Dietary Vitamin C and Folic Acid Supplementation Ameliorates the Detrimental Effects of Heat Stress in Japanese Quail

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ABSTRACT We evaluated the effects of vitamin C (L-ascorbic acid) and folic acid supplementation on performance, carcass characteristics and concentrations of the oxidative stress markers [malondialdehyde (MDA), homocysteine], adrenocorticotropic hormone (ACTH), vitamins C, E, A, B-12 and folic acid, and mineral status in broiler Japanese quail (Coturnix coturnix japonica) exposed to high ambient temperature (34°C, 8 h/d, 0900–1700 h). The birds (n = 150; 10-d-old) kept at 34°C were fed a basal diet (HS group) or the basal diet supplemented with 250 mg of L-ascorbic acid/kg of diet (Vit C group), 1 mg of folic acid/kg of diet (FA group) or both (Vit C + FA group), whereas birds kept at 22°C were fed the basal diet (TN group). Supplementing heat-stressed quail with vitamin C and folic acid improved performance compared to the HS group. Effects generally were greatest in quail supplemented with both. Although supplementation did not consistently restore concentrations to those of the TN group, it increased serum concentrations of the vitamins under study. Furthermore, serum and tissue MDA, homocysteine and ACTH concentrations were lower in the supplemented groups than in the heat-stressed controls. Retention of N, ash, Ca, P, Zn, Fe, Cu and Cr were highest in the Vit C + FA group and lowest in the HS group ($P < 0.05$). The results of the study indicate that 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 Japanese quail. J. Nutr. 133: 1882–1886, 2003.

KEY WORDS: • *folic acid* • *heat stress* • *homocysteine* • *quail* • *vitamin C*

The stress of high ambient temperature may negatively influence the performance of broiler chickens by reducing feed intake, live weight gain and feed efficiency (1,2). Environmental stress causes oxidative stress and impairs antioxidant status in vivo (3–5). Significantly lower plasma concentrations of antioxidant vitamins and minerals, such as vitamins C, E and folic acid, zinc and chromium, and increased oxidative damage have been observed in stressed poultry (6,7). Studies have shown that antioxidant nutrient supplementation, especially vitamins C, E and A, zinc and chromium, can be used to attenuate the negative effects of environmental stress (8–11). 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 (10,12). 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 (12–14). 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 molecules (15). Folic acid deficiency decreases live weight gain and feed efficiency, and increases mortality, leg weakness and cervical paralysis in growing Japanese quail (10). In addition, folic acid deficiency reduces serum α-tocopherol concentration (16) and impairs homocysteine catabolism by decreasing cystathionine synthesis and inhibiting homocysteine remethylation (17). 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 (18,19). Hyperhomocysteinemia, hypomethylation of DNA and uracil misincorporation are functional indicators of folic acid status (19). 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 quail 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 (10). Combinations of antioxidant vitamins and minerals generally show greater antioxidant activity than that of each compound alone (10,20). The objective of this study was thus to investigate the effects of vitamin C and folic acid

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supplementation on performance, carcass characteristics and antioxidant and mineral status in broiler Japanese quail reared at 34°C.

MATERIALS AND METHODS

Animals, diets, experimental design and data collection. One hundred and fifty, 10-d-old Japanese quails (Coturnix coturnix japonica; Bingol College Division of Firat University, Elazig, Turkey) were used in the study. The experiment was conducted in accordance with animal welfare at the Veterinary Control and Research Institute of Elazig, Turkey. The birds were assigned according to their initial body weights, to five treatment groups, three replicates of 10 birds each. The birds were kept in cages (10 birds per cage) in temperature-controlled rooms, and the nighttime temperature for all groups was the same (18–22°C). The birds kept at 34°C for 8 h (0900–1700 h) were fed either a basal diet (high temperature, basal diet; HS group) or the basal diet supplemented with 250 mg of L-ascorbic acid/kg of diet (Vit C group), 1 mg of folic acid/kg of diet (FA group) or both (Vit C + FA group), whereas hens kept at 22°C were fed the basal diet (thermoneutral, basal diet; TN group). Doses of supplements were determined based on our previous studies (14,21). Vitamin C (Rovimix Stay-C 35; specifically produced for use as a stabilized source of vitamin C in feed) and folic acid (Rovimix 80 SD) were provided by Roche (Levent, Istanbul, Turkey). The birds were fed a starter diet until 21 d of age followed by a finishing diet from d 21 to d 42. (See **Table 1** for ingredients and the chemical composition of the basal diet.) The basal diets were formulated using NRC guidelines (22) and contained 23 and 20 g/100 g protein for starter and grower, respectively, and 12.9 MJ/kg metabolizable energy. Quail consumed feed and fresh water ad libitum.

TABLE 1

Ingredients and chemical analyses of the starter and grower diets fed to Japanese quail

Ingredient	Starter	Grower
	g/100 g	
Soybean meal	37.42	29.10
Ground corn	45.83	43.70
Wheat	8.00	18.80
Animal–vegetable fat	4.64	4.50
Dicalcium phosphate	1.20	1.02
Sodium chloride	0.31	0.31
Limestone, ground	1.68	1.76
DL-Methionine	0.17	0.09
Vitamin–mineral premix[1,2]	0.75	0.75
ME,[3] *MJ/kg*	12.9	12.9
Chemical analysis		
Crude protein (CP), *g/100 g*	23.00	20.21
Calcium, *g/100 g*	1.0	0.95
Available phosphorus, *g/100 g*	0.46	0.40
Zinc, *mg/kg*	45	40
Copper, *mg/kg*	6.0	5.6
Iron, *mg/kg*	27	26
Chromium, *μg/kg*	1.5	1.3

[1] The vitamin premix provides the following (per kg): all-*trans*-retynyl acetate, 1.8 mg; cholecalciferol, 0.025 mg; all-*rac*-α–tocoperol acetate, 1.25 mg; menadione (menadione sodium bisulfate, 1.1 mg; riboflavin, 4.4 mg; thiamine (thiamine mononitrate), 1.1 mg; vitamin B-6, 2.2 mg; niacin, 35 mg; Ca-pantothenate, 10 mg; vitamin B-12, 0.02 mg; folic acid, 0.55 mg; *d*-biotin, 0.1 mg.

[2] The mineral premix provides the following (per kg): manganese (from manganese oxide), 40 mg; iron (from iron sulfate), 12.5 mg; zinc (from zinc oxide), 25 mg; copper (from copper sulfate), 3.5 mg; iodine (from potassium iodide), 0.3 mg; selenium (from sodium selenite), 0.15 mg; choline chloride, 175 mg.

[3] ME, metabolizable energy.

Feed intake and body weight were determined at weekly intervals. Weight gain and feed efficiency of birds were calculated. For carcass evaluations, at 42 d of age, nine birds (three per replicate) randomly chosen from each treatment group were slaughtered. The birds were not deprived of feed before slaughter, and carcasses were cleaned by removing feathers (wet), feet and visceral organs. Cold carcass weights were calculated after carcasses were kept at +4°C for 18 h. Cold carcass yield was calculated as the cold carcass weight divided by the body weight at slaughter.

At the end of the study, six birds (two per replicate) from each treatment were individually caged to determine retention and excretion of dietary nutrients at 34°C. Nutrient retention was the amount retained by each bird per day and was calculated on the basis of feed consumption and the calculated analysis of the nutrient in the feed. Excreta of hens were collected for 3 d.

Laboratory analyses. 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 × *g* for 10 min and serums were collected. Serum malondialdehyde (MDA), homocysteine, ACTH and vitamins C, E, A, B-12 and folic acid concentrations were determined. Lipid peroxidation was assessed as thiobarbituric acid-reactive substance (TBARS) concentrations in serum, liver, heart and kidney by the method of Placer et al. (23). Values are reported as the concentration of MDA. Serum homocysteine concentration was determined by ELISA (El_x-800) using a commercially available kit (Axis, Oslo, Norway). Serum folic acid, vitamin B-12 and ACTH concentrations were determined by Automated Chemiluminescence Systems (Diagnostic Products, Gwynedd, UK) using a commercially available kit (Immulite 2000, DPC, Los Angeles, CA). All measurements were performed in a single assay to avoid interassay variation. Serum vitamin A (24) and vitamin E (25) concentrations were measured by reverse-phase HPLC using a Shimadzu UV-vis SPD-10 AVP detector and a CTO-10 AS VP column (Shimadzu, Tokyo, Japan). Serum vitamin C concentrations were determined spectrophotometrically by the colorimetric method (phosphotungstic acid) (26).

Chemical analyses of the diet and excrement samples were performed using AOAC procedures (27). Excrement N was chemically analyzed according to the method of Terpstra and de Hart (28). To determine concentrations of Ca, Zn, Cu, Fe and Cr, diet and excreta were dry-ashed (27). Concentrations of minerals in the diet and excrement samples were measured using an atomic absorption spectrometer (Shimadzu AA-660). Calibrations for the mineral assays were conducted with a series of mixtures containing graded concentrations of standard solutions of each element.

Statistical analyses. Sample size was calculated based on a power of 85% and a *P* value of 0.05. We considered a 10% improvement as satisfactory. Given that assumption, a sample size of 10 per treatment was calculated. The data were analyzed using the GLM procedure of SAS software (29). Significant differences ($P < 0.05$) among the means were determined using Duncan's new multiple range test.

RESULTS

Supplemental vitamin C and folic acid increased live weight and feed intake, and improved feed efficiency ($P < 0.05$) in heat-stressed birds (**Table 2**). Performance and carcass qualities were also higher ($P < 0.05$) in treated groups compared with the HS group and generally were greatest in the Vit C + FA group (Table 2). Separately or in combination, supplemental vitamin C and folic acid increased serum concentrations of vitamins C, E, A, folic acid and vitamin B-12 ($P < 0.05$) but decreased MDA, homocysteine and ACTH concentrations ($P < 0.05$) (**Table 3**). Serum concentrations of vitamins C, E, A, B-12 and folic acid were greater ($P < 0.05$), and those of MDA, homocysteine and ACTH were lower ($P < 0.05$) in the TN group than in the HS group. Retention rates for N, ash, Ca, P, Zn, Cu, Fe and Cr were highest in the TN group. Supplementing the diet with vitamin C and folic acid increased the retention of minerals, which was highest in the Vit C + FA group and lowest in the HS group

TABLE 2

Effects of supplemental vitamin C and folic acid on performance of Japanese quail reared under conditions of heat stress (34°C) for 32 d[1,2]

Item	Treatment[3]					SEM
	TN	HS	Vit C	FA	Vit C + FA	
Live weight, *g*						
Initial	46.0	46.1	46.3	47.0	46.4	4.3
Final	175[a]	152[c]	158[c]	157[c]	168[b]	8.2
Total feed intake, *g*	509[a]	490[d]	498[c]	495[c]	500[b]	2.1
Feed efficiency, *g gain/g feed*	0.24[a]	0.21[c]	0.22[b]	0.22[b]	0.24[a]	0.01
Cold carcass wt, *g*	118[a]	101[e]	108[c]	104[d]	110[b]	2.6
Cold carcass yield, *g cold carcass wt/100 g live wt*	68.2[a]	65.5[c]	67.6[a,b]	66.4[b]	67.3[b]	1.3

[1] Live weight, total feed intake and feed efficiency values are means, $n = 3$. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] Cold carcass weight and cold carcass yield values are means, $n = 9$. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[3] TN, thermoneutral (22°C) control (basal) diet; HS, heat stress, control (basal) diet; Vit C, heat stress, control diet + 250 mg of ascorbic acid/kg of diet; FA, heat stress, control diet + 1 mg of folic acid/kg of diet; Vit C + FA, heat stress, control diet + 250 mg of ascorbic acid/kg of diet + 1 mg of folic acid/kg of diet.

($P < 0.05$). On the other hand, the excretion of minerals, which was lowest in the TN group, was lower in the treated groups than in the HS group ($P < 0.05$; **Table 4**).

DISCUSSION

Significant negative effects on live weight gain, feed intake, feed efficiency and carcass traits, as well as on antioxidant and mineral status occur in quail exposed to the high ambient temperature of 34°C. In the present study, vitamin C and folic acid supplementation with increased feed intake improved the growth rate, indicating that the two supplements alleviate the negative effects of the heat stress. Growth rate and feed intake decrease when the ambient temperature rises above the thermoneutral zone (2,30). The beneficial effects of dietary ascorbic acid supplementation under stressful conditions reported in the present study are in agreement with previous reports (8,9,31). Sahin and Kucuk (14) reported that supplemental vitamin C increases performance and yields better carcass traits in broilers reared under conditions of heat stress (32°C). At such temperatures, corticosteroid secretion increases (32). Kutlu and Forbes (31) reported that ascorbic acid reduces the synthesis of corticosteroid hormones in birds. Similarly Sahin et al. (33) reported low concentrations of ACTH in quail reared at 32°C and fed a diet supplemented with vitamin C.

By decreasing synthesis and secretion of corticosteroids, vitamin C alleviates the negative effects of stress (10). Stress increases folic acid and vitamin C requirements, indicating that both should be supplemented in birds living in stressful

TABLE 3

Effects of supplemental vitamin C and folic acid on serum metabolites of Japanese quail reared under conditions of heat stress (34°C) for 32 d[1]

Item[3]	Treatment[2]					SEM
	TN	HS	Vit C	FA	Vit C + FA	
MDA						
Serum, *μmol/L*	0.58[d]	2.02[a]	1.13[b]	1.55[b]	0.81[c]	0.4
Liver, *nmol/g*	1.92[d]	3.46[a]	2.50[b]	2.71[b]	2.25[c]	0.2
Heart, *nmol/g*	0.91[d]	1.78[a]	1.58[b]	1.55[b]	1.33[c]	0.08
Kidney, *nmol/g*	1.35[d]	2.46[a]	2.12[b]	2.18[b]	1.76[c]	0.2
Homocysteine, *μmol/L*	15.8[e]	23.8[a]	19.2[b]	18.8[c]	17.0[d]	0.8
ACTH, *pmol/L*	2.82[c]	3.03[a]	2.99[b]	2.98[b]	2.88[b,c]	0.4
Vitamin C, *μmol/L*	43.5[c]	33.8[e]	60.5[b]	38.2[d]	68.2[a]	3.5
Vitamin E, *μmol/L*	1.95[a]	1.18[d]	1.51[c]	1.42[c]	1.78[b]	0.3
Vitamin A, *μmol/L*	1.57[a]	1.08[c]	1.31[b]	1.30[b]	1.48[a,b]	0.4
Folic acid, *nmol/L*	198[a]	132[e]	159[d]	178[c]	186[b]	4.2
Vitamin B-12, *pmol/L*	303[a]	255[e]	273[d]	282[c]	288[b]	4.8

[1] Values are means, $n = 9$. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] TN, thermoneutral (22°C) control (basal) diet; HS, heat stress, control (basal) diet; Vit C, heat stress, control diet + 250 mg of ascorbic acid/kg of diet; FA, heat stress, control diet + 1 mg of folic acid/kg of diet; Vit C + FA, heat stress, control diet + 250 mg of ascorbic acid/kg of diet + 1 mg of folic acid/kg of diet.
[3] MDA, malondialdehyde; ACTH, adrenocorticotropic hormone.

TABLE 4

Effects of supplemental vitamin C and folic acid on nitrogen and mineral retention in Japanese quail reared under conditions of heat stress (34°C) for 32 d[1]

Item	Treatment[2]					SEM
	TN	HS	Vit C	FA	Vit C + FA	
Retention[3]						
Nitrogen, *g/d*	3.3[a]	2.0[d]	2.6[c]	2.5[c]	2.9[b]	0.1
Ash, *g/d*	14.6[a]	8.2[d]	11.1[c]	10.7[c]	12.0[b]	0.5
Calcium, *g/d*	3.8[a]	1.8[d]	2.6[c]	2.7[c]	3.0[b]	0.2
Phosphorus, *g/d*	0.30[a]	0.12[d]	0.20[c]	0.18[c]	0.25[b]	0.003
Zinc, *mg/d*	2.2[a]	1.08[d]	1.53[c]	1.43[c]	1.69[b]	0.1
Copper, *mg/d*	0.35[a]	0.21[d]	0.29[b,c]	0.26[c]	0.31[b]	0.02
Iron, *mg/d*	15.3[a]	7.6[d]	9.5[c]	9.0[c]	12.3[b]	1.2
Chromium, *μg/d*	85[a]	42[d]	68[b]	62[c]	69[b]	6
Excretion[3]						
Nitrogen, *g/d*	0.8[d]	1.8[a]	1.3[b]	1.4[b]	1.1[c]	0.03
Ash, *g/d*	7.2[d]	11.6[a]	8.8[b]	9.2[b]	8.1[c]	0.50
Calcium, *g/d*	1.8[d]	2.8[a]	2.4[b]	2.5[b]	2.1[c]	0.04
Phosphorus, *g/d*	0.28[d]	0.63[a]	0.51[b]	0.49[b]	0.36[c]	0.005
Zinc, *mg/d*	7.9[d]	10.9[a]	9.3[b]	9.5[b]	8.3[c]	0.3
Copper, *mg/d*	2.0[d]	2.6[a]	2.4[b]	2.4[b]	2.2[c]	0.09
Iron, *mg/d*	25.7[d]	38.3[a]	33.5[b]	34.8[b]	31.0[c]	1.2
Chromium, *μg/d*	52[d]	93[a]	72[b]	73[b]	66[c]	4

[1] Values are means, $n = 6$. Means in a row with superscripts without a common letter differ, $P < 0.05$.
[2] TN, thermoneutral (22°C) control (basal) diet; HS, heat stress, control (basal) diet; Vit C, heat stress, control diet + 250 mg of ascorbic acid/kg of diet; FA, heat stress, control diet + 1 mg of folic acid/kg of diet; Vit C + FA, heat stress, control diet + 250 mg of ascorbic acid/kg of diet + 1 mg of folic acid/kg of diet.
[3] The values were calculated on the basis of dry matter.

conditions. McDowell (10) reported that the need for folic acid 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 (10) and its deficiency causes severe defects in DNA replication and repair (18,19). Folic acid is also required for the methylation of homocysteine to form methionine (10). In the present study, folic acid supplementation improved the performance variables (Table 2). Similar to results of the present study, Wong et al. (34) reported that folic acid supplementation at 0.30–0.36 mg/kg of diet increases body weight and feed efficiency in Japanese quail.

In the present study, serum MDA, homocysteine and ACTH concentrations decreased, whereas vitamins C, E, A, B-12 and folic acid concentrations increased when dietary vitamin C and folic acid were supplemented (Table 3). The higher concentrations of homocysteine and MDA in the control group and their reduction in the treated groups are consistent with a previous report (16). Free radicals such as $O_2^{\cdot -}$ and HO• are formed during conditions of heat stress, thus reducing membrane integrity because of peroxidation of PUFA in the cell membrane (3,4,6). It was previously reported that the concentrations of antioxidant vitamins (vitamins A, C and E) in the serum and liver decrease with heat stress (33). In agreement with our results, patients with folic acid or vitamin B-12 deficiency have an elevated serum homocysteine level that is reduced by folic acid supplementation (35). Folic acid deficiency lowers the concentrations of methyl tetrahydrofolate, the main methyl donor for methylation of homocysteine to methionine. Failures in remethylation also elevate the concentration of homocysteine (10). Vitamin B-12 and folic acid are required for remethylation of homocysteine to methionine (36–38). Folic acid has been defined as an effective free-radical scavenger (39). Greater TBARS concentrations in the liver of folic acid–depleted rats have been reported (16,40).

Environmental stress increases mineral excretion (41). El-Husseiny and Creger (42) 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 (43,44). 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 (14) 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.

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 (10,45). In addition, antioxidant activity was reported to be more efficient when antioxidants are used in combination (20).

In conclusion, the results of the present study suggested 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 performance of broiler Japanese quail. Furthermore, Japanese quail have been used as a model for cardiovascular disease and cancer (46,47). The results of this study may also be applicable to the prevention of cancer and cardiovascular diseases, which have been associated with increased oxidative stress.

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