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Effects of vitamin C and folic acid supplementation on serum paraoxonase activity and metabolites induced by heat stress in vivo

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Abstract

This study investigated the effects of vitamin C (L-ascorbic acid) and folic acid supplementation on the levels of enzymes activities such as paraoxonase (PON1) and arylesterase and metabolites in the plasma of heat-stressed Japanese quails (*Coturnix coturnix japonica*) kept at 34°C. A total of 150 Japanese quails (10 days old) were randomly assigned to five treatment groups, with three replicates of 10 birds each. The birds, which were kept at 34°C (09.00 to 17.00; for 8 hours) were fed either a basal diet (high-temperature basal diet, HS group) or the basal diet supplemented with either 250 mg of L-ascorbic acid/kg of diet (Vit C group), 1 mg of folic acid/kg of diet (FA group), or 250 mg of L-ascorbic acid plus 1 mg of folic acid/kg of diet (Vit C + FA group), whereas birds kept at 22°C were fed a basal diet (thermo-neutral-basal diet, TN group). Supplemental vitamin C and folic acid significantly increased paraoxonase, arylesterase, creatine kinase, lactic dehydrogenase, and alkaline phosphatase activities compared with the HS group. Serum activities of aspartate aminotransferase and alanine aminotransferase were not influenced by dietary vitamin C or folic acid ($P > 0.05$). Serum cholesterol, triglyceride, high density lipoprotein (HDL)–cholesterol, and glucose concentrations decreased, whereas total protein and albumin concentrations increased with dietary vitamin C and folic acid supplementation ($P < 0.05$) compared with the HS group. However, the combination of vitamin C and folic acid provided the greatest results. Serum values were also greatest for PON1 and arylesterase and lowest for cholesterol, trygliceride, HDL-cholesterol and glucose ($P < 0.05$) for the TN group. The results of the study show that, separately or in combination, vitamin C and folic acid supplementation improved increased PON1 and arylesterase; the results also suggest that vitamin C and folic acid, especially in combination, may offer a potential protective management practice in

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preventing heat-stress–related depression in the performance of broiler quails.

Keywords: Quails; Heat stress; Vitamin C; Folic acid; PON1; Arylesterase; HDL-cholesterol

1. Introduction

Environmental stress has been demonstrated to cause an increase in oxidative stress and an imbalance in antioxidant status [1–3]. It has been reported that plasma antioxidant vitamins and minerals such as vitamin C, E, folic acid, and zinc levels declined and oxidative damage increased in stressed poultry [4,5]. Several studies have shown that antioxidant nutrient supplementation, especially vitamin C, vitamin E, vitamin A, and zinc are effective in protecting the oxidation of DNA, LDL low density lipoprotein (LDL), and protein in vitro and in vivo, and that such nutrients could be included in the diet to prevent the negative effects of environmental stress [6–8]. Morever, it has been reported the effects of environmental stress such as cold ambient temperature reduced plasma protein concentration, antioxidant enzymes activities such as paraoxonase (PON1) [9], and markedly increased blood glucose and cholesterol concentrations [10]. Environmental factors that alter PON1 activity including stress, which has been reported to depress PON1 activity [11]. The role of PON might be meaningful because oxidized LDL promotes secretion of the potent endothelial constrictor, endothelin [12] induced by environmental stress. A number of ways are available to alleviate the negative effects of high environmental temperature on the performance of poultry in terms of feed consumption, body weight gain and feed efficiency. As it is expensive to cool the buildings in which animals are housed, such methods are focused mostly on dietary manipulation. It is known that vitamin C and folic acid are used for reducing the negative effects of environmental stress in the diets of poultry because of the reported benefits of vitamin C and folic acid supplementation on poultry reared under heat or cold stress [3,5,6,13]. Poultry have an ability to synthesize vitamin C, but this ability is inadequate under stress conditions such as low or high environmental temperatures, humidity, high productive rate, and parasite infestation [6,14]. Pardue and Thaxton [15] have documented evidence that particular environmental stressors can alter ascorbic acid use or synthesis in avian species. A number of studies reported a beneficial effect of vitamin C supplementation on glucose, urea, triglycerides, cholesterol protein and albumin concentrations, and alkaline phosphatase [13].

The relationship between vitamin C and folic acid has been proposed in a way that vitamin C appears to have an important effect on the use and perhaps absorption of folic acid, which is postulated to function as an antioxidant [16]. As it is known that vitamin C is active in altering the form of folic acid to the tetrahydro derivate, a reduced form. Use of folic acid is impaired when vitamin C is deficient. A combination of antioxidant vitamin and minerals shows greater antioxidant ability against oxidative damage [6].

The objective of the present study was therefore to investigate the effects of vitamin C and folic acid supplementation on PON1, arylesterase, creatine kinase, lactic dehydrogenase, alkaline phosphatase activities and metabolites, aspartate aminotransferase, and alanine aminotransferase activities and cholesterol, triglyceride, HDL-cholesterol, glucose, total protein, and albumin concentrations of Japanese quails reared under heat stress (34°C).

2. Methods and materials

2.1. Animals, Diets, Experimental Design, and Data Collection

A total of 150 10-day-old Japanese quails (*Coturnix coturnix japanica*) provided from Bingol Collage Division of Firat University, Elazig, Turkey, were used in the study. The birds were assigned, according to their initial body weights, to five treatment groups, which included three replicates of 10 birds each. The quails were placed in two temperature-controlled rooms (22° and 34°C; optimal and high temperature). The birds kept at 34°C temperature (09.00 to 17.00, for 8 hours) were fed either a basal diet (high-temperature basal diet, HS group) or the basal diet supplemented with either 250 mg of L-ascorbic acid/kg of diet (Vit C group), 1 mg of folic acid/kg of diet (FA group), or 250 mg of L-ascorbic acid plus 1 mg of folic acid/kg of diet (Vit C + FA group), whereas hens kept at 22°C only fed a basal diet (thermo-neutral-basal diet, TN group). 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 a commercial company (Roche, Levent, Istanbul, Turkey). The birds were fed a starter diet until 21 days of age followed by a finishing diet from day 21 to day 40. 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. The birds were fed a starter diet until 21 days of age, followed by a finishing diet from day 21 to day 42. The basal diets were formulated using NRC (National Research Council) [17] guidelines and contained 23–20% (starter-grower) protein and 3100 kcal/kg ME. The diets and fresh water were offered ad libitum.

Serum creatine kinase, lactic dehydrogenase, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase activities, as well as cholesterol, triglyceride, HDL-cholesterol, glucose, total protein, and albumin concentrations were measured using a biochemical analyzer (Olympus AU-600, Tokyo, Japan). Paraoxanose (PON1; Basal and NaCI-stimulated) and arylesterase activities in serum samples was determined by the method of Juretic et al. [18].

2.2. Statistical Analyses

The data were analyzed using the GLM procedure of SAS (SAS, Cary, NC) [19]. Significant differences ($P < .05$) among treatment means were determined using the Duncan new multiple range test.

Table 1
Ingredients and chemical analyses of the starter and grower diets fed to Japanese quails reared under heat stress (34°C)

Ingredients	Starter	Grower
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
Salt	0.31	0.31
Limestone, ground	1.68	1.76
DL-Methionine	0.17	0.09
Vitamin*-mineral† premix	0.75	0.75
ME, kcal/kg	3100	3100
Chemical Analysis		
CP	23.00	20.21
Calcium, %	1.0	0.95
Available phosphorus, %	0.46	0.40

* Premix (Rovimix 124/V) supplied for 2 kg: vitamin A, 15,000 IU; cholecalciferol, 3 IU; vitamin E, 15 IU; menadione, 2.5 mg; vitamin B1, 1 mg; vitamin B_2; 10 mg; niacin, 70 mg; d-pantotheenic acid, 20 mg; vitamin B_{12}, 4 mg; folic acid, 2 mg; biotin, 0.1 mg.

† Premix (Remineral CH) supplied for 2 kg: Mn, 80 mg; Fe, 25 mg; Zn, 50 mg; Cu, 7 mg; iodine, 0.3 mg; Se, 0.15 mg; choline cloride, 350 mg.

3. Results

The effects of supplemental dietary vitamin C and folic acid during heat stress on enzyme activities of quails are shown in Table 2. Supplemental vitamin C and folic acid significantly increased PON1, arylesterase, creatine kinase, lactic dehydrogenase, and alkaline phosphatase activities ($P < 0.01$) in heat-stressed birds. The enzyme activities were also higher ($P < 0.05$) with each supplemental treatment group compared with the HS group, being the highest with the combination of vitamin C and folic acid treatment (Table 2). However, serum activities of aspartate aminotransferase and alanine aminotransferase were not influenced by dietary vitamin C or folic acid ($P > 0.01$). The effects of vitamin C and folic acid supplementation on serum cholesterol, triglyceride, HDL-cholesterol, glucose, total protein, and albumin are shown in Table 3. Separately or in combination, supplemental vitamin C and folic acid increased serum total protein and albumin ($P < 0.05$) but decreased cholesterol, triglyceride, HDL-cholesterol, and glucose concentrations ($P < 0.01$). Serum values were also greatest for vitamin total protein and albumin ($P < 0.05$) and lowest for cholesterol, triglyceride, HDL-cholesterol, and glucose concentrations ($P < 0.05$) for the birds kept under thermaneutral temprature (TN) compared with heat-stressed birds.

4. Discussion

In the present study, significant reduction enzymes activities except aspartate aminotransferase and alanine aminotransferase were observed when birds were kept at 34°C compared

Table 2
Effects of supplemental vitamin C and folic acid on enzyme activities in Japanese quails reared under heat stress (34°C)

Item	Treatment*				Vit C + FA	SEM†
	TN	HS	Vit C	FA		
Paraoxonase, U/L						
Basal	651^{a}	283^{d}	425^{c}	390^{c}	$596^{a,b}$	38
NaCI-stimulated	886^{a}	372^{d}	619^{c}	568^{c}	$776^{a,b}$	65
Arylesterase, kU/L	77^{a}	26^{d}	41^{c}	36^{c}	54^{b}	5
Creatine phosphokinase	3858^{a}	1943^{d}	2562^{c}	2632^{c}	3051^{b}	87
Lactic dehydrogenase	260^{a}	125^{d}	163^{c}	159^{c}	182^{b}	12
Alkaline phosphatase	351^{a}	143^{d}	215^{c}	203^{c}	262^{b}	20
Alanine aminotransferase	15	17	14	16	15	4
Aspartate aminotransferase	152	158	152	163	156	12

Values are means ($n = 9$).
a, b, c, d : Mean values within a row with no common superscript differ significantly ($P < 0.05$).
* 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 + control diet + 250 mg of ascorbic acid/kg of diet plus 1 mg of folic acid/kg of diet.
† SEM valus are pooled standard errors.

with quails kept under therma-neutral temperature (22°C). In addition, when vitamin C and folic acid supplements were included in the diet of quails under heat stress, these parameters were improved, particularly with the combination of the two supplements. Results of the present study are in agreement with the findings of several researchers [5,9,20]. Sahin et al. [5] reported that, whereas serum activities of aspartate aminotransferase and alanine ami-

Table 3
Effects of supplemental vitamin C and folic acid on serum metabolites of Japanese quails reared under heat stress (34°C)

Item	Treatment*				Vit C + FA	SEM
	TN	HS	Vit C	FA		
Cholesterol, nmmol/L	4.3^{d}	5.8^{a}	5.5^{b}	5.6^{b}	5.0^{c}	0.1
Triglyceride, nmmol/L	1.8^{d}	2.6^{a}	2.3^{b}	2.3^{b}	2.1^{c}	0.09
HDL-cholesterol, nmmol/L	0.8^{d}	1.3^{a}	1.1^{b}	1.1^{b}	1.0^{c}	0.05
Glucose, mg/ml	0.9^{c}	1.8^{a}	1.5^{b}	1.5^{b}	$1.4^{a,b}$	0.09
Total protein, mg/ml	3.5^{a}	2.6^{d}	2.9^{c}	2.8^{c}	3.1^{b}	0.09
Albumin, mg/ml	1.9^{a}	1.1^{d}	1.5^{c}	1.5^{c}	1.7^{b}	0.05

Values are means ($n = 9$).
a ,b ,c ,d : Mean values within a row with no common superscript differ significantly ($P < 0.05$).
* 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 + control diet + 250 mg of ascorbic acid/kg of diet plus 1 mg of folic acid/kg of diet.

notransferase remained unchanged, increasing both dietary vitamin C and vitamin E caused an increase in serum activity of alkaline phosphatase. Folic acid has been recently defined as an antioxidant [21], and a deficiency of this substance caused an increase in products of lipid peroxidation [22,23]. Henning et al. [24] reported reduced levels of glutathione and α-tocopherol and impairment in the activities of antioxidant enzymes such as Cu-Zn superoxide dismutase and glutathione peroxidase, observed as an compromised antioxidant defense system in rats deficient in methyl/folic acid. Folic acid scavenges free radicals very efficiently, and greater concentrations of lipid peroxidation products in livers of folic acid–depleted rats was reported [16,25].

Results of the present study indicated that both vitamin C and folic acid supplementation resulted in an increase PON1, arylesterase, creatine phosphokinase, lactic dehydrogenase, and alkaline phosphatase. It is well known that antioxidant enzyme activities such PON1 decrease when ambient temperature goes above or below the thermoneutral zone [5,9]. Some researchers reported that the effects of PON1 on LDL oxidation appear to be independent of the fuction of antioxidant vitamins, and vitamin C has been shown to inhibit LDL oxidation [12,20,26]. Any reduction in oxidative stress related to vitamin C consumption may preserve PON1 activity [20]. The HDL effect on the protection of LDL from oxidation mainly attributable to PON1, is more prolonged than the effects of antioxidant vitamins [20,26,27]. A previous study of PON1 depression by ambient temperature are consistent with lower PON activity associated with current heating in the present study and previous one [9]. Our study also demonstrated a correlation between PON1 activity and HDL levels. As known, HDL has a role in preventing LDL oxidation in vitro [26,28]. The HDL transport cholesterol from periferal tissue [29], and antioxidant [30] or antiinflammatory effects [31] are possibly related to its antiatherogenic capacity.

It is well known that heat stress increases MDA (malondialdehyde) concentration as a lipid peroxidation indicator [1,5]. Antioxidant systems (glutathione peroxidase, superoxide dismutase, PON1, 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 [1]. It has also been reported that serum glucose, cholesterol, and trygliceride decreased, whereas total protein, albumin concentrations increased when dietary vitamin C and folic acid were supplemented [5]. The higher values for glucose, cholesterol, and tryglicerides in the control group and reduction in treatment groups are in agreement with previous studies [5,10]. Similar to our results, Sahin et al. [5] 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 [13] reported that vitamin C supplementation increased plasma protein concentration whereas blood glucose and cholesterol concentrations 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 gluconeogenesis [6]. Increases in concentrations of glucose may be attributed to increased glucocorticoid secretion, which increases glucogenesis [6]. Dietary vitamin C may reverse these changes, presumably by reducing the secretion and/or synthesis of glucocorticoids.

Results of the present study showed similar trends for effects of vitamin C to those of folic acid for all parameters measured. In addition, for most parameters measured, the magnitude of the results was greater when both vitamin C and folic acid were supplemented, indicating a possible additive effect of the two supplements. The similar effects of vitamin C and folic acid on most of the parameters could be due to the similarity between the role of vitamin C and folic acid as antistress agents. These to compounds may act by different mechanisms or may have complementary roles, as additive effects of them were found. To the our best knowledge, this is the first report of PON1 values to evaluate the effect of folic acid and vitamin C supplementation on parameters measured in heat-stressed Japanese quails. The presence of both vitamin C and folic in the diet of this study resulted in an increase in serum concentration of PON1 and arylesterase activities in heat-stressed birds, indicating that vitamin C and folic acid act as antioxidants, alleviating the detrimental effects of heat stress. Concentrations of antioxidant vitamins (vitamin A, C, and E) of the serum and liver decrease with heat stress [5]. As seen in Table 3, the largest differences observed among the study groups were those between the TN group and the treatment groups. Although an alleviation is expected, it is not anticipated that these differences would be completely normalized with the supplementations. It should be noted that the purpose of studies such as as the present study is to evaluate the beneficial effects of different supplements.

In conclusion, taken together, vitamin C and folic acid supplementation (either separately or in combination) resulted in an increase in paraoxonase and arylesterase activity, as well as in a decrease in serum cholesterol, trygliceride, and glucose concentrations. The results of the present study suggest that vitamin C and folic acid have similar effects and that a combination of the two supplements results in an additive effect.

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