperature exceeds 30°C, signs of heat stress (HS) appear sensitive to temperature-associated environmental challenges, especially heat stress. High ambient temperature adversely affects poultry production and health (Lara and Rostagno, 2013). Poultry is the most sensitive to HS owing to its low ability to dissipate body heat. It has been suggested that modern poultry genotypes produce more body heat than earlier strains owing to their higher metabolic activity (Deeb and Cahaner, 2002 and Settar *et al.*, 1999). The adverse effects of HS (34 ± 2 °C) on performance variables were evident, as reflected by decreased feed intake egg production and egg weight and worsened feed conversion. Also, HS deteriorated eggshell quality indices significantly eggshell weight, eggshell thickness (Sahin *et al.*, 2018 and Liu *et al.*, 2020)

The reactive oxygen metabolites production has been stimulated by deficiencies of natural protective substances or excess exposure (Miller *et al.*, 1993) that which resulted in increased oxidative damage to important biological macromolecules such as lipids, proteins and DNA, affecting their normal function and consequently leading to reduced performance or disease (Valko *et al.*, 2007). Increasing oxidative stress by environmental temperature and ethological stress need to increase the requirement of antioxidant supplementation (Ariana *et al.*, 2011). In particular, the antioxidant effects of phytochemicals have been implicated as stress alleviation agents (Sahin *et al.*, 2008; Tuzcu *et al.*, 2008 and Sahin *et al.*, 2011). Some nutritional strategies focus on alleviating the negative effects of heat stress through supplementation with medicinal herbs (natural extracts with antioxidant potential). The main aim is to satisfy the special needs of animals, and this has proven advantageous during heat stress (Lin *et al.*, 2006). Many research's Ramadan *et al.*, (2003) and Papuc *et al.*, (2007) and (2008) reported that plant polyphenols can be used as natural preservatives, inhibiting oil peroxidation by capturing free radicals, chelating metals ions and inhibit lipoxygenases.

Green tea (*Camellia sinensis*) is rich in flavonoids and other polyphenols that have been shown to possess a wide range of biological and pharmaceutical benefits, including anti-carcinogenic, anti-oxidative, and hypolipidemic activities (Buschman, 1998; Gramza-Michałowska *et al.* 2016 and Rodrigues *et al.* 2016) anti-bacterial (Nakayama *et al.* 2012; Kawarai *et al.* 2016), anti-aging (Li *et al.* 2016), anti-cancerous (Shih *et al.* 2016). These beneficial effects are may be attributed to polyphenols (Trevisanato and Kim, 2000). Current researches suggest that tea treatment could affect the egg quality (Wei *et al.*, 2012), egg production performance

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(Panja2007) and reduced egg yolk triglyceride contents (Ariana *et al.,* 2011).

Among the experimental studies on the relation of tomato powder consumption and stress prevention, Sahin *et al.,* (2011) reported inverse association between tomato intake or blood lycopene level and stress. Also, Selim *et al*., (2013) reported using 0.5 and 1% of tomatoes puree in broiler diets recorded the best antioxidant status included plasma total antioxidant capacity, malondialdehyed. Also, it increased consumed feed and depressed the total bacteria count of intestine at 40 day of broiler chicks. (Jaafari *et al*., 2006) reported that, 50 or 100 kg dried tomato pulp (DTP) per ton diet had improved egg production and egg mass. But 150 kg DTP per ton diet decreased feed efficiency. Tomato powder supplementation increased egg production persistency and increased carotenoids and vitamin A contents in egg yolk (Akdemir *et al*., 2012). Also, Rotolo *et al*., (2010) reported that tomato extract diet resulted in a significant lycopene carry over and the intensity of egg yolk color was influenced by dietary lycopene supplementation.

Carotenoids are used in physiological processes as antioxidants, but also have a protective and recycling role for other fat soluble antioxidants like vitamins E and A (Surai and Speake, 1998). Rosa *et al*. (2012) reported that using 6 mg canthaxanthin/kg diet improve egg and chicks production of broiler breeders and decreasing the rate of oxidation of egg yolk. Ali *et al.* (2012) observed that adding 2 mg / kg diet canthaxanthin in lying hens diet increased **t**otal antioxidants capacity in plasma while decreased LDL, HDL, total cholesterol and total lipids in both plasma and egg yolk. Also, canthaxanthin increased T3 and estrogen hormones and protect it from free radical and consequently increased egg number. In this respect, Ali *et al.,* (2018) recorded that, usin**g** canthaxanthin increased significantly egg number by 23.35%, egg mass and shell thickness. Also, **canthaxanthin** has been used as a feed additive to resist oxidative stress caused by numerous factors including but not limited to high environmental temperatures (Ma *et al.,* 2005), high stocking densities (Simitzis *et al*., 2012), and pathological conditions (Georgieva *et al.,* 2006). These results disagree with those obtained by others who found that canthaxanthin supplementation had no effect on laying hens performance (Zhang *et al.,* 2011 and Rosa *et al.,* 2012).

Vitamin E is the most active natural antioxidant used in animal feed, to improve performance, to strengthen immunological status, and to increase the vitamin E content of food of animal origin and thus increase the vitamin E intake of consumers (McDowell, 1989; Sunder *et al.,* 1997and Flachowsky, 2000).

Therefore, this study aimed to investigate the effect of Green Tea (G), Tomatoes Puree (T), Canthaxanthin (C) and their combination on egg production and egg quality of laying hens fed on standard diet and compared with Vitamin E (E) during summer season.

MATERIALS AND METHODS

This study was conducted to investigate the effects of natural antioxidants sources on laying performances and egg quality compared with dietary vitamin E during summer season. The experimental setup was arranged during the extreme the period from April to August. The recorded ambient temperature and relative humidity in laying hens house during the fifth periods (Table 1) showed that, the average temperature during the experiment was (29.6 – 40.4 °C) and the relative humidity percent was (36.2 – 56.4 %). Two hundred and seventy 20 weeks old Babcock Brown that reached 40% egg production were distributed randomly into 9 treatments. Each treatment contained 30 birds in 6 replicates. Hens were kept in previously cleaned and fumigated cages of wire floored batteries in an open system house under similar conditions of management. Water offered *ad-libitum* and feed (in mash form) was offer 115 g/ hen/ day during the experimental period. The experimental diets and their chemical composition are presented in Table (2).

Table 1. Ambient temperature and relative humidity recorded during the trial periods

Period	Temperature °C		Relative humidity (%)	
	Minimum	Maximum	Minimum	Maximum
1st (22-25wk)	24	35	32	47
2nd (26-29wk)	27	39	35	49
3th (30-33wk)	30	40	36	57
4th (34-37wk)	33	44	38	64
5th (38-41wk)	34	44	40	65
Overall period	29.6	40.4	36.2	56.4

Table 2. Composition and calculated analysis of the experimental diet.

Ingredients	%
Yellow corn	62.07
Soybean meal (46%)	17.50
Corn gluten meal (60%)	9.50
Limestone	8.50
Di-Ca Phosphate	1.50
Vit. & Min. Premix *	0.30
NaCl	0.40
DL-methionine	0.10
L lysine hydrochloride	0.08
Choline chloride (60%)	0.05
Total	100
Calculated analysis **	
CP %	18.5
ME Kcal/ kg	2828
CF%	2.89
EE%	2.82
Ca %	3.545
Available P%	0.41
Lysine %	0.82
Methionine %	0.49
Meth. +Cys.%	0.81

***Supplies per3 kg premix: Vit. A 10000000 IU, Vit. D3 2000000 IU, Vit. -E 10000 mg, Vit. K3 2000 mg, Vit. B1 1000 mg, Vit. B2 5000 mg, Vit. B6 15000 mg, Vit. B12 10 mg, Pantothenic acid 10000 mg, Nicotinic acid 30000 mg, Folic acid 1000 mg, Biotin 50 mg, Copper 4000 mg, Iodine 1000 mg, Manganese 60000 mg, Zinc 50000 mg, Selenium 100 mg and Iron 30000 mg, cobalt 100mg**

**** According the Egyptian Regional Centre for Food and Feed (RCFF, 2001).**

The first treatment performed as control treatment and fed corn- soybean meal layer. Treatments 2, 3, 4 and 5 fed control diet supplemented with Vitamin E (E), Green Tea extract (G), Tomatoes Puree (T) or Canthaxanthin (C), respectively. Treatments 6, 7 ,8 and 9 fed diets contain G with T (G+T), Green Tea with Canthaxanthin (G+ C), Tomatoes Puree with Canthaxanthin (T+C) and Green Tea with Tomatoes Puree with Canthaxanthin (G+T+C), respectively. The examined feed additives vitamin E at level of 100 mg/ 100 kg diets, 20% aqueous extract of green tea adding by 4 ml/ 100 kg diets, tomato puree supplemented with 5g/ kg diet and canthaxanthin added with 4 mg/ kg diet.

Green tea extract prepared as follows: two hundred grams of dried leaves of green tea was socked in 1 liter of distilled water at 90° C for 10 min. then filtered 2 times by wallman No. 1 pilter paper. The extracted volume was adjusted to 1 L, and kept in freezer at -18° C until time of mixing to food. The active components of green tea aqueous extract were determined GC analysis (Table 3).

The antioxidants content in pastry zed Tomato puree was evaluated as descripted by (Selim *et al*., 2013) the determined value of lycopene in T was 155mg/kg. Canthaxanthin was provided by BASF Germany

Egg production

Egg number and egg weight were recorded daily. Daily average of egg weight was determined, excluding abnormal eggs and recorded on replicate basis. Egg mass was calculated as the percentage of egg number multiplied by the average egg weight. Feed consumption were fixed 115 gm /hen / day during all the experimental period. The average egg production was adjusted for mortality.

Egg quality measurements

Egg quality measurements were determined at the end of experiment. Five eggs from each replicate were collected, individually weighted and stored overnight at room temperature for subsequent measurements. Eggshell thickness without shell membrane was measured with a micrometer (Digimatic micrometer, Series 293-330, Mitutoyo, Japan). Albumen height was measured using Egg multi-tester (QCM$^+$ Technical Services and Supplies Ltd., York, England). Albumen height and egg weight, was calculated as previously described (Eisen *et al.*, 1962). Egg yolk color was measured by comparing to Roche yolk color fan (Hoffman-La Roche Ltd., Basel, Switzerland).

At the end of the experimental period, four egg yolk samples from each treatment were separated. Yolk composites from each treatment were prepared by separating the yolk material from the vitelline membrane and blending gently. Total lipids were extracted with chloroform: methanol (2:1 vol/vol) from 1 g of yolk, according to the procedure of Folch *et al.* (1957) as modified by Washburn and Nix (1974). Yolk triglycerides Mm/L and total antioxidant capacity (TAOC) Mm/L were measured by the colorimetric method (ERBA CHEM-5, Beijing Biochemical Instrument Company, Beijing, China) during three periods zero time, after 7 days, and after 10 days of egg storage.

Finally, all treatments were economically evaluated by calculating the net revenue per unit of total feed cost.

Data were analyzed using the GLM procedure of SAS software (SAS, 2001) as a completely randomized design. Differences among treatments were assessed using Duncan (1955) multiple range tests (P<0.05). The statistical model performed was as follow:

$$Y_{ik} = \mu + T_i + e_{ik}$$

Where, **Y_{ik} = An observation, μ = Overall mean, T_i = Effect of treatments (i = 1, 2….9), e_{ik} = random error**

RESULTS AND DISCUSSION

Analysis of Green tea:

The analysis of the active component for green tea extract are listed in Table (3). The total phenols concentration was 88.15 %.

Table 3. The Main components of Green Tea extract:

No.	Component	r.t	Area sum%
1	2-Hexanol,(S)	3.172	7.62
2	Isobenzofuran-1(3H)-one, 3,6, 7-trimethoxy	5.248	1.47
3	8-Carboxy-3-methylflavone	5.522	1.73
4	2-Hexenal,5-(1-ethoxethoxy)	7.292	1.76
5	2,3Dihydroxybenzoic acid	10.25	1.52
6	Quercetin 3,5,7,4,- pentamethyl ether	10.782	0.54
7	3'- benzyloxxy- 5,6,7,4- tetramethoxyflavone	11.561	0.38
8	9-Octadecenol-12- yonic acid, methyl ester	11.867	0.94
9	2- Heptadecanol	12.024	1.92
10	2- Nonadecanne 2,4- dinitrophenylhydrazine	12.281	1.09
11	Carvacrol	12.088	2.13
12	1(3H)- isobenzofuranone, 6,7- dimethoxy-3- (2-2 methoxyphenyl)-2-oxoethyl)	13.02	2.32
13	Hexadecanoic acid, methy ester	13.186	4.1
14	Caffeine	13.312	21.18
15	€-9- Octadecenoic acid ethyl ester	13.862	3.89
16	3-(3,4- Dimethoxyphenyl)-4- methylcoumarin	14.307	0.55
17	7,8,4 Trimethoxyisoflavone	14.461	5..52
18	10-Hydroxy-5,7-dimethoxy 2,3-dimethyl 1,4- anthracenedione	14.924	1.04
19	Scopoletin	15.077	1.15
20	2,5-Dimethoxy-2'-hydroxychalcone	15.208	1.03
21	Heptadecane, 9-hexyl	15.929	1.25
22	Ouabagenin acetate	16.307	0.68
23	Phytanic acid	16.78	2.31
24	Serverogenin acetate	17.272	6.85
25	2- Decanol	17.851	3.07
26	2- Nonadecanone, 1-[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]	18.131	0.88
27	Hexadecanone, 1-[2,2dimethyle-1,3-dioxolan-4-yl) methoxyl]	18.545	4.74
28	Glafenin	19.202	3.37
29	2,3,3',4'- tetramethoxy-5- (3- methoxyprop-1-	19.824	1.48
30	Oleic acid,3- (octadecylox) propyl ester	20.193	3.67
31	Monobehenin	20.634	3.49

Laying Hen Performance:

Egg production:

The average of number of produced egg and egg weight during 5 periods of 4 wks each, are shown in Table (4). The present results showed significant enhancer effect of all experimental treatments. The overall mean of produced egg number of all treatments was higher than of control group. Furthermore, the combination between more than antioxidant source resulted in better values of egg production. Mixing G, T and C recorded the highest overall egg production value 25.49 eggs followed by mixing T+ C (25.14 eggs). Within examined periods, mixture of G, T and C group recorded the highest egg production during period 2 (26.4), 3 (26.75) and 4 (26.47) eggs, followed by layers in T+C group (26.05, 26.48 and 26.16 eggs for the same periods, respectively). Mixing T and C improve both egg production 25.14 eggs and egg weight 60.26g and recorded top values compared with Control group. Consequently, egg mass and feed conversion ratio recorded values (Table 5) showed the same treads of the three examined antioxidants or T+C showed the best FCR value (2.13) compared with all other treatments. There was gradual increase in egg mass by increasing age up to 37 wks of age then slightly decreased during the period 38-41 wks of age. On the other side value of FCR were improved weekly up to 37 wks. The presented results confirmed the synergistic effect between antioxidant sources, whereas mixtures recorded better values than single supplementation.

Table 4. Effect of treatments on egg number and egg weight.

Treatments	Egg No (egg / hen)						Average egg weight (g)					
	1st (22-25wk)	2nd (26-29wk)	3th (30-33wk)	4th (34-37wk)	5th (38-41wk)	Over all	1st (22-25wk)	2nd (26-29wk)	3th (30-33wk)	4th (34-37wk)	5th (38-41wk)	Over all
Control	20.32^{e}	23.38^{d}	23.92^{f}	24.03^{f}	23.37^{de}	23.00^{g}	55.13^{b}	56.96^{c}	57.07^{bc}	59.70^{d}	60.72^{b}	57.92^{d}
E	21.83^{bc}	25.58^{b}	25.98^{bc}	25.83^{abc}	24.02^{cd}	24.65^{c}	58.15^{a}	58.03^{c}	61.45^{a}	62.28^{b}	59.80^{b}	59.94^{a}
G	20.85^{de}	23.50^{d}	24.08^{f}	24.25^{ef}	23.85^{cd}	23.31^{f}	56.43^{ab}	58.92^{abc}	58.07^{bc}	62.77^{b}	60.87^{b}	59.40^{abc}
T	21.33^{cd}	24.52^{c}	24.97^{e}	24.93^{de}	23.55^{ef}	23.86^{e}	56.33^{ab}	58.62^{bc}	58.67^{b}	62.12^{b}	61.08^{b}	59.36^{abc}
C	21.33^{cd}	24.75^{c}	25.30^{de}	25.10^{cd}	23.78^{f}	24.05^{ed}	57.02^{ab}	58.62^{bc}	57.88^{bc}	60.15^{d}	60.40^{b}	58.81^{bc}
G+T	21.77^{bc}	25.47^{b}	25.82^{cd}	25.93^{abc}	24.18^{bc}	24.63^{c}	57.43^{a}	60.87^{a}	55.87^{c}	65.07^{a}	59.80^{b}	59.81^{a}
G+C	21.43^{cd}	25.28^{b}	25.72^{cd}	25.52^{bcd}	23.43^{cde}	24.28^{d}	56.63^{ab}	58.58^{bc}	57.72^{bc}	60.82^{cd}	59.77^{b}	58.70^{cd}
T+C	22.17^{ab}	26.05^{a}	26.48^{ab}	26.18^{ab}	24.80^{ab}	25.14^{b}	57.37^{a}	60.55^{ab}	57.87^{bc}	62.72^{b}	62.82^{a}	60.26^{a}
G+T+C	22.75^{a}	26.40^{a}	26.75^{a}	26.47^{a}	25.08^{a}	25.49^{a}	57.37^{a}	59.08^{abc}	59.62^{ab}	61.65^{bc}	60.55^{b}	59.65^{ab}
Mean of SE	±0.22	±0.14	±0.20	±0.28	±0.22	±0.10	±0.61	±0.71	±0.81	±0.39	±0.48	±0.30
Probability	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.05	0.010	0.0010	0.0001	0.001	0.0001

$^{a-f}$= Means in the same column with different superscripts, differ significantly ($P<0.05$)
E= Vit. E ; G=Green Tea ; Tomato Puree = T ; C= canthaxanth

Table 5. Effect of treatments on egg mass and feed conversion ratio.

Treatments	Egg mass per hen per day (kg)						Feed conversion ratio					
	1st (22-25wk)	2nd (26-29wk)	3th (30-33wk)	4th (34-37wk)	5th (38-41wk)	Over all	1st (22-25wk)	2nd (26-29wk)	3th (30-33wk)	4th (34-37wk)	5th (38-41wk)	Over all
Control	1.12^{e}	1.33^{d}	1.36^{e}	1.43^{d}	1.42^{b}	1.33^{e}	2.88^{a}	2.42^{a}	2.36^{a}	2.25^{a}	2.27^{a}	2.43^{a}
E	1.27^{ab}	1.48^{b}	1.60^{a}	1.61^{b}	1.44^{b}	1.48^{b}	2.54^{de}	2.17^{c}	2.02^{e}	2.00^{d}	2.24^{a}	2.20^{d}
G	1.17^{d}	1.38^{c}	1.39^{de}	1.52^{c}	1.45^{b}	1.38^{d}	2.74^{b}	2.33^{b}	2.31^{ab}	2.11^{b}	2.22^{a}	2.34^{b}
T	1.20^{cd}	1.43^{b}	1.46^{c}	1.55^{c}	1.44^{b}	1.42^{c}	2.68^{cb}	2.24^{c}	2.20^{c}	2.08^{c}	2.24^{a}	2.29^{bc}
C	1.21^{bcd}	1.45^{b}	1.46^{c}	1.51^{c}	1.44^{b}	1.41^{c}	2.65^{bcd}	2.22^{c}	2.19^{c}	2.13^{c}	2.24^{a}	2.29^{bc}
G+T	1.25^{abc}	1.55^{a}	1.44^{cd}	1.69^{a}	1.45^{b}	1.47^{b}	2.58^{cde}	2.08^{d}	2.23^{bc}	1.91^{d}	2.23^{a}	2.21^{d}
G+C	1.21^{bcd}	1.48^{b}	1.49^{bc}	1.55^{c}	1.40^{b}	1.42^{c}	2.65^{bcd}	2.17^{c}	2.17^{cd}	2.08^{c}	2.30^{a}	2.28^{c}
T+C	1.27^{ab}	1.58^{a}	1.53^{b}	1.64^{ab}	1.56^{a}	1.52^{a}	2.53^{de}	2.04^{d}	2.10^{de}	1.96^{e}	2.07^{b}	2.14^{e}
G+T+C	1.30^{a}	1.56^{a}	1.60^{a}	1.63^{ab}	1.52^{a}	1.52^{a}	2.47^{e}	2.06^{d}	2.02^{e}	1.97^{e}	2.12^{b}	2.13^{e}
Mean of SE	±0.02	±0.02	±0.02	±0.02	±0.02	±0.01	±0.04	±0.03	±0.03	±0.03	±0.03	±0.02
Probability	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

$^{a-f}$= Means in the same column with different superscripts, differ significantly ($P<0.01$)
E= Vit. E; G=Green Tea; Tomato Puree= T ; C= canthaxanth

The overall egg numbers, egg weight, egg mass and FCR for hens fed experimental diets recorded significant effect compared to control group. Hens fed the triple mixture G+T+C recorded the highest value of egg number 25.49, it improved by 10.81 % over control. The highest value of egg weight recorded by T+C group 60.26 g, which was higher than control by 4.04 %. Egg mass for hens fed T+C and G+T+C recorded significantly the highest value for 1.52 kg for each compared with control, its improves by 14.28 % than control. Also, the best feed conversion values significantly recorded by hens fed T+C and G+T+C compared with control, they improved by 12.35 and 11.93 %, respectively compared with control group.

Among the experimental treatments, the double and triple mixture showed superior performance than the single additives. Also, T+C and G+T+C mixtures showed the best values and competed with vitamin E which reported as the most active natural antioxidant used in animal feed (McDowell, 1989; Sunder *et al*., 1997 and Flachowsky, 2000). This superiority effect by adding mixture may be due to the C, which may be enhance the total antioxidant effect. The obtained results agree with Ali *et al.,* (2018) who recorded significantly increasing egg number, egg mass by the mixture containing C more than single supplementation. This may also due to the multi effect of carotenoids in physiological processes as an antioxidant, a protective and recycle role for other fat soluble antioxidants like vitamins E and A (Surai and Speake, 1998) and protect hormones like estrogen from free radical and consequently increased egg number (Ali *et al*., 2012). Rosa *et al*. (2012) reported that using 6 mg canthaxanthin/kg diet improve egg and chicks production of broiler breeders and Ali *et al*., (2012) observed that adding 2 mg canthaxanthin / kg diet in lying hens diet increased T3 and estrogen hormones compared to control diet. Also results showed that, T and G improved production parameters more than control but T showed better values than G. In this respect, tea polyphenol plays roles such as anti-oxidizing (Gramza-Michałowska *et al.,* 2016 and Rodrigues *et al*., 2016), could affected on egg production (Uuganbayar *et al.*, 2005 and Panja 2007) 0.5% green tea extract decreased feed consumption and feed conversion ratio compared with control group in laying hens (Ariana *et al.,* 2011). Also, egg production and egg mass increased with inclusion levels up to 100 g/kg diet for a commercial strain, Hy-line W36 (Jaafari *et al.,* 2006). Generally, 1 % of T supplementation increased consumed feed at 40 day of broiler chicks (Selim *et al*., 2013).

On contrary, average feed intake, egg weight, laying rate and feed conversion ratio were significantly decreased by dietary inclusion of either 2% or 3% G powder to laying hens however, other group fed 1% G powder achieved significantly higher average egg weight, had little effect on the laying rate and feed conversion ratio (Xia *et al*., 2018). This improvement could be attributed to the content of essential oils which has many properties such as antimicrobial, antifungal and antioxidant activities; also, they could improve the bird's utilization of dietary nutrients (Williams and Losa, 2001). The wide range of biological and pharmaceutical benefits of G, including anti-carcinogenic, anti-oxidative, and hypolipidemic activities due to its content of flavonoids and other polyphenols that have been shown by Buschman (1998).

Also, Ali *et al.,* (2012 and 2018) recorded the addition of C increased significantly egg number and egg mass. On the contrary, (Zhang *et al.,* 2011 and Rosa *et al.,* 2012) who found that C supplementation had no effect on laying hens performance.

Egg quality:

The effect of experimental treatments on parameters of egg quality is shown in Table (6) and (7). The recorded values of external egg quality parameters (Table 6) showed non-significant effect of experimental treatments except shell %. Hens fed on control, G, or mixture of G+C recorded significant higher shell weight % compared with those fed on mixture of T+C. Results show that the average values of egg length, egg diameter, shape index, shell thickness and shell weight were almost constant for all treatments and there were no significant differences among treatments due to feeding laying hens on diets.

The determined values of internal egg quality parameters (Table 7) showed significant effect of experimental treatments on albumen height, yolk color score and yolk index compared to control group. Eggs of T+C group showed the highest values of albumen height, yolk color, and yolk index (7.08 mm, 10.67and 42.22, respectively). General improvement of yolk color was correlated with using C in the diet. Carotenoids are used in physiological processes as an antioxidant, but also have a protective and recycling role for other fat soluble antioxidants like vitamins E and A (Surai and Speake, 1998). Grashorn and Steinberg (2002) found that the deposition rate of canthaxanthin is of roughly 40% of dietary intake in yolks with strict linearity. Polarity of carotenoids also affected absorption and accumulation in chickens. The rest parameters of internal egg quality the albumen diameter, albumen weight, the albumen percent, the yolk height, the yolk diameter, the yolk weight and the yolk percent had no significant different.

Table 6. Effect of treatments on external egg quality parameters.

Treatments	Egg length (cm)	Egg diameter (cm)	Egg shape index	Shell thickness (mm)	Shell weight (g)	Shell (%)
Control	5.55	4.33	78.09	0.318	7.17	12.50[a]
E	5.55	4.37	78.69	0.328	7.17	12.08[ab]
G	5.55	4.37	78.70	0.326	7.50	12.73[a]
T	5.63	4.35	77.27	0.310	7.17	11.74[ab]
C	5.60	4.35	77.72	0.318	7.33	11.94[ab]
G+T	5.65	4.48	79.39	0.307	7.67	12.12[ab]
G+ C	5.62	4.43	78.97	0.310	7.50	12.63[a]
T+ C	5.57	4.48	80.62	0.292	7.00	11.08[b]
G+T+ C	5.52	4.37	79.19	0.290	7.00	11.64[ab]
Mean of SE	±0.08	±0.053	±0.89	±0.009	±0.23	±0.37
Probability	NS	NS	NS	NS	NS	0.06

a-b= Means in the same column with different superscripts, differ significantly (P<0.06)

E= Vit. E ; G=Green Tea; Tomato Puree = T ; C= canthaxanthin

NS= Non significant

Table 7. Effect of treatments on internal egg quality.

Treatments	Albumen parameter				Yolk parameters					
	High (mm)	Diameter (cm)	Weight (g)	Percent (%)	High (cm)	diameter (cm)	Color score	Weight (g)	Index	Percent (%)
Control	5.21[c]	7.60	33.33	57.90	1.58	4.28	4.33[d]	16.17	37.08[c]	28.13
E	5.00[c]	8.40	36.33	60.86	1.60	4.30	4.00[d]	16.33	37.20[c]	27.38
G	4.52[c]	8.52	35.67	60.43	1.57	4.32	5.17[cd]	16.67	36.33[c]	28.37
T	5.55[bc]	8.30	36.33	59.38	1.58	4.27	6.17[c]	17.50	37.14[c]	28.63
C	4.88[c]	8.62	37.83	61.46	1.65	4.22	9.17[b]	16.33	39.14[abc]	26.60
G+T	6.37[ab]	7.83	39.00	61.75	1.70	4.13	6.17[c]	16.50	41.14[ab]	26.12
G+ C	5.16[c]	8.42	30.00	51.03	1.60	4.20	9.67[ab]	15.67	38.15[bc]	26.49
T+ C	7.08[a]	8.25	39.33	62.28	1.73	4.10	10.67[a]	17.00	42.22[a]	26.95
G+T+ C	5.00[c]	8.30	36.67	60.96	1.63	4.18	8.67[b]	16.50	39.08[abc]	27.43
Mean of SE	±0.34	±0.32	±2.08	±3.10	±0.05	±0.06	±0.44	±0.45	±1.20	±0.75
Probability	0.0001	NS	NS	NS	NS	NS	0.0001	NS	0.01	NS

a-d= Means in the same column with different superscripts, differ significantly (P<0.01)

E= Vit. E G=Green Tea Tomato Puree = T C= canthaxanthin NS= Non significant

Regarding the general effect of treatments, the mixture of additives G+C, G+T, T+C and G+T+C were improved egg quality compared to single additives. These results in agreement with those reported by Ali *et al.* (2018) who reported improved yolk color score by using C to laying feed. The increase in yolk color may be due to increase in the C in the yolk (Ali *et al.,* 2018) and increased lycopene content when fed T. This results agree with Knoblich *et al*., (2005) who also reported increase in the lycopene content of egg yolk from laying chicken fed tomato seed and peels which affected the visual appraisal of egg yolk pigmentation and altered the carotenoid content. In this respect, Habanabashaka *et al.*, (2014) observed increased significantly in yolk color and yolk carotenoid content (including lycopene) with increasing levels of tomato west meal (0, 3, 6, 9 % in laying diets) therefore hen can be a way of increasing the lycopene content of poultry eggs. Also, tomato has been identified as the major source of lycopene (Agarwal *et al.,* 2001). Surai, (2012) reported that with laying hens, 80% of the total body C is located in the ovary. If so, dietary C supplementation should increase the C concentration and antioxidant status of the ovary. Also, previous researches Yamane *et al.* (1999) and Biswas *et al.* (2000) showed an improvement in egg quality by increasing albumen thickness when feeding tea extracts to laying hens. Biswas *et al.* (2000) recorded a reduction in egg yolk fat. Ariana *et al*., (2011) reported that green tea supplementation improved internal egg quality measurements, yolk weight and index. Also, they recorded that, 200 IU α-tocopheryl acetate/kg on feed was more effective than other treatments to improve yolk weight and index. On contrary, significantly decreased the eggshell thickness and egg shell strength with improved the albumen height when supplemented Green tea powder for laying diet (Xia *et al.,* 2018). Which may be affected the egg quality (Kojima and Yoshida 2008 and Wei *et al.,* 2012).

Egg analysis:

Results in Table (8) shows that Triglycerides (TG) and Total Antioxidants Capacity TAOC in yolk content during three periods zero time, after 7 days and after 10 days of egg storage.

Yolk triglycerides (TG) significant decreased during three period of egg storage. All supplemented treatment decreased yolk TG significant at zero, 7 and 10 days of egg storage compared with control treatments. On the other hand, all supplementations were recorded significant increase in yolk TAOC for compared control treatments during zero and 7 days of storage.

Table 8. Effect of treatments on triglycerides and total antioxidant capacity on egg analysis parameters.

Treatments	Triglycerides (TG) mm/L			Total Antioxidants capacity (TAOC) mm/L		
	0 day	7 day	10 day	0 day	7 day	10day
Control	304.03[a]	302.42[a]	251.13[a]	1.54[e]	1.20[d]	0.59
E	238.71[abc]	209.68[bc]	164.52[b]	1.75[abc]	1.64[abc]	0.72
G	281.13[ab]	196.77[bcd]	177.10[b]	1.79[ab]	1.63[abc]	0.57
T	234.68[bc]	208.06[bc]	139.19[b]	1.63[de]	1.66[abc]	0.78
C	251.13[abc]	154.03[d]	140.68[b]	1.69[bcd]	1.49[abcd]	0.72
G+T	205.16[c]	185.97[bcd]	149.84[b]	1.65[cde]	1.34[cd]	0.66
G+ C	247.74 [abc]	219.35[b]	150.65[b]	1.80[ab]	1.37[bcd]	0.64
T+ C	225.32 [bc]	206.94[bc]	143.18[b]	1.85[a]	1.73[a]	0.65
G+T+ C	215.97[bc]	162.90[cd]	131.61[b]	1.79[ab]	1.68[ab]	0.84
Mean of SE	±20.00	±15.80	±15.22	±0.04	±0.10	±0.10
Probability	0.05	0.002	0.001	0.0002	0.01	NS

[a-e]= Means in the same column with different superscripts, differ significantly ($P<0.01$).

E= Vit. E; G=Green Tea ; Tomato Puree = T ; C= canthaxanthin NS= Non significant

In this respect Ariana *et al*., (2011) reported that egg yolk triglyceride contents were reduced by green tea supplementation or adding 200 IU α-tocopheryl acetate/kg on laying diets. (Xia *et al*., 2018) Green tea powder treatment significantly decreased the contents of cholesterol crude fat and malonaldehyde and increased the content of vitamin E of eggs. That may be attributed to polyphenols such as epigallacathechin-3-gallate, which are known to possess powerful anti-oxidative and anticarcinogenic properties (Trevisanato and Kim, 2000). Habanabashaka *et al.*, (2014) observed a reduction in triglycerides of the egg yolk from the birds fed 6% and 9% tomato west meal with increased in yolk carotenoid content (including lycopene) when increasing levels of tomato west meal in laying diets which may be due to the antioxidants effects of carotenoids (Surai and Speake, 1998). Also, Selim *et al*., (2013) reported using 0.5 and 1% of tomatoes puree in broiler diets recorded the best antioxidant status included plasma total antioxidant capacity and malondialdehyed. The feeding of tea extracts to laying hens decreasing triglyceride in egg yolk in a short-term experiment (Biswas *et al*., 2000; Unganbayer *et al*., 2005; Koo and Noh, 2007and Yamane *et al.*, 1999). Rosa *et al*., (2012) reported that using 6 mg canthaxanthin/kg diet decreasing the rate of oxidation of egg yolk. Ali *et al*., (2012) observed that adding 2 mg / kg diet canthaxanthin in laying hens diet increased total antioxidants capacity in plasma while decreased total lipids in both plasma and egg yolk.

Economic efficiency

Data presented in Table (9) shows that the economic efficiency and money return per hen at the end of experimental period as affected by different dietary treatments.

Table 9. Effect of experimental treatments on economic efficiency of egg production.

Treatments	FI/hen (kg)	Price /kg feed (LE)	Fed cost /hen (LE)	Egg No./hen	Total revenue (LE)[a]	Net revenue (LE)[b]	E.E[c]	REE[d] (%)
Control	3.22	5.20	16.74	23.00	28.75	12.01	2.31	100
E	3.22	5.26	16.94	24.65	30.81	13.88	2.64	114
G	3.22	5.25	16.91	23.31	29.14	12.23	2.33	101
T	3.22	5.29	17.03	23.86	29.83	12.79	2.42	105
C	3.22	5.26	16.91	24.05	30.06	13.16	2.51	109
G+T	3.22	5.34	17.19	24.63	30.79	13.59	2.55	110
G+ C	3.22	5.31	17.10	24.28	30.35	13.25	2.50	108
T+ C	3.22	5.35	17.23	25.14	31.43	14.20	2.65	115
G+T+ C	3.22	5.40	17.39	25.49	31.86	14.47	2.68	116

E= Vit. E ; G=Green Tea ; Tomato Puree= T; C= canthaxanth

Total price for feeds was calculated according to the price of different ingredients available in ARE.

[a]) Assuming that the selling price of one egg is 1.25 L.

[b]) Net revenue = Total revenue /hen – total feed cost/hen.

[c]) Economical Efficiency (EE) =Net revenue per unit feed cost.

[d]) Relative Economical Efficiency (REE) Assuming that the E.E of the control diet = 100.

Generally, egg production and feeding cost are the most important factors which involved in the achievement of maximum efficiency of egg production. Data showed that feeding laying hens on diets contained all treatments improved both net revenue, economical efficiency and relative economic efficiency values compared to the control diet. The highest values recorded by laying hens fed diets containing T+C and G+T+C which were 115 and 116 %, respectively compared with those fed VitE (114%).

CONCLUSION

All examined antioxidant feed additives improved egg production, egg quality, TG, TAOC and economical efficiency of Babcock Brown hens. Also, a mixture of Tomatoes Puree + Canthaxanthin and Green Tea extract + Tomatoes Puree + Canthaxanthin recorded the best values of previous parameters, and could be used as another strategy in the same level as Vitamin E as antioxidant system of laying hens through summer season as natural antioxidant sources.

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تعزيز النظام المضاد للأكسده للدجاج البياض بإستخدام مضادات أكسده مختلفه خلال موسم الصيف

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هذه الدراسه تهدف لدراسة تاثير مصادر طبيعيه من مضادات الأكسده علي أداء الدجاج البياض وجودة البيضه والكفاءة الإقتصاديه بالمقارنه بفيتامين هـ. تم تقسيم 270 دجاجه عمر 20 أسبوع من نوع بابوك البني مرحلة إنتاج 40% الي 9 معاملات . كل معاملة تحتوي علي 30 طائر مقسم الي 6 مكرارت. المعاملة الأولى عليقة كنترول بدون إضافة والمعاملة الثانية عليقة كنترول مع إضافة فتيامين هــ بمعدل100ملجم / كجم علف والمعاملة الثالثة عليقة كنترول مع إضافة مستخلص الشاى الأخضر بمعدل 4 ملليتر/كجم علف والمعاملة الرابعة عليقة كنترول مع إضافة بيورية الطماطم بمعدل 5جم/كجم والمعاملة الخامسة عليقة كنترول مع إضافة الكنزازنثين بمعدل 4ملجم /كجم علف والمعاملة السادسة: عليقة كنترول مع إضافة خليط مستخلص الشاى الأخضروبيورية الطماطم بنفس نسب الإضافة والمعاملة السابعة: عليقة كنترول مع إضافة خليط مستخلص الشاى الأخضر والكنزازنثين بنفس نسب الإضافة والمعاملة الثامنة: عليقة كنترول مع إضافة بيورية الطماطم والكنزازنثين بنفس نسب الإضافة والمعاملة التاسعة: عليقة كنترول مع إضافة خليط مستخلص الشاى الأخضر وبيورية الطماطم والكنزازنثين بنفس نسب الإضافة. أظهرت النتائج تحسن معنوى لكلا من معدل التحويل الغذائي وعدد البيض ووزن البيضة وكتلة البيض و درجة اللون للصفار للدجاج البياض نتيجه للإضافات مقارنة بمجموعة الكنترول. الدمج بين اكثر من مصدر مضاد للاكسدة حسن إنتاج البيض وجودة البيض وتحاليل الصفار وكان احسنهم الخليط الثلاثى لمستخلص الشاى الأخضر و بيورية الطماطم والكنزازنثين والمخلوط الثنائي لبيوريه الطماطم والكنزازنثين. المجموعه المغذاه علي خليط بيورية الطماطم والكنزازنثين سجل أعلي قيم لإرتفاع البياض ولون الصفار ودليل الصفار. سجلت كل الإضافات للدجاج البياض إنخفاضا معنويا للدهون الثلاثيه وإرتفاعا معنويا لمحتوي مضادات الأكسده في تحليل صفار البيض مقارنة بمجموعة الكنترول. الإضافات المفرده او الثنائيه او الثلاثيه حسنت من إنتاج وجودة البيض و محتوي الصفار من الدهون الثلاثية ومضادات الأكسده والكفاءة الاقتصاديه. مخلوط بيوريه الطماطم والكنزازنثين والمخلوط الثلاثي لمستخلص الشاى الأخضر وبيوريه الطماطم والكنزازنثين كانا أحسنهم في التقديرات و يمكن ان يستخدما كبديل لفيتامين هــ كمصدر مضاد أكسده للدجاج البياض خلال موسم الصيف.