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EFFECTS OF VITAMIN C AND/OR FOLIC ACID SUPPLEMENTATIONS IN ALLEVIATING THE NEGATIVE EFFECTS OF HEAT STRESS IN LOCAL LAYING HENS

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SUMMARY

This study was performed to evaluate the effect of vitamin C and/or folic acid on performance, some blood constituents, oxidative stress marker (malondiladehyde (MDA) and some physiological measurements: body temperature, respiration rate, blood pH and immune response to sheep red blood cells (SRBC's) of Al-Salam laying hens under hot environmental temperature of summer months in Egypt. A total of one hundred and twenty, 30-week old Al-Salam hens were randomly selected and distributed into four equal groups (thirty hens each, in three replicates, ten hens each). Birds of the 1st group served as control, while those of the 2nd group were fed on a diet supplemented with vitamin C (250 mg/kg diet). The 3rd group received a diet supplemented with folic acid (1 mg/kg diet); whereas, the 4th group was given a diet supplemented with vitamin C (250 mg/kg diet) plus folic acid (1 mg/kg diet). All groups were put under study for 16 weeks. Supplementing heat-stressed laying hens with vitamin C and folic acid improved performance compared to their controls. The effects were more pronounced in laying hens supplemented with both vitamins. Digestibility of dry matter, organic matter, crude protein and ether extract were highest in the vitamin C and/or folic acid groups and lowest in the control group ($P<0.05$). Retention of Ca, P, Zn and Cu were highest in the vitamin C + folic acid group and lowest in the control group ($P<0.05$). Furthermore, serum MDA, cholesterol and glucose concentrations decreased, whereas, serum total protein, albumin, globulin, calcium and phosphorus concentrations increased with dietary vitamin C and folic acid supplementation ($P<0.05$) compared to the control group. Body temperature, respiration rate and blood pH were lower in the vit. C + folic acid group and higher in the control group. The results show that heat exposure reduced antibody titer against sheep red blood cells (SRBC's). However, vitamin C and/or folic acid supplementations enhanced humoral antibody response against SRBC's. The results of the study indicate that vitamin C and folic acid supplementations attenuate the decline in performance and antioxidant status caused by heat stress. Such supplementation may offer protection against heat stress related depression in performance of local laying hens.

Keywords: Vitamin C, folic acid, immunity, chicks, performance, blood constituents

INTRODUCTION

The stress of high ambient temperature may negatively influence the performance of broiler chickens by reducing feed intake live weight gain and feed efficiency (Donkoh, 1989 and Siegel, 1995). Environmental stress causes oxidative stress and impairs antioxidant status *in vivo* (Halliwell and Gutteridge, 1989; Klasing, 1998 and Sahin *et al.,* 2001). Significantly lower plasma concentration of antioxidant vitamins and minerals, such as vitamin C, E and folic acid, zinc and chromium and increased oxidative damage have been observed in stressed poultry (Feenster, 1985 and Sahin *et al.,* 2002). Studies have shown that antioxidant nutrient supplementation, especially vitamin C, E and A, zinc and chromium, can be used to attenuate the negative effects of environmental stress (Kafri and Cherry, 1984; Njoku, 1986; McDowell, 1989 and Mowat, 1994). Several methods are available to alleviate the negative effects of high environmental temperature on performance of poultry. Because of the high cost and impracticality of cooling animal buildings, interest in dietary manipulations has increased. Although poultry can synthesize vitamin C, synthesis is inadequate under stressful conditions such as low or high environmental temperature, high humidity, high egg production rate and parasite infestation (McDowell, 1989; Mowat, 1994 and Sykes, 1978). Previous reports have revealed a beneficial effect of vitamin C supplementation on growth rate, egg production, egg shell strength and thickness in stressed laying hens and broilers (Sykes, 1978; Pardue and Thaxton, 1986 and Sahin and Kucuk, 2001). Folic acid supplementation may also be useful for poultry under high stress conditions. Folic acid and its derivatives are involved in many reactions in which single carbon units are incorporated into large moleculaes (Pond *et al.,* 1995). Folic deficiency decreases live weight gain and feed efficiency, and increase mortality, leg weakness and cervical paralysis in growing Japanese quail (McDowell, 1989). In addition, folic acid deficiency reduces serum α-tocopherol concentration (Huang *et al.,* 2001) and impairs homocysteine catabolism by decreasing cystathionine synthesis and inhibiting homocysteine remethylation (Miller *et al.,* 1994). Folic acid is required in the methylation of homocysteine to form methionine and in the biosynthesis of amino acids and deoxynucleotides needed for DNA replication and repair (Selhub *et al.,* 1996 and Tapiero *et al.,* 2001). Hyperhomocysteinemia, hypomethylation of DNA and Uracil misincorporation are functional indicators of folic acid status (Tapiero *et al.,* 2001). High homocysteine levels have also been associated with increased oxidative stress. Given that low concentrations of folic acid under stress conditions have been reported, higher dietary folic acid levels may be required for laying hens exposed to high ambient temperatures.

Vitamin C appears to have a role on the utilization and perhaps absorption of folic acid. Tissue levels and urinary excretion of vitamin C are affected in folic acid-deficient animals (McDowell, 1989). Combinations of antioxidant vitamins and minerals generally show greater antioxidant activity than that of each compound alone (McDowell, 1989 and Gallo, 1980). Thus, the objective of this stud was to investigate the effects of vitamin C and/or folic acid supplementation on performance, digestibility coefficients and antioxidant status in local laying hens reared under summer conditions.

MATERIALS AND METHODS

The present study was carried out at the Poultry Research Center, Faculty of Agriculture, Mansoura University.

Birds, diets and experimental design:

Thirty-week old 120 local laying hens (El-Salam strain) were divided equally into four groups. Each group was divided into three replicates each consists of 10 hens. The birds were fed either a basal diet containing 16.4% CP and 2700 Kcal/kg ME or the basal diet supplemented with either 250 mg of L-ascorbic acid/kg of diet, 1 mg of folic acid/kg of diet, or 250 mg of L-ascorbic acid plus 1 mg of folic acid/kg of diet. Vitamin C (Rovimix® STAY-C® 35; specifically produced for use as stabilized source of vitamin C in feed) was provided by a commercial company (Roche, Levent-Istanbul, Turkey) and folic acid was supplied from Pharaonia Pharm, Alexandria, Egypt. The experimental diets were supplied to meet the nutrient requirements of the Ministry of Agriculture Decree (1996). Ingredients and chemical composition of the basal diet are shown in Table 1. Small amounts of the basal diet were first mixed with the respective amounts of vitamin C and folic acid as a small batch, then with a larger amount of the basal diet until the total amount of the respective diets were homogeneously mixed.

Table 1. Composition and calculated nutrient composition of the basal diet

Ingredients	%
Yellow corn	66.0
Soybean meal (44%)	24.0
Sodium chloride	0.30
Limestone	7.59
Dicalcium phosphate	1.71
Premix*	0.30
DL-Methionine	0.10
Total	**100**
Calculated analysis	
Crude protein %	16.43
ME/Kcal/kg	2750
Crude fiber %	3.20
Ether extract %	2.70
Calcium %	3.33
Phosphorus (avail) %	0.45
Phosphorus (total) %	0.66
Methionine %	0.39
Lysine %	0.86

* Premix contain per 3 kg: Vit. A 10, 000, 000 IU, Vit. D_3 2,000,000 IU, Vit. E 10,000 mg,Vit. K3 1,000 mg, Vit. B_1 1, 000 mg, Vit. B_2 5000 mg, Vit. B_6 1500 mg, Vit. B_{12} 10 mg, Niacin 3000 mg, Pantothenic acid 1000 mg, MnO 60, 00 mg, ZnO 50, 000 mg, Fe_2 SO_4 30,000 mg, $CuSO_4$ 4000 mg, Calcium iodide 300 mg, Co 100 mg, Choline chloride 250 mg, $CaCO_3$ carrier till 3000 g.

The birds were kept in floor pens. Water and diets were offered *ad libitum* throughout the experiment. The birds had a light period for 17 h per day. During the experiment, the inside temperature and humidity were measured four times a day (6.00, 12.00, 18.00 and 24.00). Average relative humidity was 75% and the mean value of daily temperature in the hen house was ranged between 30-38^{o}C. The experiment was carried out between June and September (2006).

Performance variables:

Body weights were recorded at the beginning and at the end of the study to determine body weight changes. During the experimental period, egg number, and egg weight were recorded daily per hen. The average daily egg production and the daily feed consumption per replicate were calculated for fortnight intervals. The value of feed conversion ratio (feed consumption/egg mass) was calculated.

Digestion Trials:

At the end of the experimental period, 46 weeks of age, a total number of 12 hens, 3 from each treatment were randomly taken for carrying digestion trials to estimate the nutrients digestibility, and determine retention and excretion of dietary calcium , phosphorus, zinc and copper. Feed and water were offered *ad- libitum*, excreta was collected quantitatively every 24 hours, during a three days collection period. Proximate analysis of the feed and dried excreta were done following the methods of A.O.A.C. (1990). Fecal nitrogen was determined according to Jakobsen *et al.* (1960). Mineral retention = mineral in feed intake gm/hen/day -mineral in excreta output gm/hen/day.

Physiological measurement:

Body temperature was measured by using digital thermometer ($\pm$ 0.1^{o}C). After inserting the thermometer probe into the cloaca the temperature was allowed to stabilize 1 minutes before the reading was recorded. The respiration rate was counted by observing the abdominal movements for one minute. Body temperature and respiration rate were recorded two times weekly during the experiment .

Some blood constituents:

At the end of the study, blood samples were collected from nine birds (three per replicate) randomly chosen from each treatment group.

Three-ml blood sample was taken in a heparinized tube from the brachial vein of each chosen bird. Blood pH was determined by using digital electric pH meter (JENCO model No. 608 U.S.A.) immediately after blood samples collection. Blood samples were centrifuged at 3000 rp for 10 min. and serums were collected. Serum total protein, albumin, globulin cholesterol, glucose, calcium, phosphorus , alkaline phosphatase (ALP) and creatinene kinases were calorimetrically determined using commercial kits . Serum malonidialdehyde (MDA) as a lipid peroxidation indicator was assessed as thiobarbituric acid-reactive substance (TBARS) concentration in serum by the method of Placer *et al.* (1966). Serum was separated to measure

triodothyronine (T_3) hormone level, Radioimmunoassay (RIA) kits (diagnostic products Corporation Los Angeles, U.S.A.) were used for the assays.

Immunization and titration:

Sheep red blood cells (SRBC) were used as test antigens to quantitatively analyze specific antibody responses as a measure of humoral immunocompetence. Three birds from each treatment group were immunized i.v. via a wing vein with 0.5 ml of 10% SRBC suspension prepared in 0.9% sterile saline. At 3, 6 and 9 days post immunization, blood samples were collected to determine the primary antibody response Antibody levels were quantitated using a micro titration hemeagglutination technique (Van der Zijpp and Leenstra, 1980).

Statistical analysis:

Data were analyzed by analysis of variance using the general linear model Procedure (Proc GLM; SAS Institute, 1996). Duncan's multiple range test was used to test the significance ($P < 0.05$) of mean differences (Duncan, 1955).

RESULTS

Laying performance:

The data presented in Table 2 revealed better values for all experimental groups when compared to the control group. Average of initial body weight was similar between groups ($P < 0.05$). However, folic acid or vitamin C alone or their combination compared with the control group resulted in higher body weight change % ($P < 0.05$), egg number, egg weight, egg mass, egg production, feed intake, and improved feed efficiency ($P < 0.05$). The highest values of performance were obtained when vitamin C was supplemented together with folic acid.

Table 2. Effects of supplemental vitamin C and/or folic acid on performance of laying hens reared during summer season

Item	Treatments					
	Control	Vit. C	Folic acid	Vit. C + FA	SEM	P-value
Initial body weight (g)	1503.33	1500.00	1501.67	1503.33	3.67	0.991
Final body weight (g)	1683.33	1700.00	1716.67	1708.33	5.82	0.219
Body weight change %	10.69 [b]	11.76 [a]	12.52 [a]	12.00 [a]	0.24	0.015
Egg number/hen/16 wks	62.67 [c]	64.80 [b]	66.07 [b]	70.53 [a]	0.88	0.0001
Average egg weight (g)	48.93 d	49.34 [c]	49.53 [b]	49.77 [a]	0.09	0.0001
Egg mass kg/h/16 wk	3.01 d	3.19 [c]	3.27 [b]	3.51 [a]	0.49	0.0001
Egg production (%)	52.22 [c]	54.00 [b]	55.06 [b]	58.78 [a]	0.74	0.0001
Feed intake/h/d	87.73 [b]	93.05 [a]	93.84 [a]	93.84 [a]	0.90	0.011
Feed conversion	3.43 [a]	3.50 [a]	3.44 [a]	3.21 [b]	0.04	0.007

[a, b, c] Means within each row with different superscripts are significantly different ($P < 0.05$).

Digestibility coefficients of nutrients:

Digestibility of dry matter (DM), organic matter (OM), crude protein (CP), ether extract (EE) were higher with vitamin C or/and folic acid supplementation ($P < 0.05$) (Table 3); however, no effect on crude fiber was detected between groups ($P < 0.05$).

Supplementing the diet with vitamin C and folic acid increased the retention of minerals (Ca, P, Zn and Cu) which was highest in the vit. C + FA group and lowest in control group ($P < 0.05$). On the other hand, the excretion of minerals was lower in the treated groups than in the control group ($P < 0.05$; Table 3).

Table 3. Effects of supplemental vitamin C and/or folic acid on nutrient digestibility and mineral retention and excretion of laying hens reared during summer season

Item	Treatments					
	Control	Vit. C	Folic acid	Vit. C + FA	SEM	P-value
Nutrient digestibility:						
Crud protein %	74.33 ^{b}	79.57 ^{a}	80.70 ^{a}	81.03 ^{a}	1.08	0.024
Crude fiber %	21.17	22.73	21.70	22.20	0.459	0.737
Ether extract %	73.33 ^{b}	76.17 ^{a}	77.33 ^{a}	77.10 ^{a}	0.561	0.008
NFE %	73.03	73.97	74.37	73.53	0.825	0.876
Retention:						
Calcium (g/h/d, DM)	2.1 ^{b}	2.7 ^{a}	2.6 ^{ab}	2.77 ^{a}	0.106	0.079
Phosphorus (g/h/d, DM)	0.20	0.26	0.24	0.29	0.014	0.125
Zinc (mg/h/d, DM)	1.00 ^{c}	1.87 ^{ab}	1.63 ^{bc}	2.50 ^{a}	0.182	0.004
Copper (mg/h/d, DM)	0.23 ^{b}	0.30 ^{ab}	0.28 ^{ab}	0.36 ^{b}	0.018	0.033
Excretion:						
Calcium (g/h/d, DM)	3.17 ^{a}	2.40 ^{b}	2.41 ^{b}	2.27 ^{b}	0.126	0.014
Phosphorus (g/h/d, DM)	0.69 ^{a}	0.48 ^{b}	0.52 ^{b}	0.35 ^{c}	0.039	0.001
Zinc (mg/h/d, DM)	10.67 ^{a}	8.00 ^{ab}	8.50 ^{ab}	6.67 ^{b}	0.556	0.047
Copper (mg/h/d, DM)	2.87 ^{a}	2.17 ^{a}	2.30 ^{ab}	1.83 ^{a}	0.167	0.023

$^{a, b, c}$ Means within each row with different superscripts are significantly different ($P < 0.05$).

Body temperature and respiration rate:

Supplemental vitamin C and folic acid decreased significantly body temperature and respiration rate ($P < 0.05$) in heated-stressed birds compared to the control group (Table 5).

Serum constituents:

Separately or in combination, supplemental vitamin C and folic acid increased serum concentrations of total protein, albumin globulin, calcium , phosphorus and T3 ($P<0.05$) but decreased cholesterol, glucose and MAD concentrations ($P<0.05$) (Table 4) compared to the control group, while, no effect on alkaline phosphatase and creatinene kinases were detected between groups ($P< 0.05$).

Blood pH:

In general, blood pH of treated groups (vitamin C and/or folic acid) was significantly decreased as compared to the control group (Table 5).

Antibody titer against SRBC's:

The influence of heat stress and vitamin C and folic acid supplementation on antibody titer against SRBC is shown in Table 5. Throughout the days post

immunization, control group produced the lowest antibody levels, whereas vitamin C or folic acid alone or their combination groups had higher SRBC antibody levels as compared to the control group.

Table 4. Effects of supplemental vitamin C and/or folic acid on some blood constituents of laying hens reared during summer season

Item	Treatments					
	Control	Vit. C	Folic acid	Vit. C + FA	SEM	P-value
Total protein (mg/dl)	3.03 [b]	4.10 [a]	4.27 [a]	4.17 [a]	0.156	0.0001
Globulin (mg/dl)	2.33	2.37	2.40	2.43	0.071	0.975
Albumin (mg/dl)	0.70 [b]	1.73 [a]	1.87 [a]	1.73 [a]	0.146	0.001
Glucose (mg/dl)	254.33 [a]	243.33 [ab]	241.33 [ab]	230.00 [b]	3.198	0.027
Cholesterol (mg/dl)	192.33 [a]	127.00 [b]	139.67 [b]	135.00 [b]	7.98	0.001
Calcium (mg/dl)	10.53 [b]	11.23 [a]	11.33 [a]	11.27 [a]	0.128	0.064
Phosphorus (mg/dl)	7.33	8.13	8.00	8.00	0.130	0.101
MDA (nmol/ml)	2.77 [a]	2.13 [b]	2.07 [b]	1.87 [b]	0.408	0.009
ALP(IU/L)	71.33	70.43	70.53	70.80	0.404	0.897
Creatinine (mg/dl)	0.953	0.983	1.00	1.06	0.242	0.519
T3 (mg/100ml)	0.792[c]	1.200[b]	1.33[a]	1.30[a]	0.174	0.001

[a, b] Means within each row with different superscripts are significantly different (P<0.05).

Table 5. Effects of supplemental vitamin C and/or folic acid on body temperature, respiration rate, blood pH and immune response of laying hens reared during summer season

Item	Treatments					
	Control	Vit. C	Folic acid	Vit. C + FA	SEM	P-value
Body temperature	42.30 [a]	41.30 [b]	40.97 [b]	41.13 [b]	0.175	0.004
Respiration rate	82.00	78.00	77.67	77.00	0.890	0.180
Blood pH	7.77 [a]	7.57 [b]	7.47 [b]	7.57 [b]	0.036	0.001
Antibody titer (Days post immunization)						
3 days	3.67 [b]	5.00 [a]	5.33 [a]	6.33 [a]	0.336	0.011
6 days	4.33 [b]	6.00 [a]	7.00 [a]	7.33 [a]	0.405	0.008
9 dats	3.33 [b]	4.67 [a]	4.67 [a]	5.33 [a]	0.261	0.017

[a, b,] Means within each row with different superscripts are significantly different (P<0.05).

DISCUSSION

Significant negative effects on egg weight, egg production, feed intake and feed conversion and some blood constituents as well as on nutrient digestibility and immune response to SRBC occurred in the experimental laying hens when exposed to the high ambient temperature (Tables 2,3,4, and 5) . In the present study, vitamin C and folic acid supplementation increased feed intake and improved the productive performance indicating that the two supplements alleviated the negative effects of the heat stress. Performance and feed intake decrease when ambient temperature rises above the thermoneutral zone (Siegel, 1995 and Ensminger *et al.,* 1990). The reduced feed intake in the present study (Table 2) during summer season may be caused by a

direct effect on various regions of the brain acting on feed intake control mechanism. Also, the blood flow and the motility of the intestine may be decreased, which may resulted in an increase of food passage time and delayed the thermogenic effect of food intake (Van-Handel-Hruska *et al.,* 1997). El-Tantawy *et al.* (1998) found that feed consumption was lower in high environmental temperature by about 36-43%. At such temperatures, corticosteroid secretion increased (Brown and Nestor (1973). Kutlu and Forbes (1993) reported that ascorbic acid reduces the synthesis of corticosteroid hormones in birds. By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress (McDowell, 1989). Stress increases folic acid and vitamin C requirements, indicating that both should be supplemented in birds living in stressful conditions. McDowell (1989) reported that the need for folic aid is greater for animals with greater growth or production rates because of its role in DNA synthesis. Folic acid plays an important role in amino acid and DNA metabolism (McDowell, 1989) and its deficiency causes severe defects in DNA replication repair (Selbub *et al.,* 1996 and Tapier *et al.,* 2001).

Folic acid is also required for the methylation of homocysteine to form methionine (McDowell, 1989). In the present study, folic acid supplementation improved the performance variables (Table 2). Similar to results of the present study, Wong *et al.* (1977) reported that folic acid supplementation at 0.30-0.36 mg/kg of diet increased body weight and feed efficiency in Japanese quail. Tollba *et al.* (2007) reported that folic acids supplementation to laying hens during the environmentally high temperature stress at summer months, improved egg production, egg mass, feed conversion and lowered mortality rate as compared to the respective control hens.

Environmental stress increases mineral excretion (Smith and Teeter, 1987). El-Husseiny and Creger (1981) reported significantly lower rates of retention of minerals such as Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn in broilers subjected to environmental stress. High environmental temperature significantly decreases the true digestibility of protein and amino acids in broilers (Wallis and Balnave, 1984 and Zuprizal *et al.,* 1993). The effects of ascorbic acid and folic acid on the retention of nitrogen and minerals likely are attributable to the protection of the pancreas from oxidative stress. Sahin and Kucuk (2001) reported that utilization of dry matter, crude protein and an ether extract in broiler quails kept at high ambient temperature is significantly decreased and that such negative effects were restored by vitamin C supplementation.

It is well known that heat stress increases MDA concentration as a lipid peroxidation indicator (Halliwell *et al.,* 1989 and Sahin *et al.,* 2002). Antioxidant systems (glutathione peroxidase, superoxide dismutase, and vitamins E, C and A) are important in scavenging free radicals and their metabolic products, as well as in maintaining normal cellular physiology restoring depletion of various antioxidants in stressed poultry (Halliwell *et al.,* 1989). The results also revealed that serum glucose and cholesterol decreased, whereas total protein, albumin, globulin, calcium and phosphorus concentrations increased when vitamin C and folic acid were dietary supplemented. Similar to our results, Sahin *et al.* (2002) reported that serum glucose, triglycerides, and cholesterol concentrations decreased, whereas protein and albumin concentrations increased when both dietary vitamin C and vitamin E were increased. Similarly, Kutlu and Forbes (1993) reported that vitamin C supplementation

increased plasma protein concentration whereas blood glucose and cholesterol concentration markedly decreased in heat-stressed (36°C) broilers. A likely mechanism by which vitamin C causes a reduction in corticosterone concentration is through inhibitory effect of vitamin C on glucocorticoid synthesis, and it has been postulated that the improved performance of poultry results from a decrease in protein-derived glucogenesis (McDowell, 1989). Dietary vitamin C may reverse these changes, presumably by reducing the secretion and/or synthesis of glucocorticoids.

Thyroid gland is involved in control of growth and development and exerts primary control of metabolic rate. Any treatments like heat stress that changes metabolic rate affect thyroid activity (May and McNaughton, 1980). The present study showed that T_3 level was reduced significantly during heat stress. This means that thyroid hormone is an important factor in response to heat stress. Exogenous thyroid hormone has a shorter survival time during heat stress (Fox, 1980; Bowen *et al.,* 1984). Also, thyroid size and thyroid activity was reduced by high temperature and increased by low temperature in chickens (Huston *et al.,* 1962). The present study explained that vitamin C or/and folic acid supplementation influenced thyroid activity. These results are in agreement with those of Abd El-Wahab *et al.* (1975) who showed that at high temperatures, thyroid activity was reduced. Supplemental vitamin C and folic acid has been shown to attenuate these negative responses in poultry to heat stress by increasing thyroid activity.

Chicken like all birds, is a homothermic, it keeps its body temperature at a relatively constant level by thermoregulation. The body temperature of chicken depending on bird size, environmental temperature, age and sex (Struckie, 1986). The rise in body temperature in response to high environmental temperature in the present study was also reported previously (El-Gendy and Washburn, 1995 and Osman, 1996). They considered that rectal temperature is a good indicator of both heat stress and acclimation. The elevation of blood pH which is concomitant with the increase in respiration rate in the present study, was previously interpreted by Balnvave and Gorman (1993). The current study indicated that vitamin C and folic acid supplementation stabilised body temperature, respiratory rate and blood pH.

Previous studies showed that *in vitro* heat stress suppress the activity of T- and B-lymphocytes and macrophages (Atta, 1996). Higher antibody titer against SRBC at 6 days post immunization in vitamin C and folic acid supplemented birds may explain the benefits of vitamin C and folic acid supplementation on humoral immune response, especially during heat stress. Dietary supplementation of vitamin C at 1000 ppm increased antibody response to SRBC that were suppressed by heat stress (Pardue *et al.,* 1985), also Tollba *et al.* (2007) reported that immune response such as hemagglutination-inhibition (HI) titer were ($P < 0.05$) increased by folic acid addition compared to control group during summer month's conditions in Egypt.

The enhancement of immune response via vitamin C or folic acid supplementation may be due to their antioxidant property. These vitamins protect immature lymphocytes from damage by free radical due to oxidation (Amaky-Anim *et al.,* 2000).

Vitamin C and folic acid positively affected all variables measured in the present study. In addition, for most variables, the magnitude of the effect was greater when both were supplemented as compared to either compound given alone. Although ascorbic acid does not appear to be needed for normal folate metabolism, lower

ascorbic acid concentrations occur in folate deficiency and utilization of folic acid is impaired in ascorbate deficiency, suggesting an interaction between the vitamins (McDowell, 1989 and Lewis *et al.,* 1982). In addition, antioxidant activity was reported to be more efficient when antioxidants are used in combination (Gallo-Torres, 1980).

In conclusion, the results of the present study suggest that both vitamin C and folic acid have alleviative effects and that the combination of the two supplements resulted in an enhanced effect against oxidative stress. Supplementing a combination of vitamin C and folic acid may offer a potential protective management practice in preventing heat stress depression in the productive performance of local laying hens.

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تأثير اضافة فيتامين ج وحمض الفوليك فى تقليل التأثيرات الضارة للاجهاد الحرارى فى الدجاج البياض المحلى

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أجريت هذه الدراسة لمعرفة تأثير فيتامين "ج" مع/أو حمض الفوليك على الأداء الإنتاجى وبعض مكونات الدم ومضادات الأكسدة (المالونالدهيد) وبعض القياسات الفسيولوجية (درجة حرارة الجسم . معدل التنفس . حموضة الدم) والاستجابة المناعية لكرات الدم الحمراء للأغنام. لسلالة السلام البياض تحت ظروف المناخ الحار خلال شهور الصيف فى مصر.

وقد استخدم ١٢٠ دجاجة بياضة سلام عمر ٣٠ أسبوع تم توزيعها عشوائيا إلى أربعة مجموعات متساوية كل منها ٣٠ دجاجة فى ٣ مكررات (كل منها ١٠ دجاجات) وتم تربيتهم تحت ظروف متماثلة. المجموعة الأولى عليقة المقارنة والثانية تم إضافة فيتامين "ج"(٢٥٠ملليجرام/كليو جرام علف) والثالثة تم إضافة حمض الفوليك (١ مليجرام/كيلو جرام علف) والرابعة تم إضافة فيتامين "ج" مع حمض الفوليك وذلك لمدة ١٦ أسبوع من يونيه إلى سبتمبر أثناء فصل الصيف الحار ٢٨-٣٨ درجة مئوية ودرجة الرطوبة نسبية ٥٧% وكانت النتائج كالآتى:

إضافة فيتامين "ج" وحمض الفوليك إلى الدجاج البياض المجهد حراريا أدى إلى تحسين الأداء الإنتاجى بالمقارنة بمجموعة المقارنة. عامة التأثيرات كانت أعلى فى الدجاج البياض التى يتغذى على كل من فيتامين "ج" وحمض الفوليك.

معاملات الهضم للمادة الجافة . المادة العضوية . البروتين الخام . المستخلص الإثيرى أعلى من المجاميع التى تتغذى على فيتامين "ج" مع/أو حمض الفوليك وكانت أقل فى مجموعة المقارنة. الاملاح المحتجزه (كالسيوم . فوسفور . زنك . نحاس) كانت اعلى فى مجموعة فيتامين ج مع حمض الفوليك وكانت اقل فى مجموعة الكنترول.

تركزات السيرم من المالونالدهيد . الكوليسترول والجلوكوز كانت قليلة فى حين أن البروتين الكلى . الألبيومين . الجلوبيلين . الكالسيوم والفوسفور زادت مع إضافة فيتامين "ج" وحمض الفوليك بالمقارنة مع مجموعة الكنترول بينما اتحاد فيتامين "ج" مع حمض الفوليك أعطى أعلى نتائج.

درجة حرارة الجسم . معدل التنفس وحموضة الدم كانت أقل فى مجاميع فيتامين "ج" أو مع حمض الفوليك وكانت أعلى فى مجموعة المقارنة.

أظهرت النتائج أن التعرض للإجهاد تقلل الأجسام المضادة لكرات الدم الحمراء للأغنام بينما إضافة فيتامين "ج" وحمض الفوليك زادت الاستجابة المناعية لكرات الدم الحمراء للأغنام.

دلت النتائج فى هذا البحث أن إضافة فيتامين "ج" أو حمض الفوليك منفردين أو متحدين تعدل من الانخفاض فى الأداء الإنتاجى ومضادات الأكسدة الناتج من الإجهاد الحرارى. مثل هذه الإضافات يمكن أن تعطى حماية ضد الإجهاد الحرارى المرتبط بالانخفاض فى الأداء الإنتاجى للدجاج البياض المحلى.