

Effect of Dietary Vitamin E on the Performance, Plasma and Egg Yolk Vitamin E Levels and Lipid Oxidation of Egg in Heat Stressed Layers

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Abstract

This study was conducted to investigate the effects of dietary vitamin E levels on the concentration of vitamin E of egg yolk and plasma, TBARS (thiobarbituric acid reactive substance) of egg yolk during refrigerated storage of 1 or 42 days and productive performance of laying hens exposed to heat stress (30 °C). Twenty four week old, 128 Lohman LSL hybrids layers divided into 8 groups. Half of the groups were kept at normal poultry-house conditions (20 °C) and the other half were exposed to heat stress (30 oC). In both poultry-houses, former groups were fed on basal diets and 2nd, 3rd, and 4th groups were fed on the vitamin added rations which were 45, 65, 85 IU/kg vitamin E respectively.

Supplementation of vitamin E caused a very significant increase in the egg yields. Egg production was significantly greater ($P < 0.01$) with 85 IU/kg vitamin E (81%) as compared to control hens. On the other hand the average egg yields were significantly decreased (approximately 14%) by the heat stress. A higher concentration of vitamin E reduced TBARS values in eggs. Concentration of vitamin E in the yolk and plasma linearly increased as the dietary vitamin E increased.

Key words: Laying hen, TBARS, vitamin E, egg production, heat stress

INTRODUCTION

Vitamin E is the most active natural antioxidant used in animal diets. It exhibits an antioxidant activity at low concentration and prooxidant activity at high concentration [1]. Bollengier-Lee et al. [2-3] speculated that vitamin E protected the liver from lipid peroxidation and damage to cell membranes. Tengerdy [4] reported that vitamin E supplementation could reduce the negative effects of corticosterone induced by stress. Jiang et al. [5]; Surai et al. [6] and Meluzzi et al. [7] reported that dietary α -tocopherol increases the content of vitamin E in the egg yolk in a dose dependent manner. Halliwell and Gutheridge [8] and Yu [9] reported that vitamin E was as an excellent biological chain-breaking antioxidant that protects cell and tissue from lipoperoxidative damage induced by free radicals. It was found that hens supplemented with dietary α -tocopherol had a significant reduction in TBARS values, as an indicator of lipid peroxidation, in eggs and liver tissue [10]. Surai et al. [11] indicated that an increased vitamin E supplementation of the maternal diet can substantially increased vitamin E concentration in the developing tissues of the chick and significantly decreased their susceptibility to lipid peroxidation.

Chan and Decker [12] found that chickens cannot synthesize vitamin E; therefore, vitamin E requirements must be met from dietary sources. It has been known that heat stress has negative effect on laying hens feed intake by reducing in laying hens exposed to heat stress [13]. Putpongsiriporn et al. [14] and Lin et al. [15] reported that feed intake, egg production, egg weight and shell quality were decreased in heat-stressed birds. Therefore, the purpose of this experiment was to determine the

effects of four dietary doses of vitamin E in the yolk and plasma, on lipid stability of fresh and stored eggs and on performance in laying hens exposed to heat stress.

MATERIAL AND METHODS

Trial was designed to determine the effects of dietary vitamin E levels on the concentration of vitamin E of egg yolk and plasma, TBARS (thiobarbituric acid reactive substance) of egg yolk during refrigerated storage of 1 or 42 days and productive performance of laying hens exposed to heat stress (30 oC). The experimental design consisted of a 2 x 4 factorial arrangement using two heat level and four dietary treatments. Twenty four week old, 128 Lohman LSL hybrids layers were used this experiment. Birds were randomly assigned to 8 groups at equally (n=16), each of which included 4 cages (50 x 46 x 46 cm) with four animals. Half of the groups (4 groups) were kept at normal poultry-house conditions (20 oC) and the other half exposed to heat stress (30 oC and 60% RH). In both poultry-houses, the first groups were fed on basal diets (contained 6 IU vitamin E/kg, 0E) (Table 1), and 2, 3, 4 th, groups were fed on the vitamin added rations at 45 (45 E), 65 (65 E), 85 (85 E) IU/kg vitamin E (Rovimix® E- 50 SD) respectively.

During this study, hens were fed and watered ad libitum. Egg production, feed conversion ratio and feed consumptions were recorded daily from each cage. Egg weight was recorded biweekly. Birds were weighted at the beginning and end of the study in order to calculate live weight changes. At the end of this research, blood samples (3 ml) were taken from the branchial vein of two hens from each replicate cage. These samples were then, transferred to heparinized tubes and placed on ice.

Plasma samples were prepared by centrifugation of blood at 1500 g for 5 min, frozen immediately and stored at -20°C until analyzed. Also, yolks were sampled from two hens of each cage (totally eight yolks from each group) in order to determine TBARS (thiobarbituric acid reactive substance) and vitamin E contents. All samples were stored 4°C until the analyses. The α -tocopherol content in plasma and yolk was determined by HPLC [16, 17- 18] and the TBARS were measured using the method described by Cherian et al. [10].

Differences between groups were analysed by one-way and two way analysis of variance (ANOVA), using the statistical package SPSS for Windows [19], version 10.0. Statistical differences between means were tested using Duncan's multiple range tests [20].

Table 1. Composition of the diets used in the experiments.

Ingredient	(%)	Chemical Composition	(%)
Maize	35	Dry matter	90
Wheat	17.5	Crude Protein	16
Wheat bran	12.5	Crude fiber	6
Soya bean meal	17	Crude ash	12
Sunflower meal	5	Ash dissolved in HCL	1
Meat bone meal	2.5	Lysine	0.7
Marble meal	7.5	Methionin	0.33
Limestone	2.4	Calcium	3.4
NaCl	0.3	Phosphorus	0.7
Vitamin Premix ¹	0.2		
Mineral Premix ²	0.1		

¹ Supplied per kilogram of diet: 6400 IU Vit. A, 1600 IU Vit. D, 6 IU Vit. E, 3000 mg K₂, 1500 mg Bl. 4000 mg B₂, 0.4 mg Nicotinamide, 4.8 mg Cal-D, 2 mg B6, 160 mg choline, 0.8 mg D-Biotin, 0.28 mg Ca

² Supplied per kilogram of diet: 80 mg Mn, 90 mg Zn, 50 mg Fe, 12 mg Cu, 1.5 mg Se, 2.5 g sodium chloride.

RESULTS

The effects of dietary treatment were significant ($P < 0.01$) on feed intake in birds in normal poultry house and the highest feed intake was determined in birds fed 45 IU vitamin E/kg. However, feed intake of the groups was not significantly ($P > 0.05$) different in birds exposed to heat stress. The changes in feed intake was significant ($P < 0.01$) between the heat and normal condition groups. Feed intake reduced significantly in heat stress group compared to normal condition group (Table 2).

In the present study, the dietary treatment had no effects on feed conversion ratio in both poultry houses. It was found that feed conversion rate did not change significantly ($P > 0.05$) in both groups, heat stressed and normal conditions (Table 2). The egg weight of groups did not change in heat stressed and not heat stressed hens. Egg production in laying hens in both poultry conditions (heat stress and normal) increased significantly ($P < 0.05$) with the supplementation of dietary vitamin E.

At overall effects evaluation, exposure of hens to high temperature resulted in a significant ($P < 0.01$) decrease in egg production and egg weight (Table 2).

The results of this study showed that supplementing diets of laying hens with a relatively high concentration of vitamin E (85 IU/kg) can reduce the detrimental effect of heat stress upon egg production. The effects of dietary treatment were not significant on weight changes in birds in both poultry houses. It was determined that in the animals kept under heat stress weight change was significantly different ($P < 0.05$) compared to those in normal condition. The interactions between heat stress and vitamin E were significant in terms of feed intake and egg production.

The effects of vitamin E on the thiobarbituric acid reactive substance (TBARS) values of egg yolk during the storage period

Table 2. Influence of Heat Stress and Dietary Vitamins E on Performance of Laying Hens

	Vitamin E (IU kg ⁻¹)	Feed Intake (g)	Feed conversion rate (kg:kg ⁻¹)	Egg production (%)	Weight change ¹ (%)	Egg weight (g)
Heat stress	0 E	99.40	1.99	68.00 b ³	-13.20	54.90
	45 E	100.30	2.05	67.80 b	-16.80	58.00
	65 E	99.50	2.02	68.70 b	-17.00	56.20
	85 E	99.50	1.97	78.40 a	-15.20	57.30
	SED ⁴	11.5	0.70	10.10	1.09	3.3
	P	NS	NS	**	NS	NS
Normal condition	0 E	114.60 ^c	2.00	82.00 ^d	3.20	62.20
	45 E	119.309 ^a	2.30	83.70 ^c	6.00	63.60
	65 E	116.304 ^b	1.94	88.10 ^a	6.40	63.30
	85 E	116.50 ^b	1.88	85.40 ^b	5.75	66.40
	SEM	12.00	0.50	9.80	1.23	3.70
	P	**	NS	**	NS	NS
OVERALL EFFECTS						
Heat	Heat stress	99.80	2.00	70.72	-15.55	56.60
	Normal	116.68	2.03	84.80	5.33	68.87
	P	**	NS	**	**	**
Vitamin E (IU kg ⁻¹)	0	107.00	1.99 ^b	75.00 ^c	-10.00	58.55 ^c
	45	105.05	2.18 ^a	75.75 ^c	-10.80	60.80 ^{ab}
	65	107.90	2.02 ^b	78.40 ^b	-10.60	59.75 ^b
	85	108.00	1.93 ^c	81.90 ^a	-9.45	61.85 ^a
	P	NS	**	**	NS	**
Heat x Vitamin E		*	NS	*	NS	NS

NS not significant *: $P < 0.05$ **: $P < 0.01$

^{a,b,c} Column means with no common superscript differ significantly

¹ Weight change during the 10- week experimental period

(1 and 42 days) were presented in Table 3. The dietary treatment and storage time affected TBARS values in both heat stressed and normal condition hens. Inclusion of vitamin E resulted in a significant ($P<0.01$) reduction in the TBARS values in eggs at 1 and 42 storage in heat stressed hens and normal hens (Table 3). Pooled data show that TBARS values of eggs of the heat-distressed hens were higher than the normal hens. Level of vitamin E significantly reduced TBARS values in yolk. It was found that supplementation of vitamin E causes to an increase in the deposition of α -tocopherol and thus inhibits the chain reaction of peroxidation in yolk of hens exposed to heat stress. The interaction between heat stress x day and heat stress x days x vitamin E was important with respect to TBARS.

Plasma α -tocopherol levels were increased by increasing the level of dietary vitamin E in heat stressed and normal hens. The greatest accumulation of α -tocopherol was observed in the group with 85 E (Table 4). It was observed that the plasma vitamin E levels of heat stressed groups were significantly lower than the normal condition group at overall effects evaluation. The interaction between heat stress x vitamin E was important in terms of plasma α -tocopherol levels (Table 4).

Table 5 shows the α -tocopherol contents of fresh and stored eggs. The dietary treatment and storage time had effects on α -tocopherol of egg yolk in both heat stressed and normal condition hens. Supplementation of laying hen's diet with vitamin E was a novel way of increasing the intrinsic concentration of vitamin E in eggs. The greatest concentration of vitamin E in yolk was obtained by increasing vitamin E. The content of yolk vitamin E was significantly ($P<0.01$) decreased during the refrigerated storage (from 1 to 42 days) in all dietary treatments in heat stressed and normal condition hens. Pooled data show that at the egg yolk, α -tocopherol contents of the group exposed to heat stress were lower than those of the normal group. At each sampling time, the yolk concentration of α -tocopherol increased depended on the dose in hen diets. The interactions between vitamin E x days were important with respect to egg α -tocopherol levels.

DISCUSSION

Feed intake did not change by supplemental vitamin E level in the present study. Controversial results were reported by Puthongsiriporn et al. [14] in accordance with our study.

Table 3. Influence of Dietary Vitamins E and Days of Refrigerated Storage on TBARS Values of the Egg Yolk (mg malondialdehyde per kg).

Storage days	Normal hens		Heat distressed hens	
	1 days	42 days	1 days	42 days
0 E	0.04 ^c	0.06 ^a	0.04 ^c	0.07 ^a
45 E	0.04 ^c	0.05 ^b	0.04 ^c	0.07 ^a
65 E	0.03 ^d	0.05 ^b	0.03 ^d	0.05 ^b
85 E	0.03 ^d	0.04 ^c	0.03 ^d	0.05 ^b
SEM	0.001		0.001	
P	**		**	
<i>OVERALL EFFECTS</i>				
Heat	Heat distressed hens		0.05	
	Normal hens		0.04	
Days of storage	P		*	
	1		0.04	
Vitamin E level (IU kg ⁻¹)	42		0.06	
	P		**	
Heat x Days	0		0.06 ^a	
	45		0.05 ^b	
Heat x Days x Vit E	65		0.04 ^c	
	85		0.04 ^d	
	P		**	
	*			
	*			

* $P<0.05$ ** $P<0.01$ NS: Not significant ^{a-f}: Means with no common superscript differ significantly

Table 4. Influence of Dietary Vitamins E on α -tocopherol Content ($\mu\text{g/g}$) of the Plasma of Hens.

	Heat-distressed hens	Normal hens
0E	2.39 ^d	2.58 ^c
45 E	3.57 ^c	5.53 ^b
65 E	4.29 ^b	7.40 ^a
85E	5.56 ^a	7.49 ^a
SEM	0.048	0.068
P	**	**
<i>OVERALL EFFECTS</i>		
Heat	Heat distressed hens	3.95
	Normal hens	5.75
Vitamin E level (IU kg ⁻¹)	P	**
	0	2.48 ^d
Heat x Vitamin E	45	4.55 ^c
	65	5.84 ^b
	85	6.52 ^a
	P	**
	*	

NS: not significant ** $P<0.01$ ^{a,b,c}: Column means with no common superscript differ significantly

Table 5. Influence of Dietary Vitamins E and Days of Refrigeration on α -tocopherol content ($\mu\text{g/g}$) of the Egg Yolk.

Storage days	Normal hens		Heat distressed hens	
	1 day	42 day	1 day	42 d
0 E	18.30 ^{de}	14.10 ^e	13.70 ^{fg}	9.80 ^g
45 E	45.40 ^c	36.00 ^{cd}	34.60 ^d	29.30 ^{de}
65 E	72.90 ^b	67.00 ^{bc}	77.00 ^b	67.30 ^c
85 E	100.60 ^a	92.60 ^{ab}	95.30 ^a	86.60 ^{ab}
SEM	0.257		0.210	
P	**		**	

OVERALL EFFECTS

Heat	Heat distressed hens	51.70
	Normal hens	55.86
	P	**
Days of refrigerated storage	1 d	57.22
	42 d	50.33
	P	**
Vitamin E level (IU kg ⁻¹)	0	13.97 d
	45	36.32 c
	65	71.05 b
	85	93.77 a
	P	**
<i>Vitamin E x Days</i>	*	

NS: not significant **:P<0.01 a,b,c : Column means with no common superscript differ significantly

However, supplementation of dietary vitamin E (500 mg/kg) caused a significant increase ($P<0.01$) in feed intake of hens [2] and in Japanese quail [21] exposed to heat stress. The dietary treatment had no effects on feed conversion ratio in not heat stressed and control hens in the present study. Similarly, Jerry et al. [22] reported that supplementation of vitamin E did not affect feed conversion in male turkey. Feed conversion ratio did not change significantly ($P>0.05$) in either of the groups, heat stressed and not heat stressed, at overall effects evaluation. The results of these trials were in line with the data suggesting the supplementation of vitamin E in laying diets for reducing the negative effects of heat stress reported by Bollenger-Lee et al. [2] and Şahin et al. [21].

Vitamin E supplementation increased egg production in heat stressed hens in this experiment. These results agreed with the findings of Puthongsiriporn et al. [14] and Bollenger-Lee et al. [2-3] who showed that supplementation of vitamin E significantly increased egg production in laying hens exposed to heat stress. Similarly, Şahin et al. [21] reported that dietary supplementation of vitamin E increased egg production in Japan quail exposed to heat stress.

At the present study, TBARS values of eggs of the heat-distressed hens were higher than not-heat-distressed hens. Level of vitamin E significantly reduced TBARS values in yolk. The present data, were in accordance with the findings of Cherian et al. [10], Puthongsiriporn et al. [14] and Franchini et al. [23]. It was found that supplementation of vitamin E leads to an increase deposition of α -tocopherol and thus inhibits the chain reaction of peroxidation in yolk of hens exposed to heat stress. Puthongsiriporn et al. [14] reported that this result is due to the antioxidant property of vitamin E.

Plasma α -tocopherol levels of heat stressed and not heat stressed hens increased linearly with the increment of vitamin E level in this study. These results were similar to those reported by Franchini et al. [23]. Puthongsiriporn et

al. [14] reported that supplementation of vitamin E increased level of α -tocopherol in plasma of hens. The proportions of α -tocopherol in egg yolk were increased by dietary vitamin E level. Same situations were reported by earlier findings of Cherian et al. [10], Puthongsiriporn et al. [14] and Franchini et al. [23]. The content of yolk vitamin E significantly ($P<0.01$) decreased during the refrigerated storage (from 1 to 42 days) in all dietary treatments in heat stressed and normal hens. These results were in accordance with the findings of Franchini et al. [23]. However, Cherian et al. [10] reported that there was no effect of 42-d of storage on α -tocopherol content of eggs.

In conclusion, vitamin E supplementation at different levels (45, 65 and 85 IU/kg of diet) had no significant effects on feed intake under heat stress, however, it improved egg production in laying hens in the present study. It was observed that a higher concentration of vitamin E ameliorated lipid oxidation in eggs.

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