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EFFECTS OF DIETARY VITAMIN C AND FOLIC ACID SUPPLEMENTATION ON AMELIORATIONS THE DETRIMENTAL EFFECTS OF HEAT STRESS IN LOCAL LAYING HENS

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ABSTRACT

The study was performed to evaluate the effect of vitamin C and/or folic acid on performance, some blood constituents, oxidative stress marker (malondialdehyde (MDA), some physiological measurement (body temperature, respiration rate and blood (pH) and immune response to sheep red blood cells (SRBC's) of Al-Salam laying hens under hot environmental temperature of summer month's conditions in Egypt. A total number of one hundred and twenty, 30th weeks old Al-Salam hens were randomly selected and distributed into four equal groups, 30 birds each, in three replicates, 10 birds each. Birds in the 1st group were served as control, while those in the 2nd group were fed on a diet with vitamin C (250 mg/kg diet). The 3rd group was received a diet supplemented with folic acid (1 mg/kg diet) whereas, the 4th group was given a diet inclusive vitamin C plus folic acid. All groups were put under observation for 16 weeks. Supplementing heat-stressed laying hens with vitamin C and folic acid improved performance compared to the control group. 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 vit. C + folic acid group and lowest in the control group ($P < 0.05$). Furthermore, serum malondialdehyde (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 with the control group. However, the combination of vitamin C and folic aid provided greatest results. Body temperature, respiration rate and blood pH were lower in the vit. C + folic acid group and higher in the control group. The results showed that heat exposure reduced antibody titer against sheep red blood cells (SRBC's). However, vitamin C and/or folic acid supplementation enhanced humoral antibody response against SRBC's. The results of the study indicate that, separately or in combination, vitamin C and folic acid supplementation attenuates 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.

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-Torres, 1980). The objective of this stud was thus to investigate the effects of vitamin C and folic acid supplementation on performance, digestibility coefficient 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 used in the present study. 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. 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 experimental diet.

Ingredients	%
Yellow corn	66.0
Soybean meal (44%)	24.0
Sodium chloride	0.30
Limestone	7.59
Dicalcium phosphate	1.71
Vit.&Min.primex*	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
Methinine %	0.39
Lysine %	0.86

*** Each 3 kg of vit. & Min. Mixture contains: 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.**

****According to NRC (1994)**

The birds were randomly assigned into four experimental groups, 30 hens each, and fed a basal diet (control diet), or the basal diet supplemented with 250 mg vitamin C, 1 mg folic acid or 250 vitamin C plus 1 mg folic acid/kg diet, respectively their combination . Each treatment group was divided into three replicates each consists of 10 birds. The birds were kept in floor pens. Water and the diets were offered *ad libitum* throughout the experiment. The bird house was lit for 17 h light per day. During the experiment, hen house's temperature and humidity were measured four times a day (6.00, 12.00, 18.00 and 24.00). Average ambient relative humidity inside the hen house was 75%, the mean value of daily temperature in the hen house was ranged between 30-38°C. The experiment was carried out between June to 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) were calculated.

Digestibility coefficients:

At the end of the feeding trail, three birds per treatment were randomly assigned to determine the retention and excretion of dietary nutrients. Nutrient was the amount of nutrient retained per hens which was calculated based on the availability of nutrient and feed intake. Excreta of layers were totally collected for three days. Diets and excreta were analyzed according to chemical procedures of A.O.A.C. (1990) for proximate analysis. Urinary nitrogen was determined according to Jakobson *et al.* (1960). Urinary organic matter was evaluated according matter was evaluated according to Abou-Raya and Galal (1971).

Physiological measurements:

Body temperature was measured by using digital thermometer (± 0.1°C). After inserting the thermometer probe into the cloaca the temperature was allowed to stabilize 5 minutes before the reading was recorded.

The respiration rate was counted by observing the abdominal movements for one minute.

Three-ml of heparinized blood was taken from the brachial vein of nine birds from each treatment. Blood pH was determined by using digital electric pH meter (JENCO model No. 608 U.S.A.) immediately after blood samples collection.

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. Blood samples were centrifuged at 3000 rpm for 10 min. and sera were collected. Serum total protein, albumin, globulin, cholesterol, glucose alkaline phosphatase, creatinine, calcium and phosphorus were calorimetrically determined using commercial kits following the recommendations of manufacturers. Serum malonidialdehyde (MDA). Lipid peroxidation 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 statistically analyzed using the general Linear Model Procedure (SAS, 1996). Duncan's multiple range test was used to test the significance ($P < 0.05$) of mean differences (Duncan, 1955).

RESULTS

Laying performance:

As can be seen in Table 2 better results were obtained in 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 with folic acid.

Table (2): The effects of supplemental vitamin C and folic acid on performance of laying hens reared during summer season.

Item	Treatments					
	Control	Vit. C	Folic acid	Vic. + 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), but 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): The effects of supplemental vitamin C and folic acid on nutrient digestibility and mineral retention and excretion of laying hens reared during summer season.

Item	Treatments					
	Control	Vit. C	Folic acid	Vic. + 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 ^{a}	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 with control group (Table 5).

Blood pH:

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

Serum constituents:

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

Table (4): The effects of supplemental vitamin C and folic acid on some blood constituents of laying hens reared during summer season.

Item	Treatments					
	Control	Vit. C	Folic cid	Vic.+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)	0.700 ^{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
Alkaline phosphatase (u/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

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

Table (5): The effects of supplemental vitamin C and folic acid on body weight temperature, respiration rate, blood pH and immune response of laying hens reared during summer season.

Item	Treatments					
	Control	Vit. C	Folic acid	Vic. + FA	SEM	P-value
Body weight 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
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 days	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 ($P < 0.05$).

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 had higher SRBC antibody levels as compared to control group.

DISCUSSION

Significant negative effects on egg weight, egg production, feed intake and fed conversion and some blood constituents as well as on nutrient digestibility and immune response to SRBC occur in local laying hens exposed to the high ambient temperature. In the present study, vitamin C and folic acid supplementation with increased feed intake improved the productive performance indicating that the two supplements alleviate the negative effects of the heat stress. Performance and feed intake decreased 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 decreased, which may result in an increase of food passage time and delay in the thermogenic effect of food intake (Van-Handel-Hruska *et al.,* 1997). El-Tantawy *et al.* (1998) found that the feed consumption was lower in high environmental temperature by about 36-43%. At such temperatures, corticosteroid secretion increases (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 of 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 DAN replication repair (Selhub *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 increases 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 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 (malondialdehyde) 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). It has also been reported that serum glucose and cholesterol decreased, whereas total protein, albumin, globulin, calcium and phosphorus concentrations increased when dietary vitamin C and folic acid were 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 also 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). Increases in concentrations of glucose may be attributed to increased glucocorticoid secretion, which increases 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 agreement with Abd El-Wahab *et al.* (1975) who showed that a 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 homothermia, 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. Elevation of blood pH was concomitant with increase in respiration rate in the present study, was interpreted by Balnvave and Gorman (1993). The current study indicated that vitamin C and folic acid supplementation improve 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. It reasons to protect immature lymphocytes from damage by free radical due to oxidation (Amaky-Anim *et al.,* 2000).

Vitamin C and folic acid similarly affected all variables measured in the present study. In addition, for most variables, the magnitude of the effect was greater when both were supplemented 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 vitamin C and folic acid have similar 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-related depression in the productive performance of local laying hens.

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التغذية على اضافة فيتامين ج وحمض الفوليك لتقليل 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 الإنتاجي 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 من سلالة سلام عمر ٢٨ 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"(٢٥٠مللیجرام/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 الإنتاجي 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 الإنتاجي ومضادات الأكسدة الناتج من الإجهاد الحرارى. مثل هذه الإضافات يمكن أن تعطى حماية ضد الإجهاد الحرارى المرتبط بالانخفاض فى الأداء الإنتاجي للدجاج البياض المحلى.