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Original Research Article

Productive performance, egg quality, hematological parameters and serum chemistry of laying hens fed diets supplemented with certain fat-soluble vitamins, individually or combined, during summer season

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A B S T R A C T

This present study aimed to determine the efficacy of supplementing layer diets with vitamin A (0, 8,000 and 16,000 IU/kg diet) and vitamin E (0, 250 and 500 mg/kg diet) either individually or in combination on egg production and quality, and blood hematology and chemistry of birds reared under summer conditions. A total of 135 Bovans Brown laying hens were distributed to 9 treatment groups with 5 replicates of 3 hens/pen in a 3 × 3 factorial design. A significant improvement in feed conversion ratio (FCR) was observed as supplementary vitamin A or E increased ($P \leq 0.01$). Hens fed diets supplemented with 16,000 IU vitamin A plus 500 mg vitamin E/kg diet had the best FCR among all groups. Egg quality traits were not significantly affected by the interaction of vitamin A and vitamin E levels. There was a significant increase in monocytes ($P \leq 0.01$) and a decrease in basophils counts ($P \leq 0.05$) in response to vitamin E. Significant decreases were observed in packed cell volume (PCV), thyroxine (T_4), alanine transferase (ALT), albumin, total cholesterol and total lipids (($P \leq 0.05$ or $P \leq 0.01$) $P \leq 0.01$), and increases were observed in serum concentrations of globulin ($P \leq 0.05$) and calcium ($P \leq 0.01$) due to vitamin A. The combination of 0 IU vitamin A and 500 mg vitamin E/kg diet had the highest values of PCV (40.09%) and hemoglobin (Hb) (10.33 mg/100 mL) among all groups. Vitamin E raised serum values of total protein, total cholesterol and total lipids ($P \leq 0.05$ or $P \leq 0.01$). Feed intake, FCR, PCV, Hb, lymphocytes, monocytes, eosinophils, T_4, ALT and total protein were significantly affected by the interaction of vitamins A and E ($P \leq 0.05$ or $P \leq 0.01$). The interaction of vitamins A and E was only significant with respect to serum total protein ($P \leq 0.05$). It can be concluded that layer diets supplemented with vitamins A and E had good results in alleviating the harmful impacts of high ambient temperature. The combination of 16,000 IU vitamin A and 500 mg vitamin E per kilogram diet is preferable for obtaining better production of laying hens reared under hot summer conditions.

1. Introduction

Environmental factors, such as heat stress, can impact laying hen productivity. The ideal temperature in houses of Bovans Brown layers should be around 21 to 24 °C. Negative effects on layer behavior and production are expected if the ambient temperatures exceed 28 °C (Kirunda, et al., 2001). Increasing the nutrients density

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such as limiting amino acids and vitamins is very important to alleviate the deleterious effects of high ambient temperature on feed consumption and metabolic requirements (Habibian et al., 2015). Given that heat stress may increase the excretion and mobilization of minerals and vitamins from tissues, there is a benefit to be gained from adding extra amounts of these nutrients to layer diets to avoid marginal deficiency during hot weather (Sahin et al., 2002a).

Vitamin A is a vital antioxidant that minimizes lipid peroxidation under heat stress (Abd El-Hack et al., 2015). It is essential for visual development, growth, and reproductive physiology. Supplementing higher level of vitamin A than recommended by NRC (1994) is preferable to help normal development of the layer reproductive organs and membrane integrity when birds are not reared under ideal conditions, such as heat stress (Kaya and Yildirim, 2011). Many researchers confirmed that vitamin A can improve the productive performance traits and egg quality characteristics in laying hens reared under heat stress (Lin et al., 2002; Kucuk et al., 2003). Vitamin E is well-known as an essential antioxidant that can also be used in poultry diets to have beneficial impacts during heat stress (Abd El-Hack et al., 2015). El-Mallah et al. (2011) pointed out that Hisex Brown laying hens fed diets supplemented with vitamin E at 40 IU/kg had statistical improvement in egg yield percentage (85%) compared with the control (83%). Vitamin E has a critical impact on the absorption and utilization of vitamin A. In addition, vitamin E, as an antioxidant, protects and prevents vitamin A from oxidative breakdown induced by heat stress (Kucuk et al., 2003). The data concerning the use of fat-soluble vitamins, especially vitamin A under heat stress conditions on thyroid hormones of laying hens are scanty and the previous reports on the supplementation of these vitamins in layer diets resulted in contradictory conclusions. Therefore, the objective of the present study was to evaluate dietary supplemental levels of vitamins A and E, fed separately or combined of laying hendiets on productive performance traits, egg quality traits, and blood hematology and chemistry during hot summer seasons.

2. Materials and methods

The present study was performed at Poultry Research Farm, Department of Poultry, Faculty of Agriculture, Zagazig University, Egypt. All the experimental procedures were approved by the Local Experimental Animals Care Committee, and approved by the ethics of the Institutional Committee at Faculty of Agriculture, Zagazig University, Egypt. Birds were cared for using husbandry guidelines derived from Zagazig University standard operating procedures.

2.1. Experimental design, birds, and husbandry

A 3 × 3 factorial arrangement of treatments was set-up, which included 3 levels of vitamin A (0, 8,000 and 16,000 IU/kg diet) and 3 levels of vitamin E (0, 250 and 500 mg/kg diet). A total of 135 Bovans Brown laying hens were distributed into 9 treatment groups, with 5 replicates (3 hens per pen). Hens of all experimental groups had approximately the same initial average body weight (1,660 ± 2.3 g) at the start of the experiment. The basal diet contained 17.5% CP and 2,800 kcal ME/kg, and was formulated to meet the nutrient recommendation of Bovans Brown laying hens management guidelines during the period of 42 to 54 weeks of age (Table 1). The basal diet was supplemented with vitamin A acetate (the purity was 100%) at 0, 8,000 and 16,000 IU/kg diet. Under each level of vitamin A, the diets were supplemented with dl-α-tocopherol acetate (the purity was 50%) at 0, 250 and 500 mg vitamin E/kg diet. Vitamins were bought from Multivita Company, Sixth of October Governorate, Egypt. The hens were housed in wire cages (50 cm × 50 cm × 45 cm) with nipple drinkers and trough feeders. The house was provided with open side curtain ventilation with a circulation fan. The lighting program used was 14 h of light at the beginning of the trial, with light increasing by 15 min weekly to 17 h of light. Feed and water were provided *ad libitum* throughout the experimental period. The experimental period lasted for 12 weeks (42 to 54 weeks of age). The average indoor ambient temperature (°C) and the average relative humidity (%) during the summer months are illustrated in Fig. 1.

Table 1
The basal diet composition and nutrient content (as fed basis).

Item	Content
Ingredient, %	
Maize	56.71
Soybean meal (44% CP)	28.62
Limestone	9.33
Soybean oil	3.13
Di-calcium phosphate	1.45
DL-methionine	0.16
NaCl	0.30
Vitamin-mineral premix[1]	0.30
Nutrient composition[2], %	
Crude protein	17.51
ME, kcal/kg	2,800
Lysine	0.92
Methionine	0.43
TSAA	0.73
Calcium	4.00
Phosphorus (available)	0.38

TSAA = total sulphur amino acids.
[1] Vitamin-mineral premix: each 1 kg contains vitamin D_3, 1,300 IU; vitamin A, 8,000 IU; vitamin E 4.5 IU; vitamin K, 2 mg; vitamin B_1, 0.7 mg; vitamin B_2, 3 mg; vitamin B_6, 1.5 mg; vitamin B_{12}, 7 mg; biotin 0.1 mg; pantothenic acid, 6 g; niacin, 20 g; folic acid, 1 mg; manganese, 60 mg; zinc, 50 mg; copper, 6 mg; iodine, 1 mg; selenium, 0.5 mg; cobalt, 1 mg.
[2] Calculated according to NRC (1994).

2.2. Data collection and calculations

2.2.1. Productive performance

Feed intake was recorded weekly and feed conversion ratio (FCR) (g of feed/g of egg) was calculated. Egg weight and egg number were recorded daily to calculate the egg production and egg output volume (egg number × egg weight).

2.2.2. Egg quality traits

Egg quality traits were measured monthly using 15 eggs from each treatment group. Exterior and interior egg quality parameters (percentages of yolk, albumen, shell, and egg shape index, shell thickness and Haugh unit) were determined according to Romanoff and Romanoff (1949).

2.2.3. Blood chemistry and hematological parameters

Blood samples were collected randomly from 5 birds per treatment from the brachial vein into sterilized tubes. Samples were allowed to coagulate and centrifuged at 2,328 × *g* for 15 min at 4 °C to obtain serum. Serum samples were kept at −20 °C until being analyzed. After obtaining whole blood samples, blood films were made using the slide method of Schalm (1961). Blood films were stained using Pappenheim May-Grunwald Giemsa stain. The differential count of white blood cells (WBC) was made of 3 horizontal edge fields followed by 2 fields towards the center, and followed by 2 fields in horizontal direction and then 2 fields in vertical direction to reach the edge again. The field takes a zigzag shape with right angles. Two hundred cells, with 100 cells on each

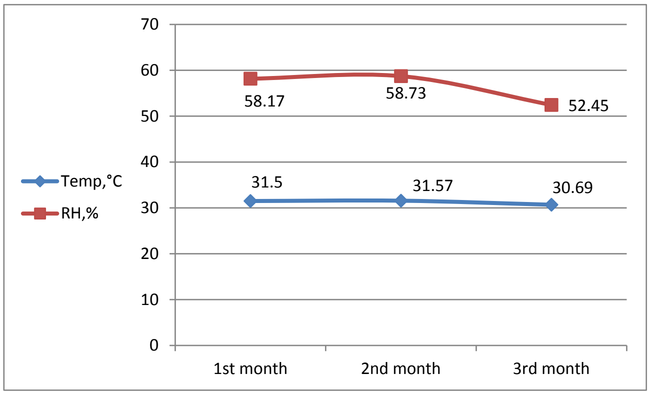

Fig. 1. Average indoor ambient temperature (Temp) and average relative humidity (RH) during the 1st (42 to 46 weeks of age), 2nd (47 to 50 weeks of age) and 3rd months (51 to 54 weeks of age).

edge of the film, were differentiated, and the percentages of heterophils, lymphocytes, monocytes, basophils and eosinophils were calculated. Blood hemoglobin (Hb) was determined with reference to Dukes and Schwarte (1931). Packed cell volume (PCV) was determined using microhematocrit tubes, where 3 tubes were used for each sample, centrifuged at 3,000 × g for 10 min and the mean of the 3 obtained readings was recorded. The following serum metabolites: triiodothyronine (T_3), thyroxine (T_4), aspartate transferase (AST), alanine transferase (ALT), total protein, albumin, total lipids, total cholesterol and calcium were determined spectrophotometrically using commercial diagnostic kits provided by Biodiagnostic Co. Giza, Egypt.

2.3. Statistical analysis

Data were statistically analyzed using the GLM procedure of SPSS. A 3 × 3 factorial arrangement of treatments was used to analyze data of layer performance, quality of eggs, hematological and biochemical blood parameters as a response to 3 levels of vitamin A and 3 levels of vitamin E. The statistical differences among the means of treatments were determined using the *post hoc* Newman–Keuls test ($P \leq 0.05$). The model used was: $Y_{ij} = \mu + A_i + E_j + AE_{ij} + e_{ij}$, where: Y_{ij} = an observation, μ = the overall mean, A_i = fixed effect of vitamin A levels, E_j = fixed effect of vitamin E levels, AE_{ij} = fixed effect of interaction between vitamins A and E levels and e_{ij} = random error associated to each observation.

3. Results and discussion

3.1. Hen productive performance

Results in Table 2 illustrate the effect of supplementing vitamin A in graded levels on productive performance traits of laying hens from 42 to 54 weeks of age. Highly significant impacts ($P \leq 0.01$) were recorded due to vitamin A addition on FI and FCR values. It is noticeable that FI of hens fed the diet enriched with 8,000 IU vitamin A/kg diet was the highest, but was depressed in those fed a higher level of vitamin A (16,000 IU/kg diet). An improvement ($P \leq 0.01$) in FCR was observed in vitamin A groups compared with the control. Vitamin A addition increased ($P \leq 0.01$) FI. Similar results were obtained by Lin et al. (2002) who confirmed an increase in FI of laying hens fed diets supplemented with vitamin A ranging from 3,000 to 12,000 IU/kg diet during heat stress. Moreover, in accordance with our results, Abdo (2009) reported a significant elevation in consumed feed due to supplementing the diet with 3,000 IU vitamin A/kg comparing with the control. On the other hand, Kaya et al. (2001) found no significant impact of supplementary vitamin A (10,000 IU/kg diet) to layer diets on FI. Albeit not significant, there was a noticeable increase in egg production and egg output in vitamin A treated groups compared with the control (Table 2). Improving egg production and egg output due to vitamin A addition (8,000 and 16,000 IU/kg diet) may be attributed to improved FI and FCR under the hot summer condition. On the other hand, Ramalho et al. (2008) postulated that adding retinyl palmitate at different doses (180, 360, 720 and 1,440 IU/kg diet) to laying quail diets did not have any significant impact on daily egg yield.

There were significant main effects ($P \leq 0.01$) in the values of FI and FCR with respect to vitamin E supplementation. It is obvious that FI was significantly ($P \leq 0.01$) decreased with increasing vitamin E level in the diet. In a converse trend, a significant ($P \leq 0.01$) improvement in FCR was observed as supplementary vitamin E increased. Improving FCR as a result to vitamin E supplementation may be explained by the antioxidant properties of vitamin E, which improves feed utilization and metabolism as well as protecting the liver and other organs from oxidative damage induced by a heat burden (Bollengier-Lee et al., 1998). Conversely, Meluzzi et al. (2000) assured that FI and FCR of Hy-Line Brown laying hens were insignificantly influenced when vitamin E was supplemented at levels of 0, 45, 90 and 180 IU/kg diet. No significant impacts were noticed on monthly egg production or egg output during the experimental period due to vitamin E supplementation. In the same time, both of egg production and egg output were

Table 2
Influences of dietary vitamins A and E supplementations and their interaction on productive performance of laying hens from 42 to 54 weeks of the age.

Item		FI, g/day	FCR, g feed/g egg	Egg production, egg/month	Egg output, g
Vitamin A, IU/kg	Vitamin E, mg/kg				
0	0	126.08 ± 4.64[a]	2.67 ± 0.058[a]	23.22 ± 1.06	1,413.49 ± 85.27
	250	105.23 ± 4.24[e]	2.53 ± 0.025[c]	23.67 ± 0.86	1,359.54 ± 67.80
	500	108.39 ± 3.87[d]	2.60 ± 0.075[b]	22.89 ± 1.13	1,298.38 ± 64.37
8,000	0	108.38 ± 2.55[d]	2.50 ± 0.049[c]	23.89 ± 0.73	1,418.03 ± 52.08
	250	117.81 ± 1.64[b]	2.45 ± 0.031[d]	24.78 ± 0.72	1,455.53 ± 57.63
	500	115.70 ± 1.73[bc]	2.51 ± 0.053[c]	24.67 ± 0.64	1,415.24 ± 60.97
16,000	0	114.57 ± 2.50[bc]	2.60 ± 0.021[b]	23.67 ± 1.03	1,403.07 ± 67.60
	250	110.74 ± 1.63[c]	2.52 ± 0.045[c]	24.11 ± 0.67	1,438.61 ± 62.60
	500	106.58 ± 4.22[e]	2.35 ± 0.012[e]	25.33 ± 0.67	1,539.09 ± 49.60
Significance					
Vitamin A		**	**	NS	NS
Vitamin E		**	**	NS	NS
Vitamin A × Vitamin E		**	**	NS	NS

FI = feed intake; FCR = feed conversion ratio.
[a-e] Means in the same column within each classification bearing different letters are significantly different ($P \leq 0.05$).
NS = not significant, **: $P \leq 0.01$.

Table 3
Influences of dietary vitamins A and E supplementation and their interaction on some egg quality traits of laying hens from 42 to 54 weeks of the age.

Item		Egg shape index	Albumen, %	Yolk, %	Shell, %	Shell thickness, mm	Haugh unit
Vitamin A, IU/kg	Vitamin E, mg/kg						
0	0	75.40 ± 0.007	64.40 ± 0.78	25.70 ± 0.94	10.53 ± 0.35	0.40 ± 0.013	77.07 ± 3.43
	250	74.90 ± 0.007	63.93 ± 0.50	25.28 ± 0.49	10.79 ± 0.11	0.37 ± 0.013	74.10 ± 1.71
	500	74.30 ± 0.005	64.60 ± 1.13	24.58 ± 0.80	10.82 ± 0.37	0.37 ± 0.010	65.42 ± 2.40
8,000	0	74.70 ± 0.007	63.71 ± 0.65	25.31 ± 0.61	10.98 ± 0.34	0.39 ± 0.013	76.72 ± 3.27
	250	74.90 ± 0.004	63.20 ± 0.48	26.02 ± 0.43	10.78 ± 0.18	0.38 ± 0.010	78.95 ± 4.97
	500	75.20 ± 0.006	62.85 ± 0.63	26.59 ± 0.58	10.56 ± 0.23	0.36 ± 0.013	74.72 ± 3.05
16,000	0	73.60 ± 0.007	55.11 ± 8.05	26.09 ± 0.49	18.80 ± 8.17	0.39 ± 0.010	79.00 ± 4.25
	250	72.60 ± 0.004	63.39 ± 0.53	25.96 ± 0.43	10.65 ± 0.21	0.39 ± 0.010	69.78 ± 1.01
	500	73.00 ± 0.004	64.41 ± 0.47	25.24 ± 0.38	10.34 ± 0.31	0.39 ± 0.010	77.71 ± 3.78
Significance							
Vitamin A		**	NS	NS	NS	NS	NS
Vitamin E		NS	NS	NS	NS	*	NS
Vitamin A × Vitamin E		NS	NS	NS	NS	NS	NS

NS = not significant, *: $P \leq 0.05$ and **: $P \leq 0.01$.

numerically enhanced as a result to vitamin E addition comparing with other diets (Table 2). Similarly, Grobas et al. (2002) found no significant influence of dietary vitamin E supplementation at levels from 13 to 263 mg/kg diet on improving egg production of ISA brown laying hens. On the contrary, Bollengier-Lee et al. (1998) claimed that laying hens fed diets enriched with vitamin E at doses from 125 to 500 mg/kg diet produced more eggs and attributed this positive effect to facilitating the release of vitellogenin from the liver and increasing its level in the blood. Jiang et al. (2013) demonstrated that feeding laying hens diets supplemented with vitamin E had a positive effect on egg production.

With regard to the interactive effect of vitamins A and E levels, it is noticeable that only FI and FCR statistically ($P \leq 0.01$) differed due to this combination. Birds fed the diet free of the 2 vitamins (0 vitamin A × 0 vitamin E) consumed more feed (126.1 g/d) than other experimental groups, and had the worst FCR (2.67). Hens fed diets supplemented with 16,000 IU vitamin A plus 500 mg vitamin E/kg diet had the best FCR when compared with other treatment groups (Table 2). It was confirmed that the combination of vitamin A and vitamin E increased feed consumption of Japanese quails reared under heat stress conditions (Sahin and Kucuk 2001). The trials performed by Lin et al. (2002) revealed that the interactive impact of vitamin A and vitamin E was beneficial on productive performance of laying hens exposed to heat stress. Abdo (2009) revealed that the average values of egg yield and egg output were significantly improved in hens fed diets enriched with 2 levels of vitamins A and E either individual or in combination.

3.2. Egg quality traits

No significant influences were observed due to supplemental vitamins A and E or their interaction on the egg quality traits with the exception of significant ($P \leq 0.01$) impacts of vitamin A on egg shape index and of vitamin E on eggshell thickness ($P \leq 0.05$). Increasing the level of vitamin A was associated with a significant decrease in egg shape index value. The same trend was also observed with elevating vitamin E level in the diet from 0 to 500 mg/kg diet which accompanied with a depression in values of eggshell thickness. Yolk and shell percentages, shell thickness, and Haugh unit score increased as a result of supplemental vitamin A, though those differences were not significant (Table 3). The stimulatory effects of supplemental vitamin A on the development and growth of female reproductive system may be the reason for the observable improvement in Haugh unit (Brody, 1993). Our results are in partially agreement with those reported by Ramalho et al. (2008) who observed an improvement in egg quality traits, when laying hens were fed diets supplemented with vitamin A in the form of retinyl palmitate at levels of 600, 1,200, 2,400 and 4,800 IU/kg. Abdo (2009) found that eggshell percentage was maximized by dietary addition of vitamin A at the level of 10 IU/kg diet. On the contrarily, Bárdos et al. (1996) observed no significant effect of vitamin A on egg quality traits of laying Japanese quail. Yuan et al. (2014) found that vitamin A supplementation at a dose of 45,000 IU/kg diet significantly depressed eggshell thickness of broiler breeders. Our results disagree with those reported by Abdel-Fattah and Abdel-Azeem (2007) who found that feeding laying quail with a diet supplemented with different dietary levels of vitamin E had the best egg quality traits comparing with the control. Radwan et al. (2008) demonstrated that supplementing 200 mg vitamin E/kg to layer diets caused a numeric increase in the egg shape index value and eggshell percentage. On the other hand, Jiang et al. (2013) pointed out that Haugh unit scores were not statistically impacted by dietary vitamin E supplementation. With regards to the insignificant results on egg quality traits due to the interaction of vitamin A and vitamin E levels in the present study, it could be partially explained by results of Grobas et al. (2002) who assured that high concentration of dietary vitamin A declines vitamin E absorption. Frigg and Broz (1984) assured that the antagonism between vitamins A and E may be due to the competition during digestion, the antagonism is depressed markedly when vitamin E is administered parenterally.

3.3. Blood hematology

The thermoneutral region for most poultry species ranged between 18 and 20 °C as stated by Ensminger et al. (1990). When ambient temperature goes above this range, a wide range of changes was noted in biochemical and hematological blood parameters (Altan et al., 2000 and Sahin and Kucuk, 2001). During the high ambient temperature, there were no significant effects of vitamin A on any of the blood hematological parameters except for PCV values, which were statistically ($P \leq 0.01$) depressed in response to vitamin A supplementation (Table 4). It has been reported that vitamin A deficiency is usually combined with impaired immune response (Friedman et al., 1991). The authors confirmed the major role of vitamin A in improving the immune function of chicken. On the other hand, Yuan et al. (2014) theorized that vitamin A supplementation to broiler breeder diets at level of 135,000 IU/kg declined the proliferation of blood lymphocytes.

Only the counts of monocytes ($P \leq 0.01$) and basophils ($P \leq 0.05$) were significantly affected by vitamin E supplementation. The highest level of vitamin E (500 mg/kg diet) increased monocyte

Table 4
Influences of dietary vitamins A and E supplementation and their interaction on blood hematological parameters of laying hens at 54 weeks of age.

Item		PCV, %	Hb, mg/100 mL	Differentiation of white blood cell types					
				Heterophils	Lymphocytes	H/L ratio	Monocytes	Basophils	Eosinophils
Vitamin A, IU/kg	Vitamin E, mg/kg								
0	0	36.94 ± 1.53^{b}	9.99 ± 0.24^{b}	20.44 ± 2.28	73.47 ± 2.36^{b}	0.29 ± 0.040	3.54 ± 0.43^{bc}	0.30 ± 0.21	2.25 ± 0.20^{bc}
	250	39.02 ± 0.42^{ab}	10.26 ± 0.27^{ab}	23.03 ± 2.69	70.14 ± 2.32^{cd}	0.34 ± 0.057	4.22 ± 0.57^{ab}	0.11 ± 0.11	2.50 ± 0.58^{b}
	500	40.09 ± 1.05^{a}	10.33 ± 0.31^{a}	21.61 ± 2.07	70.89 ± 2.24^{cd}	0.31 ± 0.037	3.96 ± 0.14^{b}	0.00 ± 0.00	2.04 ± 0.22^{c}
8,000	0	35.37 ± 1.28^{c}	9.84 ± 0.18^{b}	22.47 ± 2.49	70.52 ± 2.34^{cd}	0.33 ± 0.060	3.43 ± 0.25^{c}	0.17 ± 0.17	3.41 ± 0.46^{a}
	250	31.91 ± 1.50^{d}	10.20 ± 0.09^{ab}	17.60 ± 1.83	76.60 ± 2.11^{a}	0.24 ± 0.031	$4.03 \pm 0\ .46^{b}$	0.00 ± 0.00	1.77 ± 0.25^{d}
	500	37.20 ± 1.65^{b}	9.88 ± 0.35^{b}	22.99 ± 3.47	69.89 ± 3.96^{d}	0.36 ± 0.078	4.47 ± 0.41^{ab}	0.05 ± 0.04	2.60 ± 0.42^{b}
16,000	0	38.19 ± 1.23^{ab}	10.34 ± 0.27^{a}	21.51 ± 1.52	71.53 ± 1.58^{c}	0.31 ± 0.029	4.57 ± 0.34^{a}	0.16 ± 0.12	2.24 ± 0.27^{bc}
	250	36.98 ± 1.67^{b}	10.03 ± 0.24^{ab}	20.41 ± 0.71	73.17 ± 1.31^{b}	0.28 ± 0.015	2.97 ± 0.27^{d}	0.00 ± 0.00	3.45 ± 0.55^{a}
	500	34.07 ± 1.13^{c}	9.39 ± 0.29^{c}	22.94 ± 3.18	70.49 ± 4.34^{cd}	0.36 ± 0.077	4.69 ± 0.86^{a}	0.00 ± 0.00	1.88 ± 0.36^{d}
Significance									
Vitamin A		**	NS	NS	NS	NS	NS	NS	NS
Vitamin E		NS	NS	NS	NS	NS	**	*	NS
Vitamin A × Vitamin E		**	*	NS	*	NS	**	NS	**

PCV = packed cell volume; Hb = hemoglobin; H/L = the ratio of heterophils to lymphocytes.
[a-d] Means in the same column within each classification bearing different letters are significantly different ($P \leq 0.05$).
NS = not significant, *: $P \leq 0.05$; **: $P \leq 0.01$.

count by 11.90% comparing with the control. Conversely, a significant ($P \leq 0.05$) decrease in basophil count was recorded with increasing vitamin E level. In line with our findings, El-Sebai (2000) reported that vitamin E supplementation to broiler diets caused a significant increase in WBC count by a 4.65% comparing with the control. Perez-Carbajal et al. (2010) assured that vitamin E has antioxidant and immunomodulatory proprieties which positively influence the chicken immune response through improving the phagocytic function of macrophages.

In the present study, all hematological parameters were significantly altered due to the interaction of vitamins A and E levels. The combination between 0 IU vitamin A and 500 mg vitamin E/kg diet had the highest value of PCV (40.09%) and Hb (10.33 mg/100 mL) comparing with other groups. The highest count of lymphocytes was observed in blood of hens fed diets enriched with 8,000 IU vitamin A plus 250 mg vitamin E/kg diet. The interaction of 8,000 IU/kg vitamin A and 0 mg/kg vitamin E had the highest count of monocytes compared with other treatment groups. Hens fed diets supplemented with 16,000 IU vitamin A combined with 250 mg vitamin E/kg increased the count of eosinophil cells in its blood than those fed other diets. The aforementioned positive results may be due to the synergistic effect between the 2 fat-soluble vitamins which appeared in improved immunity. It has been established that vitamin E acts as a protector of vitamin A in poultry diets and that the combination of both vitamins E and A levels enhances the proliferation of lymphocytes (Haq and Bailey, 1996). Where, vitamins A and E are essential micronutrients for health (Otten et al., 2006). The common circulating forms of vitamin A and vitamin E include α- and γ-tocopherols for vitamin E (Ford et al., 2006), retinol for vitamin A, and β-carotene for provitamin A (Tanumihardjo et al., 2016). These fat-soluble compounds have been implicated in the normal function of multiple physiological systems (Gagne et al., 2009). The major circulating form of vitamin E is α-tocopherol, whereas γ-tocopherol is the most abundant form of vitamin E in the diet (Jiang et al., 2001). Also, vitamins A and E may be beneficial for metabolic health through their anti-oxidant effects (Martins Gregório, et al., 2016).

3.4. *Thyroid hormones and liver enzymes activities*

Thyroid hormones are considered as the key controllers of metabolic heat production which is necessary for the maintenance of high and constant body temperature in homoeothermic birds (Danforth and Burger, 1984). Bobek et al. (1980) reported that Japanese quail subjected to ambient temperature of 34 to 35 °C had a continuous decrease in T_4 concentration and a continuous increase in T_3 concentration, during the first 6 h of heat exposure. In the current study, significant decreases ($P \leq 0.01$) in T_4 concentration and ALT activity were observed as a result of vitamin A supplementation under heat stress condition. In a converse trend, the activity of serum AST significantly increased ($P \leq 0.01$) as the level of vitamin A supplementation increased (Table 5). It is well-known that vitamin A plays a major role in regulating the secretion of thyroid hormones, liver enzyme in addition to its role in normal growth and development. Sahin and Kucuk, (2001) observed that vitamin A supplementation increased serum concentrations of T_4 and T_3 compared with the control group. Conversely, Kaya et al. (2001) found that plasma concentrations of T_3 and T_4 were not significantly impacted by vitamin A supplementation in laying hens.

No significant impacts were recorded for vitamin E addition on thyroid hormones and liver enzymes activities excepting for serum ALT which statistically ($P \leq 0.01$) differed without a definite trend. Serum concentrations of T_3 and T_4 were not significantly increased due to vitamin E supplementation. Abdel-Fattah and Abdel-Azeem (2007) reported that serum concentrations of T_3 and T_4 increased with elevating vitamin E level up to 250 ppm. This is because of the positive impacts of vitamin E on alleviating the harmful influences of heat stress. Several researchers confirmed that heat stress reduces blood concentrations of T_3 and T_4 in chickens; this effect may be due to a reduction in size and secretion of thyroid gland (Sahin et al., 2002b). Abdel-Fattah and Abdel-Azeem (2007) demonstrated that vitamin E supplementation at levels from 375 to 500 mg/kg elevated plasma activity of ALT enzyme of laying hens under heat burden.

During heat stress, the concentration of T_3 was significantly reduced (Atta, 2002). This means thyroid hormones are important factors in a response to high temperature. In addition, exogenous thyroid hormones have a shorter survival time when exposed to heat stress (Bowen et al., 1984). In chicken, thyroid activity and size were decreased by high ambient temperatures and increased by low ambient temperatures (Huston et al., 1962). Birds exposed to heat stress show elevated level of corticosterone and lower levels of thyroid hormones (Mahmoud et al., 2014). In the present study, the interactive effect of vitamins A and E was significant ($P \leq 0.01$) on T_4 concentration and ALT activity. The highest values of T_4 (4.98 μg/mL) and ALT (22.33 U/L) were achieved by birds fed diets enriched with 0 IU vitamin A plus 500 mg vitamin E/kg diet and

Table 5
Influences of dietary vitamins A and E supplementation and their interaction on blood thyroid hormones and liver enzymes of laying hens at 54 weeks of age.

Item		Thyroid hormones		Liver enzymes	
		T_3, ng/mL	T_4, μg/mL	ALT, U/L	AST, U/L
Vitamin A, IU/kg	Vitamin E, mg/kg				
0	0	0.080 ± 0.01	4.38 ± 0.21^{ab}	21.00 ± 1.17^{b}	42.67 ± 2.36
	250	0.093 ± 0.01	4.98 ± 0.28^{a}	17.89 ± 0.60^{c}	40.22 ± 2.00
	500	0.175 ± 0.08	5.32 ± 0.36^{a}	20.89 ± 1.57^{b}	37.33 ± 1.81
8,000	0	0.098 ± 0.01	4.78 ± 0.22^{ab}	18.00 ± 1.90^{c}	48.11 ± 3.92
	250	0.119 ± 0.04	4.21 ± 0.18^{b}	17.78 ± 0.97^{c}	46.00 ± 2.27
	500	0.080 ± 0.01	4.27 ± 0.23^{ab}	22.33 ± 1.17^{a}	42.44 ± 2.17
16,000	0	0.089 ± 0.01	4.54 ± 0.17^{ab}	17.89 ± 0.60^{c}	46.67 ± 1.60
	250	0.093 ± 0.01	4.42 ± 0.17^{ab}	16.75 ± 1.06^{d}	48.22 ± 2.68
	500	0.170 ± 0.08	4.49 ± 0.21^{ab}	18.00 ± 1.90^{c}	46.56 ± 3.27
Significance					
Vitamin A		NS	**	**	**
Vitamin E		NS	NS	**	NS
Vitamin A × Vitamin E		NS	**	**	NS

T_3 = triiodothyronine; T_4 = thyroxine; ALT = alanine transferase; AST = aspartate transferase.
[a-d] Means in the same column within each classification bearing different letters are significantly different ($P \le 0.05$).
NS = not significant, *: $P \le 0.05$; **: $P \le 0.01$.

8,000 IU vitamin A combined with 500 mg vitamin E/kg, respectively. Sahin and Kucuk, (2001) stated that serum concentrations of thyroid hormones (T_3 and T_4) were higher ($P \le 0.01$) in birds fed diets supplemented with vitamin A plus vitamin E than those fed the control diet.

3.5. Serum metabolites

Results in Table 6 illustrate a significant reduction in serum concentration of albumin, total cholesterol as well as total lipids and an increase in serum concentrations of globulin and calcium due to vitamin A addition under summer condition. Sahin et al. (2004) recorded a significant increase in plasma concentrations of cholesterol and glucose compared with birds reared under thermoneutral conditions. Our findings agree with Kaya et al. (2001) who found that dietary vitamin A addition declined the concentration of serum cholesterol in chicks. Also, our results are consistent with those reported by Kucuk et al. (2003) who noticed that vitamin A addition increased plasma concentration of total protein in broilers and depressed cholesterol concentration.

Vitamin E supplementation in Bovans Brown hen's diet under summer ambient temperature had a positive influence ($P \le 0.01$) on the values of total protein, total cholesterol, and total lipids. This result may be attributed to the antioxidant activity of vitamin E which protects lipids from peroxidation induced by the extra heat burden. On the other hand, no significant effects were observed on other blood metabolites (albumin, globulin and calcium) as shown in Table 6. El-Sebai (2000) reported that plasma concentrations of total protein, total lipids and total cholesterol increased in experimental groups fed diets supplemented with vitamin E compared with the control. Furthermore, Sahin et al. (2002b) postulated that the treatment with vitamin E caused an elevation in serum concentrations of total protein and albumin of broilers during heat stress conditions. On the other hand, Abd El-Hack et al. (2015) assured that dietary supplementation with vitamin E at level of 250 mg/kg diet did not have any significant impact on serum total protein, albumin, globulin or total cholesterol.

Also in Table 6, the interaction of vitamins A and E was only significant ($P \le 0.05$) with respect to serum total protein values. The highest value of total protein (4.77 g/dL) was found in hens fed diets supplemented with 8,000 IU vitamin A plus 250 mg vitamin E/kg diet. Sahin et al. (2002a) reported that using a combination of vitamins A and E as a dietary supplement can alleviate bad metabolic changes related to heat stress in broilers. Abdo (2009) hypothized that supplementing vitamins at high level may cause a physiological stress on birds, which increases and activates the release of corticosterone to cope with stress. In birds suffering from heat stress, creatine kinase is freed from muscle cells into the plasma as a result to the intracellular influx of Ca, this impact may alter plasma protein fraction. In this case, vitamins A and E can modulate

Table 6
Influences of dietary vitamins A and E supplementation and their interaction on some blood constituents of laying hens at 54 weeks of age.

Item		Total protein, g/dL	Albumin, g/dL	Globulin, g/dL	Total cholesterol, mg/dL	Total lipids, g/L	Calcium, mg/dL
Vitamin A, IU/kg	Vitamin E, mg/kg						
0	0	4.64 ± 0.14^{ab}	3.02 ± 0.13	1.67 ± 0.09	148.61 ± 1.37	23.31 ± 0.66	21.62 ± 0.39
	250	4.43 ± 0.11^{b}	3.01 ± 0.20	1.50 ± 0.13	152.95 ± 2.37	27.47 ± 0.29	22.99 ± 0.48
	500	4.59 ± 0.13^{ab}	2.96 ± 0.13	1.74 ± 0.07	152.23 ± 2.33	27.17 ± 0.44	22.73 ± 0.42
8,000	0	4.41 ± 0.17^{b}	2.73 ± 0.05	1.79 ± 0.07	141.33 ± 2.13	21.13 ± 0.40	25.33 ± 0.33
	250	4.77 ± 0.05^{a}	2.90 ± 0.08	1.90 ± 0.03	149.07 ± 2.08	22.66 ± 0.58	24.09 ± 0.35
	500	4.73 ± 0.10^{a}	3.93 ± 0.07	1.76 ± 0.07	144.48 ± 2.08	22.79 ± 0.62	24.29 ± 0.43
16,000	0	4.35 ± 0.17^{b}	2.81 ± 0.10	1.68 ± 0.13	137.53 ± 2.70	22.56 ± 0.87	24.85 ± 0.43
	250	4.65 ± 0.17^{ab}	2.64 ± 0.10	1.68 ± 0.11	147.87 ± 1.35	24.03 ± 0.88	24.65 ± 0.49
	500	4.65 ± 0.11^{ab}	2.69 ± 0.11	1.79 ± 0.07	146.57 ± 1.54	24.39 ± 0.74	24.67 ± 0.43
Significance							
Vitamin A		NS	**	*	**	**	**
Vitamin E		*	NS	NS	**	**	NS
Vitamin A × Vitamin E		*	NS	NS	NS	NS	NS

[a,b] Means in the same column within each classification bearing different letters are significantly different ($P \le 0.05$).
NS = not significant, *: $P \le 0.05$; **: $P \le 0.01$.

or improve the status by minimizing intracellular influx of Ca and cell membrane permeability (Kaya et al., 2001; Ramalho et al., 2008).

4. Conclusions

From the aforementioned results and discussion, it could be concluded that the layer diets supplemented, individually or combined, with vitamins A and E had good results in alleviating the harmful impacts of summer ambient temperature on different aspects of health indices and productive performance. Our results confirm the efficiency of vitamin A in enhancing the productive performance traits. The combination of vitamins A and E (16,000 IU vitamin A plus 500 mg vitamin E per kilogram diet) is preferable for obtaining better production of laying hens reared under hot summer conditions.

Conflict of interest

The authors declare that they have no competing interests.

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