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# EFFECT OF DIETARY VITAMIN C AND E SUPPLEMENTATION ON PERFORMANCE OF LAYING HENS UNDER HIGH ENVIRONMENTAL TEMPERATURE

**By**

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**Abstract:** *An experiment was conducted to study the effects of vitamin C (L-ascorbic acid) and vitamin E ( α-tocopherol acetate ) supplementation to diets on feed intake, egg production, egg quality and plasma constituents of Lohman LSL laying hens exposed to the natural high environmental temperature during the summer in Egypt. The expermintal period was from the 20th of June to the 20th of August.*

*A total of 160, 25 weeks old Lohman LSL hens, were randomly divided into four groups of fourty birds. First group was fed a basal diet (control group). The other groups were fed the basal diet supplemented with 200 mg vitamin C/kg diet (group 2), 150 mg vitamin E/kg diet (group 3) and 200 mg of vitamin C plus 150 mg of vitamin E (group 4). The results indicated that supplementation of the diet with vitamins C and E (group 4) resulted in positive significant effects on final body weight and feed efficiency compared to the control group. Feed intake did not significantly differ among different groups. Egg weight significantly increased with vitamins supplementation. Whereas, egg production has significantly increased and mortality has lightly reduced for the birds received vitamins supplementation compared to the control, particularly for group 4. Yolk percentage was also significantly increased in vitamin treated birds, particularly for birds received vitamins C and E. Also, vitamins supplementation significantly increased shell thickness than the control group. Whereas, albumin and shell percentages, yolk index and hugh unit were not affected. Plasma albumin was significantly decreased and plasma total protein was significantly increased in groups 2 and 4. Plasma cholesterol concentration significantly decreased, whereas plasma calcium and phosphorus concentrations significantly increased with the treatments of feed with vitamins compared to the control group.*

*These results showed that supplementation of vitamin C at 200 mg/kg diet and vitamin E at 150 mg/kg diet, significantly increased egg production, egg weight, yolk percentage, shell thickness, plasma calcium and plasma phosphorus and decreased the feed efficiency, compared to the control group in laying hens during the exposure to natural high environmental temperature. Moreover, vitamins supplementation lightly reduced mortality rate. This parameters were maximal in vitamin C+E group than others.*

## INTRODUCTION

High environmental temperature in summer season in Egypt causes negative effects on laying hens. It depresses body weight, egg production, egg weight, egg quality, and increases mortality (Balnave and Muheereza, 1997 and Genedi, 2000). It also suppresses feed intake, which could cause the decline in egg production and egg weight. The ideal temperature for laying hens is a bout 20°C (North and Bell, 1990). Heat stress begins when the ambient temperature becomes higher than 27°C and is readily apparent above 30°C (Bollengier-lee *et al.*, 1999). The researchers have tried to minimize the effect of heat stress by changing the environmental condition and diets of laying hens. Vitamins and electrolytes were supplemented in layer diets to allviate the negative effects of heat stress (Puthpongsiriporn *et al.*, 2001). Supplementation of vitamin C and vitamin E in the layer diets is suggested because of their anti-stress effects (Gonjalez *et al.*, 1995). Heat stress also depress cellular-mediated immunity. This suppression may be due to the insufficient of vitamins in feed (Deyhim *et al.*, 1994). Vitamin E serves as a physiological anti-oxidant through the inactivation of free radicals (Bollengier-lee *et al.*, 1999). Vitamin E is an anti-oxidant and it may help in increaseing the immune response in chickens.

Vitamin E act as a physiological synergist and as a functioning portion of specific enzymes (Franchini *et al.*, 1991), and increases yolk precursor concentrations during exposure to heat stress which improve yolk and egg production (Utomo *et al.*, 1994). Heat stress impairs the synthesis and release of vitellogenine and that the supplementation of diet with vitamin E facilitates the release of vitellogenine that necessary for yolk formation (Bollengier-lee, *et al.*, 1999). Also, under hot conditions, birds are not able to synthesize sufficient amounts of ascorbic acid and supplemental ascorbic acid could significantly reduce the body temperature (Kutlu and Forbes 1993). Ascorbic acid is an indispensable micronutrient required to maintain the physiological processes of poultry (McDowell, 1989). Also, mortality rate observed during heat stress was low when ascorbic acid was supplied (Gonjalez *et al.*, 1995).

The present study was conducted to determine the effects of supplementation of feed with vitamin C and vitamin E on feed intake, feed efficiency, egg production, egg quality and plasma constituents in laying hens under the high temperature of the summer in Egypt.

## MATERIAL AND METHODS

**Birds and Mangement:**

One handred and sixty, 25 week old, Lohman LSL laying hens were randomly and equally divided into four groups. Hens were housed individualy in single layer cages in an open system house. The experiment was carried out in summer season of Egypt from the 20$^{th}$ of June to the 20$^{th}$ of August. Treatment groups were fed a commercial layer ration (17.5% CP and 2750 Kcal ME/Kg diet, Table 1), (control group) with supplemented 200 mg of L-ascorbic acid (vitamin C) /Kg diet, 150 mg α-tocopherol acetate (vitamin E) /Kg diet or 200 mg of vitamin C plus 150 mg of vitamin E. Feed and water were provided *ad libitum* through the experimental period. Birds were exposed to 17 hrs of light daily. Ambient temperature and relative humidity were recorded daily, and the avreages are reborted every 3 days.

Figure 1, presentes the ambient temperature during the experimental period reached to as low as 29° C and as high as 37° C with an average temperature of 32.7° C. The relative humidity reached to as low as 38% and as high as 79% with average of 57.4%.

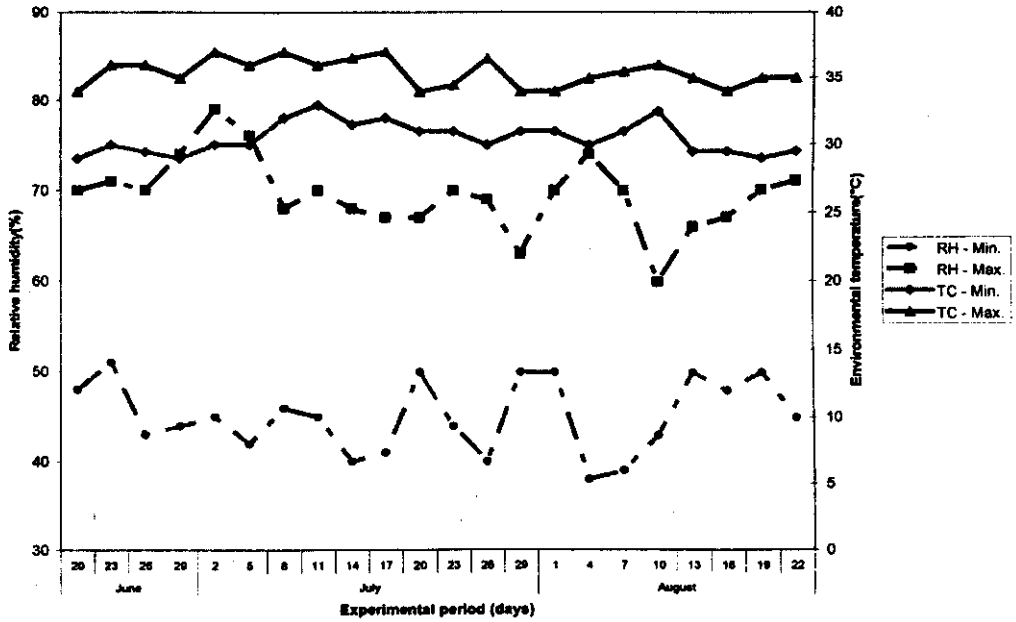


Figure (1): Durinal maximum and minimum environmental temperature (T°C) and relative humidity (RH) recorded during the experimental period.

**Measurements:**

Body weights at the begining and the end of the experiment were individualy recorded. Egg production, egg weight and mortality were also recorded daily. Feed consumption was recorded every week and feed effeciency was calculated. Egg quality measurements (egg, shell, albumin and yolk weights) were measured using 5 eggs from each treatment group at the last 2 days of each month. Albumin, yolk and shell percentages were also calculated. The shell thickness (without membrane) was determined using the micrometer in the wide tip and the middle region and then averaged. Yolk index percentage was calculated as yolk height divided by yolk diameter (Well, 1968). Haugh units were measured on five eggs per group.

Blood samples were collected, at the end of the experiment, from 5 hens per treatment (5 ml/ hen) from the brechial vein and transferred into heparanized test tube. Samples were centrifuged at 3200 rpm for 25 minutes and plasma was separated in clean dry vials and then stored at -20°C until they were analyzed. Plasma albumin (g/dl), total protin (g/dl), cholesterol

(mg/dl), calcium (mg/dl) and phosphorus (mg/dl) were determined calorimetrically using computerized spectrophotometer (Milton Roy Spectronic, 1201 ).

**Statical Analysis:**

Data were analyzed using the SAS general linear model procedure (SAS Institute, 1990). Mean values were compared using Duncan multiple rang test (Duncan, 1955) when significant differences existed. Significance was set at 5%.

## RESULTS AND DISCUSSION

**Body weight and Egg Production:**

The results of body weights and egg production for the different hen groups are presented in Table (2). Each of vitamin C or E speratly should no significant effect on body weight and feed efficiency. A combination of vitamin C and vitamin E increased body weight and feed efficiency significantly compared to the control group under high environmental temperature. Although, each of vitamin C and vitamin E significantly ameliorated egg production and lightly reduced mortality compared to the control group, the highest effect was observed for the group received a combination of vitamin C+E. In addition, the supplementation of feed with either vitamin C, E or both increased egg weight significantly compared to the control group under high environmental temperature. Heat stress induces oxidative damage through producing free radicals such as $O^{\bullet}$ and $OH^{\bullet}$ (Lin *et al.*, 2006) that caused pro-inflammatory cytokinez, IL-1, IL-6, and TNFα, (Abbas *et al.*, 2007). The pro-inflammatory cytokinez induce brain injury (Harnett *et al.*, 2007) that results in reducing feed efficiency and suppressing growth rate. Furthermore producing free radical increases the expression of vascular endothelial growth factor (VEGF) (Kanazawa, 2007). Pro- inflammatory cytokinez and VEGF causes heart failure and blood circulation disfunction leading to death (Vila *et al.*, 2007).

Vitamin C and vitamin E are effective antioxidants that protect cells from oxidative stress induced by free radicals (Bartov and Frigg, 1992). Through scavenging the free radical (Zuprizal *et al.*, 1993), transferring radical equivalents from lipid phases to a queous compartment (Halliwell and Gutteridge, 1989), or increasing the expression of antioxidant enzymes such as glutathione (Luadicina and Marnett, 1990). In general, the synergic effects between these two vitamins are particularly efficient to reduce the production of reactive oxygen species. These positive effects were evidenced by improving the growth performance, feed efficiency, egg

production and reducing mortality rate. The non-significant differences in feed intake indicate that the positive effects of vitamins C and E were mainly in feed utilization.

**Blood Parameters:**

While vitamin E had no significant effect on plasma albumin or plasma protein concentration, vitamin C speratly or in combination with vitamin E significantly reduced plasma albumin and increased plasma protein concentrations (Table 3). These results are in agreement with El-Gendi *et al.* (1999) and Sahin *et al.* (2002). However, the vitamin effects on plasma cholesterol level or plasma calcium and phosphorus were very abvious. Vitamin C, vitamin E, or their combination reduced the level of plasma cholesterol and increased the concentrations of plasma calcium and phosphorus significantly compared to the control group under high environmental temperature. Even though high environmental temperature depresses the concentration of plasma calcium (Kutlu and forbes, 1993), vitamin C improves calcium metabolism (Shahin *et al.*, 2002), resulting in increasing plasma calcium concentration. Moreover, The supplementation of diets containing polyunsaturated fatty acids with vitamin E results in reducing the cholesterol level and maximizing fat burning ( Zanini *et al.*, 2003 and Bourre and Galea, 2006).

**Egg quality:**

Vitamin C, vitamin E or their combination showed no significant effects on albumin (%), shell (%), yolk index (%), or Hugh unit (Table 4). These data are in agreement with Bollengier *et al.*, (1999), Kirunda *et al.*, (2001) and Metwally, (2005). Moreover, the percentage of egg yolk was significantly greater in vitamin treatments than in the control group, this result was observed by Ciftci *et al.*, (2005).

The current data showed that vitamin C, vitamin E, or their combination increased significantly the shell thickness compared to the control group under high environmental temperature (Table 4). This result could be due to the high plasma calcium concentration caused by vitamin supplementation (Table 3). This result is in agreement with Cheng *et al.*, (1990), who observed that shell weight per unit surface area was increased, under heat stress condition, due to vitamin C supplementation.

In conclusion, dietary supplementation of vitamin C or vitamin E separately or combined showed high ability of alleviating the negative effect of heat stress and improved egg production and egg quality. Furthermore, vitamins supplementation lightly reduced mortality and suppressed plasma

cholesterol concentration under high environmental temperature. The potential beneficial effects of vitamin C or vitamin E could be due to their high ability of attenuating the oxidative damage induced by high temperature. environmental

**Table (1): Composition and calculated analysis of the basal diet.**

| Ingredients | % |
|---|---|
| Yellow corn | 63.2 |
| Soybean meal (44%) | 26.4 |
| Wheat bran | 1.5 |
| Layer premix* | 0.3 |
| Di calcium phosphate | 1.6 |
| Limestone | 6.6 |
| Sodium chloride | 0.3 |
| DL-Methionine | 0.1 |
| Total | 100 |
| Calculated analysis | |
| ME, Kcal/Kg | 2750 |
| Crude Protein % | 17.50 |
| Crude Fiber % | 2.81 |
| Ether extract % | 2.86 |
| Calcium % | 3.24 |
| Avilable phosphorus % | 0.53 |
| Lysin % | 0.76 |
| Methionine % | 0.42 |
| Methionine + Cystine % | 0.67 |

*Each 3kg of layer premix contained:
Vit. A 10,000,000 I.U., Vit. $D_3$ 2,250,000 I.U., Vit. E 10g; Vit. $K_2$ 1g; Vit. $B_1$ 1g; Vit. 4g; Vit. $B_6$ 1.5g; Vit. $B_{12}$ 10mg; Pantothenic acid 10g; Niacin 20g; Folic acid 1g; $B_2$ Biotein 50mg; Choline chloride 500g; Iron 30g; Manganese 40g; Zinc 45g; Cupper 3g; Cobalt 100mg; Iodin 300mg; Selenium 100mg and CaCO3 to 3000g.

**Table (2): Body weight and egg production in laying hens as affected by dietary vitamin C and E supplementation during summer heat stress.**

| Parameters | Control | Vit. C | Vit. E | Vit. C+E |
|---|---|---|---|---|
| Initial body weight (g) | 1569±22.4 | 1549±24.2 | 1561±28.4 | 1544±20.6 |
| Final body weight (g) | *1648±28.6$^{b}$ | 1669±26.8$^{ab}$ | 1674±25.7$^{ab}$ | 1698±24.8$^{a}$ |
| Feed intake (g/hen/day) | 102.1±3.57 | 103.1±2.27 | 103.2±2.53 | 104.1±2.89 |
| Feed efficiency (g feed/g egg) | 2.21±0.10$^{a}$ | 1.85±0.09$^{a}$ | 1.88±0.09$^{ab}$ | 1.71±0.05$^{b}$ |
| Egg production (%) | 86.26±0.90$^{d}$ | 90.13±0.84$^{b}$ | 88.12±0.73$^{c}$ | 93.29±0.71$^{a}$ |
| Egg weight (g) | 59.7±0.57$^{b}$ | 60.6±0.42$^{a}$ | 60.8±0.62$^{a}$ | 60.8±0.59$^{a}$ |
| Mortality rate | 3/40 | 2/40 | 2/40 | 1/40 |

*Overall means, within a row, followed by different superscripts differ significantly ( $p \leq 0.05$ ).

**Table (3): Plasma constituents in laying hens as affected by dietary vitamin C and E supplementation during summer heat stress.**

| Items | Control | Vit. C | Vit. E | Vit. C+E |
|---|---|---|---|---|
| Albumin (g/dl) | *2.86±0.05a | 2.36±0.07b | 2.81±0.04a | 2.35±0.06b |
| Total protein (g/dl) | 4.31±0.07b | 4.89±0.06a | 4.40±0.07b | 4.90±0.09a |
| Cholesterol (mg/dl) | 148.42±4.62a | 126.62±5.18b | 128.41±7.24b | 123.19±7.29b |
| Calcium (mg/dl) | 9.54±0.14b | 12.72±0.17a | 12.46±0.15a | 13.11±0.19a |
| Phosphorus (mg/dl) | 7.21±0.07b | 9.18±0.09a | 8.92±0.08a | 9.36±0.12a |

*Overall means, within a row, followed by different superscripts differ significantly ( $p \leq 0.05$ ).

**Table (4): Egg components and egg quality in laying hens as affected by dietary vitamin C and E supplementation during summer heat stress.**

| Parameters | Control | Vit. C | Vit. E | Vit. C+E |
|---|---|---|---|---|
| Albumin (%) | 61.44±5.16 | 61.40±4.73 | 60.98±3.24 | 60.15±5.26 |
| Yoik (%) | 23.71±0.54d | 25.02±0.51c | 26.09±0.52b | 27.54±0.59a |
| Shell (%) | 12.11±0.26 | 12.29±0.36 | 12.33±0.27 | 12.40±0.32 |
| Shell thickness (mm) | 0.32±0.01b | 0.36±0.02a | 0.36±0.02a | 0.37±0.03a |
| Yolk index (%) | 40.72±0.73 | 42.11±0.71 | 41.20±0.67 | 42.01±0.73 |
| Hugh unit | 88.12±2.12 | 89.15±2.51 | 88.41±2.33 | 87.85±2.24 |

*Overall means, within a row, followed by different superscripts differ significantly ( $p \leq 0.05$ ).

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## الملخص العربي

# تأثير إضافة فيتامين E، C في العليقة على أداء الدجاج البياض تحت ظروف الإجهاد الحراري

**عادل عبد المنعم دسوقي**

قسم الإنتاج الحيواني – كلية الزراعة – جامعة القاهرة

أجريت التجربة لدراسة تأثير إضافة فيتامين E,C في العليقة على كمية الغذاء المستهلك و إنتاج البيض و جودة البيض و مكونات البلازما في الدجاج البياض لوهمان LSL المعرض لدرجات حرارة بيئية مرتفعة في فصل الصيف في مصر من ٢٠ يونيو إلى ٢٠ أغسطس .

تم إختيار عدد ١٦٠ دجاجة لوهمان LSL عمر ٢٥ أسبوع و تقسيمهم إلى أربعة مجاميع بكل مجموعة ٤٠ طائر. المجموعة الأولى تغذت على عليقة أساسية (مجموعة كنترول) بينما المجاميع الأخرى تم تغذيتها على العليقة الأساسية مضاف لها ٢٠٠ ملليجرام فيتامين C / كجم عليقة (المجموعة الثانية)، ١٥٠ ملليجرام فيتامين E / كجم عليقة (المجموعة الثالثة)، ٢٠٠ ملليجرام فيتامين C + ١٥٠ ملليجرام فيتامين E (المجموعة الرابعة).

**أوضحت النتائج الآتي:**

١- إضافة فيتامين E+C له تأثير معنوي موجب على وزن الجسم النهائي و معامل التحول الغذائي عن الكنترول.

٢- كمية الغذاء المستهلك بين المعاملات لم تتأثر معنويا .

٣- زاد وزن البيض معنويا بإضافة الفيتامينات .

٤- إنتاج البيض زاد معنويا ونسبة النفوق قلت بدرجة طفيفة في معاملات الإضافة مقارنة بالكنترول خاصة في مجموعة فيتامين E+C .

٥- نسبة الصفار زادت معنويا في المجاميع المعاملة بالفيتامين خاصة مجموعة فيتامين E+C .

٦- مجاميع المعاملات زادت معنويا في سمك القشرة عن مجموعة الكنترول ، بينما نسبة الألبيومين و القشرة ومعامل الصفار ووحدات هاو لم تتأثر .

٧- ألبيومين البلازما نقص معنويا و البروتين الكلي في البلازما زاد معنويا في مجاميع فيتامين E+C وC عن باقي المجاميع.

٨- تركيز الكوليسترول في البلازما نقص معنويا بينما تركيزات الكالسيوم و الفوسفور في البلازما زادت معنويا في المجاميع المعاملة بالفيتامينات مقارنة بمجموعة الكنترول .

أوضحت النتائج أن إضافة فيتامين C بمعدل ٢٠٠ ملليجرام / كجم عليقة و فيتامين E بمعدل ١٥٠ ملليجرام / كجم عليقة رفع معنويا إنتاج البيض ووزن البيض ووزن الصفار و نسبة الصفار و سمك القشرة و نسبة الكالسيوم و الفوسفور في البلازما و قلل معنويا معامل التحول الغذائي، مع إنخفاض طفيف في نسبة النفوق مقارنة بمجموعة الكنترول في الدجاج البياض خلال التعرض للحرارة المرتفعة – هذه النتائج كانت أعلى في مجموعة فيتامين E+C عن باقي المجاميع.