The effect of natural and synthetic antioxidants on performance, egg quality and blood constituents of laying hens grown under high ambient temperature

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Abstract

A total of 216 laying hens was kept at high ambient temperature (32±4°C, 60% relative humidity) from week 24 to 32 of age. Birds were divided in 8 treatments with 9 replicates of 3 hens each. The groups were fed the same basal diet and submitted to these dietary treatments: control, un-supplemented; green tea (GT), fed GT at 1 g/kg diet; brown marine algae (BMA), fed BMA at 1 g/kg diet; vitamin E (vit. E), fed vit. E at 300 mg/kg diet; GT+BMA, fed GT and BMA at 1 g/kg each; GT+vit. E, fed GT and vit. E at 1 g and 300 mg/kg, respectively; BMA+vit. E, fed BMA and vit. E at 1 g and 300 mg/kg, respectively. Feeding BMA at 0.1% increased laying rate by 1.2% and improved feed conversion ratio by 5.2% compared to the control. Vitamin E significantly increased shell thickness by 6.6% and Haugh unit by 4.6% compared to the control. In addition, BMA+vit. E or GT+vit. E increased yolk colour by 9.1 and 10.7%, and Haugh unit of stored eggs by 10.9 and 11.1%. Cholesterol of fresh eggs and plasma was significantly decreased by 16.0 and 10.4% due to supplementation with BMA, and by 19.2 and 8.1% with vit. E addition. Plasma phosphorus increased by 19.1% after vit. E+BMA supplementation. In conclusion, use of BMA or vit. E or GT in laying hens diets which grow under heat stress is recommended as it improves production performance and egg quality.

Introduction

In warmer regions, high ambient temperature is a major problem facing the egg production industry, since it adversely affects feed intake, egg production, egg quality, antioxidant status, physiological traits and, therefore, the overall profit of poultry farms (Mashaly et al., 2004; Daghir, 2008; Yoshida et al., 2011). High temperature has a negative effect on feed intake (Attiya et al., 2011) and as a consequence the amount of any particular nutrient in the diet may be insufficient to meet hen requirements. However, using dietary additives can play a significant role for improving production and quality, limiting the negative environmental impact of nitrogen and phosphorus pollution and maintaining animal health in warmer regions (Attiya et al., 2009).

Free radicals derive from metabolic processes and some dietary components. Dietary oxidized fatty acids are absorbed by the intestine, which then, initiate lipid peroxidation in the tissues (Penemetcha et al., 2000; Lobo et al., 2010). Insufficient amounts of antioxidants in the feed, e.g. vitamin E (vit. E) deficiency, may increase the incidence of diseases and toxicoses (Young and Woodside, 2001). Natural antioxidants, such as tocopherols, vitamin C, flavonoids and synthetic antioxidants, such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ethoxyquin are generally used to slow down or stop lipid peroxidation and preserve product freshness. However, synthetic antioxidants are not always beneficial for human health (Lobo et al., 2010). There are many controversies around the use of these synthetic antioxidants in foods, since both BHT and BHA seem to have tumor-promoting activities and anti-carcinogenic properties (Baszczyk et al., 2013).

Green tea (GT) phytopgenic is one of these beneficial ingredients due to its high content of pharmacologically active substances, such as catechins, flavonols, flavadiols, flavonoids and phenolic acids (Katiyar and Mukhtar, 1996; Ahmad et al., 1998; Lin et al., 1998). In addition, minor constituents, such as caffeine, theobromine, theophylline, phenolic acids and gallic acid are also present (Ahmad et al., 1998). Biswas and Wakita (2001) demonstrated that cholesterol and fat in liver and serum cholesterol were significantly reduced (P<0.05) by feeding a GT supplemented-diet, and also lower oxidative profiles. Fujiki and Suganuma (2002) observed that GT flower powder with epigallocatechin gallate could be an active substance to improve feed conversion ratio (FCR) and interfere with micelle solubility of cholesterol in the gut, and could also decrease cholesterol absorption (Raederstorff et al., 2003). Additionally, GT improved broiler meat quality (Kaneko et al., 2001). This generally resulted in the deposition of epigallocatechin and catechin in the breast and thigh meats (Kaneko et al., 2005). Uuganbayar et al. (2005) reported that egg weight and eggshell thickness significantly decreased, but feed intake increased when GT powder was supplemented at 0.5 and 1.5%, respectively, compared to negative and positive control diets. This can be correlated with the findings of Abdo et al. (2010), who noted that adding GT powder at 2% in laying hens diets could reduce cholesterol content and thiobarbituric acid value in the egg yolk.

Kojima and Yoshida (2007) found that the transfer of α-tocopherol from GT to egg yolk was sensitive to α-tocopherol intake, since 1% GT did not affect egg production traits, yolk color and fatty acid composition in the egg yolk. On the other hand, shell strength decreased with increasing GT. Furthermore, Ariana et al. (2011) concluded that incorporating antioxidant components derived from herbal plants such as GT in laying hens diets, as an alternative option to α-tocopherol acetate, can also be effective.

Marine algae are considered as a potential source of nutrients that contain greater amounts of protein, amino acids, carbohydrates, lipid, vitamins A, B, C, (especially B3), colorants, antioxidants and antimicrobial substances (Rimber, 2007; Abd El-Baky et al., 2008; Al-Harthi and El-Deek, 2011, 2012). Algae could be used with no limitation in food and feed (Becker, 2004). Mohd et al. (2000) postulated that G. changii (C. changii) contains a high composition of unsaturated fatty acids (74%), mainly omega 3 fatty acids and 26% of saturated fatty acids (mainly palmitic acid). It, also,
contains fat and water soluble vitamins and pigments such as chlorophyll (Becker, 2004; Schiavone et al., 2007; Al-Harthi, 2014). Al-Harthi and El-Deek (2012) found that brown marine algae (BMA) significantly improved egg quality and decreased egg cholesterol and this was attributed to the antioxidant properties of BMA. The objective of this study was to evaluate the effect of different natural antioxidants such as GT, BMA and synthetic vit. E on production performance, quality of fresh and sorted eggs and biochemical constituents of blood plasma of laying hens kept under high ambient temperature.

Materials and methods

This work was done in the Agriculture Research Station at Hada Al-Sham area, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia.

Birds and experimental design

A total of 216 Hy-line laying hens at 24 weeks of age were selected at similar weights, then distributed among 8 treatments, each containing 27 hens equally distributed among 9 replicates of 3 hens per replicate (cage). Hens were housed in an environmentally-controlled house and provided 520 cm² per hen. Table 1 shows the experimental diets that were formulated based on National Research Council (1994) recommendations. The groups were fed the same basal diet and were submitted to the following dietary treatments: control group, unsupplemented; GT group, supplemented with GT at 1 g/kg diet; BMA group, supplemented with BMA at 1 g/kg diet; vit. E group, fed vit. E as α-tocopherol acetate-powder at 300 mg/kg diet; GT with BMA (GT+BMA) group, fed GT and BMA at 1 g/kg of each; GT with vit. E (GT+vit. E) group, fed GT and vit. E at 1 g and 300 mg/kg, respectively; BMA with vit. E (BMA+vit. E) group, fed BMA and vit. E at 1 g and 300 mg/kg, respectively. Additives were mixed biweekly and feeds were kept in black plastic bags. The experimental period lasted from week 24 to 32 of age.

Feed in a mash form and water were provided ad libitum throughout the experimental period. Vaccinations and medical programmes were executed according to the different stages of life under supervision of a veterinarian. Laying hen houses were illuminated with a 14:10 light-dark cycle. The indoor temperature was around 32±4°C with 60% relative humidity.

Data collection

Laying rate (egg number/hen/day), egg weight (g), egg mass (g/hen/day) and feed intake (g/hen/d) were measured daily for each replicate. Feed conversion ratio was calculated as the amount of feed required to produce 1 kg of eggs. Survival rate (%) was calculated as the number of hens alive at the end of the experiment divided by the total hens at the beginning of the experiment.

At 32 weeks, 30 eggs per treatment were collected to determine egg and shell quality (Burke and Attia, 1995; Alsaffar et al., 2013). Eggs were divided into two portions of 15 eggs each. The 1st group was used to calculate fresh eggs quality (on the same day of laying), while the remaining 15 eggs were stored in the refrigerator at 5°C for 21 days and then broken for quality assessment.

In addition, 8 fresh eggs and the same number of sorted eggs per treatment were used for the determination of proteins and lipids according to AOAC (2004) methods (No. 954.01 for protein and 920.39 for lipids). Yolk cholesterol was also determined after lipid extraction with a mixture of chloroform: methanol (2:1 v/v) using the procedure described by Folch et al. (1957). Cholesterol determination was carried out in triplicate using a commercial test kit for cholesterol analysis (Sigma diagnostic cholesterol reagent procedure No 352°; Sigma Aldrich, St. Louis, MO, USA).

Blood (nine samples per treatment) were collected (5 mL), when hard shell eggs were present in the oviduct, from the brachial vein into heparinised tubes at week 32 of age. Plasma was collected after blood centrifugation at 1500 g. Total plasma proteins, lipids, cholesterol, Ca and inorganic phosphorus were determined by colorimetric methods using commercial test kits (Diamond Diagnostics, Holliston, MA, USA).

Statistical analysis

Data were analysed using the GLM procedure of SAS® (SAS, 1996) using one-way ANOVA according to the following model:

\[ y_{ij} = \mu + A_i + e_{ij} \]

where \( \mu \) is the overall mean, \( A_i \) is the effect of additives, and \( e_{ij} \) is the random error. Before analysis, all percentages were subject to logarithmic transformation (log10 \( x+1 \)) to normalise data distribution. Mean difference at \( P<0.05 \) was tested using Duncan’s multiple range test (Duncan, 1955).

Results and discussion

Table 2 shows the effect of different additives on egg production traits. The BMA, GT and vit. E did not significantly affect egg weight and feed intake.

Laying rate of hens fed diets supplemented with BMA only or with GT+BMA was significantly greater (\( P<0.05 \)) than BMA+vit. E group. Egg mass of BMA+vit. E group was significantly lower than other groups except the control one. Moreover, FCR was higher (\( P<0.05 \)) in hens fed BMA+vit. E than the group fed BMA alone. No significant differences for FCR were detected among the other groups.

Table 3 indicates data of fresh eggs quality traits. No significant effects of feed additives were observed on yolk index, yolk % and yolk colour. The group fed on vit. E had greater (\( P<0.05 \)) egg shell thickness compared to the other groups except the one supplemented with BMA only. The group fed on GT+vit. E had a lower (\( P<0.05 \)) shell thickness in comparison to the other groups with the exception of the control group. Haugh unit score was higher (\( P<0.05 \)) in the BMA fed group than in other groups except the one supplemented with vit. E alone. The control and BMA+vit. E groups showed lower (\( P<0.05 \)) Haugh unit score than other groups excluding the GT one.

Table 1. Ingredients and chemical composition of the experimental diet for laying hens from week 24 to 32 of age.

| Ingredient                  | g/kg Value |
|-----------------------------|-----------|
| Yellow corn                 | 663.3     |
| Soybean meal 48%            | 242.0     |
| Limestone                   | 75.0      |
| Dicalcium phosphate         | 13.2      |
| Vit. min. premix°           | 2.5       |
| NaCl                        | 2.5       |
| DL-methionine               | 1.5       |
| ME kcal/Kg                  | 2775      |
| CP, g/kg                    | 172.6     |
| Methionine, g/kg            | 4.4       |
| Methionine+cystine (SAA), g/kg | 7.1   |
| Lysine, g/kg                | 8.3       |
| Calcium, g/kg               | 32.0      |
| Available phosphorus, g/kg  | 36.0      |

| Available phosphorus, g/kg  | 36.0      |

ME, metabolisable energy; CP, crude protein; SAA, sulfur amino acids; °Provided the following per kg of diet: vitamin A, 12,000 IU; vitamin E, 10 IU; menadione, 3 mg; vitamin D₃, 2200 IU; riboflavin, 10 mg; Ca pantothenate, 10 mg; nicotinic acid, 20 mg; choline chloride, 300 mg; vitamin B₁₂, 10 g; vitamin B₆, 1.5 mg; vitamin B₃, 2 mg; folic acid, 1 mg; biotin, 50 μg. Trace minerals (mg/kg of diet): Cu, 55; Zn, 5; Fe, 36; Mn, 18; Se, 8.10. Antioxidant, 3 mg/kg of diet.
Table 4 reports data about stored eggs quality traits. The group fed on BMA+vit. E had greater (P<0.05) egg loss weight during storage for 21 days compared to GT, BMA and vit. E groups. Yolk colour and Haugh unit score after storage period were higher (P<0.05) with GT+vit. E and BMA+vit. E groups than in other groups. In addition, Haugh unit score was the lowest with the control and GT+BMA groups, followed by GT or vit. E group, and then by BMA group.

Albumen % in BMA and GT+BMA groups was higher (P<0.05) than the control and GT groups. Groups supplemented with BMA or vit. E had significantly higher yolk percentage than groups supplemented with vit. E + either GT or BMA.

Table 5 reports data about protein, lipid and cholesterol of fresh and stored eggs. Cholesterol of fresh egg was the lowest (P<0.05) with vit. E group. In addition, GT, BMA and GT+BMA groups had lower (P<0.05) yolk cholesterol than the control group only. However, no significant differences were detected with the other observations.

Table 6 shows data of blood plasma total protein, lipid, cholesterol, Ca and inorganic phosphorus. Supplementation of BMA with or without vit. E significantly decreased plasma total lipid compared to the control group only. Plasma cholesterol was lower (P<0.05) in BMA and vit. E groups than the control group only. BMA+vit. E group had significantly higher plasma phosphorus than the control group.

**General remarks**

Supplementation of BAM at 0.1% to laying hen diets increased laying rate by 1.2%, improved FCR by 5.2%, while decreasing yolk cholesterol by 16% and plasma cholesterol by 9.4% compared to the control group. In addition, BMA increased shell thickness by 3.4% and Haugh unit score by 4.6% of fresh eggs. Moreover, it increased Haugh unit and albumen percentage of stored eggs by 6.1 and 3.5%, respectively.

**Table 2.** Effect of green tea, brown marine algae, vitamin E and mixtures of them on the performance of laying hens from week 24 to 32 of age.

| Treatments       | Egg weight, g | Laying rate (h/d), % | Egg mass, g/h/d | Feed intake, g/hen | FCR, g feed/g eggs |
|------------------|---------------|----------------------|-----------------|--------------------|-------------------|
| Control          | 59.1          | 78.2                 | 46.2            | 115.2              | 2.49              |
| GT               | 60.1          | 78.2                 | 47.0            | 114.3              | 2.43              |
| Brown algae      | 60.2          | 79.1                 | 47.6            | 112.1              | 2.36              |
| Vit. E           | 60.1          | 78.4                 | 47.1            | 114.0              | 2.42              |
| GT+brown algae   | 60.4          | 79.0                 | 47.7            | 116.4              | 2.44              |
| GT+vit. E        | 60.3          | 78.4                 | 47.3            | 113.0              | 2.39              |
| Brown algae+vit. E | 60.3        | 74.5                 | 44.9            | 113.2              | 2.52              |
| SEM              | 0.35          | 0.89                 | 0.87            | 1.81               | 0.003             |
| P                | 0.43          | 0.05                 | 0.05            | 0.61               | 0.05              |

FCR, feed conversion ratio; GT, green tea; vit. E, vitamin E. a-dMeans within a column not sharing similar superscripts are significantly different (P<0.05).

**Table 3.** Effect of green tea, brown marine algae, vitamin E and mixtures of them on the quality of fresh eggs at 32 weeks of age.

| Treatments       | Shell thickness, µm | Yolk index | Yolk, % | Yolk colour | Haugh unit score |
|------------------|---------------------|------------|---------|-------------|-----------------|
| Control          | 351                 | 43.5       | 25.3    | 6.85        | 82.3            |
| GT               | 352                 | 43.2       | 25.2    | 6.72        | 83.4            |
| Brown algae      | 363                 | 44.1       | 25.4    | 7.11        | 86.1            |
| Vit. E           | 374                 | 45.2       | 25.6    | 7.24        | 85.4            |
| GT+brown algae   | 355                 | 46.2       | 25.6    | 7.51        | 84.9            |
| GT+vit. E        | 343                 | 45.6       | 25.1    | 6.93        | 84.3            |
| Brown algae+vit. E | 354            | 44.8       | 25.2    | 7.42        | 82.2            |
| SEM              | 5.1                 | 0.81       | 1.81    | 0.412       | 0.11            |
| P                | 0.043               | 0.234      | 0.386   | 0.543       | 0.023           |

| Treatments       | Albumen, % | Yolk, % |
|------------------|------------|---------|
| Control          | 60.1       | 25.2    |
| GT               | 60.3       | 25.1    |
| Brown algae      | 60.1       | 26.3    |
| Vit. E           | 60.1       | 26.3    |
| GT+brown algae   | 60.1       | 26.3    |
| GT+vit. E        | 60.1       | 26.3    |
| Brown algae+vit. E | 60.1  | 26.3    |
| SEM              | 0.004      | 0.005   |
| P                | 0.004      | 0.005   |

GT, green tea; vit. E, vitamin E. a-dMeans within a column not sharing similar superscripts are significantly different (P<0.05).
respectively, when compared with the control group. These results are in agreement with those reported by El-Deek et al. (2011), Al-Harthi and El-Deek (2012) and Al-Harthi (2014), who indicated that BMA can be fed at 3 to 5% in broiler and laying hen diets without adverse effects on productive performance and quality traits of meat and eggs. The positive response of BMA could be attributed to many of the beneficial components found in marine algae, such as vitamins (A, B, B12, C), antioxidants, and antimicrobial substances (Zulkifli et al., 2004; Rimber, 2007; Abd El-Baky et al., 2008; Al-Harthi and El-Deek, 2011, 2012). Moreover, BMA can be considered as a source of fat and water soluble vitamins, chlorophyll, lutein and zeaxanthin pigments, and an alternative source for n-3 fatty acids (Schiavene et al., 2007; Becker, 2004). In this regard, Al-Harthi and El-Deek (2012) found that BMA significantly improved egg quality and decreased egg cholesterol, and attributed this to antioxidants properties of BMA. Similar to the present results, Abd El-Baky et al. (2008) suggested that marine algae is a valuable source to increase shelf life of foodstuffs, rather than synthetic antioxidants such as BHT and BHA and can prevent cellular damage. In addition, they suggested that BMA could be used as a natural preservative ingredient in the food and pharmaceutical industries, according to its antibacterial activities and food colorant properties. Also, GT had a beneficial influence on Haugh unit score (3.5%) of stored eggs and yolk cholesterol of fresh eggs (12.2%) when compared to the control group. Moreover, GT+vit. E supplementation resulted in an improvement in Haugh unit score (2.4%) of fresh eggs, and yolk colour (9.1%) and Haugh unit score (10.9%) of stored eggs when compared with the control group. These results showed that GT improved the egg quality of fresh and stored eggs, and were in general agreement with the findings of Biswas and Wakti (2001), who demonstrated that liver cholesterol, fat and serum cholesterol were reduced (P<0.05) by feeding a GT supplement- ed diet. Also, GT decreased oxidative profiles and cholesterol due to decreasing cholesterol absorption (Raederstorff et al., 2003). Abdo et al. (2010) demonstrated that GT powder at 1 to 3% in laying hen diets or diets containing GT extract at 0.5 to 1.5 L/100 kg can reduce cholesterol content and thiobarbituric acid value of the egg yolk, implying its potential effect on egg quality parameters, especially during storage. Furthermore, Ariana et al. (2011) observed that feeding GT extract or powder improved FCR and decreased feed intake, serum low-density lipoprotein (LDL) and blood cholesterol, while GT extract reduced serum triglycerides and yolk cholesterol and increased HDL to cholesterol or LDL ratio. The beneficial effects of GT could be attributed to its contents of flavonoids, phenolic extracts, catechins and β-carotene. Thus, active substances of GT can act as reducing agents, hydrogen donors, singlet oxygen quenchers, superoxide radical scavengers and even as metal chelators. They also activate antioxidant enzymes, reduce α-tocopherol radicals (tocopherols), inhibit oxidases, mitigate nitrosative stress, and increase uric acid level and low molecular weight of molecules (Varilak et al., 2001).

El-Deek and Al-Harthi (2004) indicated that GT increased oviduct percentage on BW, which induced an increase of egg production and mass, but had no effect on egg quality. This

### Table 5. Effect of green tea, brown marine algae, vitamin E and mixtures of them on chemical composition of fresh and stored egg yolks at 32 weeks of age.

| Treatments                              | Fresh eggs | Stored eggs |
|-----------------------------------------|------------|-------------|
|                                         | Protein, % | Total lipid, % | Cholesterol, mg/g | Protein, % | Total lipid, % | Cholesterol, mg/g |
| Control                                 | 19.2       | 27.2         | 15.60<sup>a</sup> | 16.8       | 27.3         | 12.16          |
| GT                                      | 18.1       | 27.9         | 13.70<sup>a</sup> | 17.2       | 28.1         | 13.80          |
| Brown algae                             | 18.3       | 28.1         | 13.10<sup>b</sup> | 16.3       | 28.2         | 14.10          |
| Vit. E                                  | 18.4       | 28.2         | 12.60<sup>b</sup> | 16.7       | 27.9         | 13.10          |
| GT+brown algae                          | 18.9       | 28.3         | 13.17<sup>b</sup> | 16.9       | 28.3         | 12.18          |
| GT+vit. E                               | 19.3       | 27.3         | 14.50<sup>b</sup> | 17.8       | 27.6         | 14.10          |
| Brown algae+vit. E                      | 18.7       | 27.6         | 14.44<sup>b</sup> | 17.5       | 28.1         | 14.90          |
| SEM                                     | 0.213      | 0.144        | 0.477           | 0.641      | 0.817        | 0.451          |
| P                                       | 0.47       | 0.39         | 0.002           | 0.73       | 0.52         | 0.19           |

GT: green tea; vit. E: vitamin E. *Means within a column not sharing similar superscripts are significantly different (P<0.05).

### Table 6. Effect of green tea, brown marine algae, vitamin E and mixtures of them on plasma biochemical constituents of laying hens at 32 weeks of age.

| Treatments                              | Total protein, g/100 mL | Total lipid, g/L | Cholesterol, mg/100 mL | Calcium, mg/100 mL | Inorganic phosphorus, mg/100 mL |
|-----------------------------------------|-------------------------|------------------|------------------------|--------------------|---------------------------------|
| Control                                 | 4.00                    | 7.11<sup>a</sup> | 160.1<sup>a</sup>     | 21.70              | 7.90<sup>c</sup>               |
| GT                                      | 4.01                    | 6.92<sup>a</sup> | 155.2<sup>a</sup>     | 21.37              | 8.61<sup>c</sup>               |
| Brown algae                             | 4.03                    | 6.23<sup>a</sup> | 145.1<sup>a</sup>     | 21.50              | 8.90<sup>a</sup>               |
| Vit. E                                  | 3.89                    | 6.81<sup>a</sup> | 147.2<sup>a</sup>     | 20.81              | 9.01<sup>a</sup>               |
| GT+brown algae                          | 4.00                    | 6.39<sup>a</sup> | 148.2<sup>a</sup>     | 22.40              | 9.17<sup>a</sup>               |
| GT+vit. E                               | 3.99                    | 6.73<sup>a</sup> | 151.0<sup>a</sup>     | 20.78              | 8.93<sup>a</sup>               |
| Brown algae+vit. E                      | 4.09                    | 6.14<sup>a</sup> | 153.0<sup>a</sup>     | 22.06              | 9.41<sup>a</sup>               |
| SEM                                     | 0.37                    | 0.078            | 2.037                  | 0.233              | 0.051                           |
| P                                       | 0.53                    | 0.003            | 0.002                  | 0.19               | 0.002                           |

GT: green tea; vit. E: vitamin E. *Means within a column not sharing similar superscripts are significantly different (P<0.05).
contradiction in response to GT in literature could be attributed to varying level and source of GT as well as stress and hygienic conditions. In this regard, Carocho and Ferreira (2013) reported a controversy around the use of antioxidants in vivo and correlated their benefits to the decrease in the absorption of harmful foods. It is interesting to report, that using synthetic antioxidants of vit. E at 300 mg/kg, improved egg shell thickness by 6.6% and Haugh unit by 3.8%, respectively, of fresh eggs, and Haugh unit by 3.2% of stored eggs when compared to the control group. In addition, BMA+vit. E supplements increased yolk colour and Haugh unit of stored eggs by 10.7 and 3.2%, respectively, when compared to control group. This may be due to increasing antioxidants concentration in eggs which can decrease deterioration processes and improve quality during storage. Furthermore, beneficial effects of vit. E supplementation were noted when vit. E was given with BMA. This led to a significant decrease in total plasma lipid (13.6%) and increase in plasma phosphorus (19.1%).

The present results are in agreement with those reported by Kirunda et al. (2001), Bollegnieri-Lee et al. (1998) and Das et al. (2011), who showed that the addition of vit. E could reduce the negative impact of high ambient temperature on performance of laying hens and blood plasma parameters. On the other hand, Metwally (2005), Mohiti-Asli et al. (2010) and Ziaei et al. (2013) found that vit. E (α-tocopheryl acetate) did not affect production performance and egg quality. Moreover, it increased antibody titres and serum cholesterol concentration of laying hens exposed to 33°C.

It is interesting to note that some synergetic effects were identified. These effects were very clear in the yolk colour of stored eggs, since, the effect of BMA+vit. E was more intense (10.7%) than in the control group. While the effect of BMA or vit. E was equal to 5.9 and 3.6% compared to the control group. A similar effect was noted with stored eggs on Haugh unit. It was greater (10.9 and 11.1%) with GT+vit. E and BMA+vit. E groups, respectively, when compared with the control group. However, the effect of GT or BMA or vit. E was 3.5, 6.1 and 3.2% on Haugh unit, when compared with the control group. There was also an additive effect on plasma phosphorus. The effect of GT+BMA, GT+vit. E and BMA+vit. E was 16.1, 13 and 19.1%, respectively, greater than in the control group. The effect of GT or BMA or vit. E was 9, 12.7 and 14.1% indicating an increase in antioxidant concentration from natural and synthetic sources (accumulation), which resulted in better egg quality traits of fresh eggs, and was also retained during the storage period. These results are in agreement with those of Miura et al. (2001) and El-Deek et al. (2011), who reported that GT reduces the content of cholesterol and triglycerides by up to 27 to 50% and attributed these positive effects to the antioxidant activities of GT. In addition, Morrissey et al. (1994) and Jang et al. (2007) indicated that GT had beneficial effects on health and played an effective role against microbes like vit. E. Also, Puthpangsiriporn et al. (2001) and Metwally (2003) revealed a significant increase (P<0.05) in eggshell thickness in a hot climate due to vit. E addition at 310 mg/kg diet. Similar results were observed in Haugh unit and vit. E concentration in yolk (Yan and Kim, 2013).

Conclusions
The findings of this study justify the recommendation to use BMA at 0.1% or vit. E at 300 mg/kg or GT at 0.1%, in order of preference in diets of laying hens, which grow under heat stress. This will improve production performance and egg quality.

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