



Effects of Coenzyme Q10 and Vitamin C on Growth Performance and Blood Components in Broiler Chickens under Heat Stress

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Abstract

This experiment was carried out to study the effects of Coenzyme Q10 (CoQ10) and vitamin C (VC) on growth performance and blood biochemistry in broiler chickens under heat stress conditions. One of three levels of CoQ10 (0, 20, and 40 mg/kg of diet) and one of two levels of VC (0 and 250 mg/kg of diet) were supplemented to diets of chicks (from 1-42 d of age) in a 3 × 2 factorial arrangement. Each dietary treatment had four replicate pens (10 chicks/pen). In order to create chronic heat stress, the house temperature was set at an ambient temperature of 35±2°C for 8 hrs daily (09:00 to 17:00) between 25-42 d of age. Feed intake, body weight gain (BWG), and feed to gain ratio (F:G) were recorded at d 10, 25 and 42. At the end of experiment, two chicks/pen were randomly selected to assess blood components. CoQ10 supplementation improved BWG and F:G during 11-25 days, 26-42 days, and the whole period of the experiment ($P < 0.05$), while VC supplementation improved BWG and F:G only during 11-25 d of age. Blood glucose, cholesterol and triglycerides concentrations were reduced ($P < 0.05$) by addition of CoQ10 to the diet. Both Supplementation of CoQ10 and VC together lowered heterophil (H) count but increased lymphocyte (L) count, thereby reducing H/L ratio ($P < 0.05$). Serum concentrations of corticosterone and T4 were positively affected by dietary supplementation of CoQ10 ($P < 0.05$), but no differences were obtained with addition of VC to the diet. In conclusion, our observations demonstrated that dietary supplementation of 40 mg/kg CoQ10 or 250 mg/kg VC improves the growth performance of broiler chickens under the heat stress.

Introduction

Currently, heat stress is a major concern in the poultry industry, particularly in warm, tropical, and sub-tropical regions. Heat stress reduces feed intake, nutrient utilization, growth rate, and feed efficiency in poultry. Jiroft city in southern Iran (28°40'N 57°44'E, 650 m above sea level) has a warm sub-tropical climate during summer months (summer maximal temperatures range between 24.8-48.3°C). In this

geographical area, due to high ambient temperatures and relative humidity, poultry producers generally do not rear broiler chickens from mid-May until the end of September, which inflicts heavy economic losses on the regional industry. Therefore, recent efforts in our laboratory focus on eliminating the negative impacts of heat stress on broiler production using different strategies, such as dietary

supplementation of herbal plant powders and vitamins (Alba *et al.*, 2015; Behboudi *et al.*, 2016; Rafiee *et al.*, 2017).

Heat stress can cause several alterations in the animal body. Adaptation to heat stress requires physiological integration including the endocrine, cardiorespiratory, and immune systems (Etches *et al.*, 1995). For instance, in the studies of Borges *et al.* (2003) and Lin *et al.* (2006), heat stress increased mortality, outbreak of respiratory alkalosis, and suppressed the immune system of birds. Moreover, an increased number of heterophils and decreased number of lymphocytes in blood of broilers were observed (Cirule *et al.*, 2012). The adverse hormonal alterations including an increase in plasma concentration of corticosterone, cortisol and catecholamines, and a decrease in plasma thyroid hormones have been observed in heat stressed birds (Iqbal *et al.*, 1990; Kutlu and Forbes, 1993; Tao *et al.* 2006; Ma *et al.*, 2014). Other signature elements of the heat stress response in the birds include an increase in circulating glucose and cholesterol (Kutlu and Forbes, 1993), a reduction in lipolysis, as well as greater amino acid catabolism (Geraert *et al.*, 1996).

Several researchers have tried to combat heat stress in poultry through dietary supplementation with feed additives such as vitamins, antioxidants, minerals, and herbal plants (Lara and Rostagno 2013; Alba *et al.*, 2015). Vitamin C and CoQ10 (ubiquinone) are important bioactive compounds that exhibit strong antioxidant capacity in scavenging free radicals. Both components are synthesized in the body but their amounts become inadequate under heat stress conditions due to an increase demand for scavenging free radicals. CoQ10 is an important antioxidant in the mitochondrial electron transport chain and a constituent of various cellular membranes. CoQ10 as a nutritional supplement has been shown to yield a wide range of beneficial effects (Feher *et al.*, 2007). For example, it can capture perferri, carbon-centred lipids, lipid-peroxyl, and alkoxyl radicals, improve mitochondrial respiration and immune system, possess protective effects on liver cell, and affect vitamin E regeneration (Littarru and Tiano 2005; Feher *et al.*, 2007).

The addition of a combination of antioxidants can better minimize the harmful effects of free radicals than antioxidant monotherapy (Feher *et al.*, 2007). We hypothesize that dietary supplementation of VC and CoQ10 together

may play a critical role in the reduction of heat stress-induced reactive oxygen species. Considering the economic impact of heat stress on broiler chickens reared in hot summer seasons, this experiment assesses the effects of different levels of CoQ10, VC, and its combination on growth performance and blood components in broiler chickens under heat stress conditions.

Materials and Methods

Birds, diets and management

This experiment was approved by the animal ethical committee in the University of Jiroft. 240 1-d old Ross 308 male broiler chicks were obtained from a commercial hatchery and randomly distributed into 24 floor pens (1 m × 1.5 m) in an environmentally controlled house with free access to feed and water until 42 d of age. The experiment was conducted as a 3 × 2 factorial arrangement with three levels of CoQ10 (0, 20, and 40 mg/kg of diet) and two levels of VC (ANPRO VIT C 500, Chemifarma, Forli, Italy) (0 and 250 mg/kg of diet). The broiler chickens were allocated to one of the six treatment groups, with four replicates of 10 birds each. Feed ingredients and nutrient composition of the basal diet are shown in the Table 1. The basal diet was formulated to meet or exceed all the nutrient recommendations for the starter, grower, and finisher phases published in the Ross rearing guideline (Aviagen 2007). Both VC and CoQ10 were included in addition to the basal diet.

Light from compact fluorescent bulbs with an intensity of 15 lux were continuously used. Temperature was initially set at 32°C for the first 3 d of age, then decreased to 24°C by the end of the third week. In order to create chronic heat stress, the house temperature was set at an ambient temperature of 35±2°C for 8 h daily (from 09:00 AM until 17:00 PM) from 26-42 d of age. During the remaining hours (from 18:00 PM until 08:00 AM), the temperature was maintained on 22±2°C. The mean relative humidity was 50±10% during the course of study.

Growth performance

On d 0, 10, 25 and 42, broiler chickens were weighed by pen, and average body weight gain (BWG), feed intake (FI), and feed to gain ratio (F:G) were determined. The birds were observed daily, and the BW of dead broilers and date of death were recorded.

Table 1. Composition (as-fed basis) and calculated analysis of the basal diet for starter (0-10 d), grower (11-25 d) and finisher (26-42 d) periods[‡]

Ingredients (%)	Starter (0-10 d)	Grower (11-25 d)	Finisher (26-42 d)
Corn	53.00	54.81	60.63
Soybean meal, 44% CP	39.00	36.24	30.85
Sunflower oil	3.38	5.00	4.84
Limestone	1.43	1.35	1.30
Dicalcium phosphate	1.81	1.29	1.21
Vitamin premix [†]	0.25	0.25	0.25
Mineral premix [‡]	0.25	0.25	0.25
Sodium chloride	0.32	0.29	0.29
DL-Methionine	0.24	0.29	0.25
L-Lysine HCl	0.32	0.23	0.13
<i>Nutrient content</i>			
ME (Kcal/kg)	3025	3150	3200
Crude protein (%)	22	21	19
Methionine + cystine (%)	1.37	0.95	0.86
Lysine (%)	1.43	1.30	1.09
Threonine (%)	0.94	0.9	0.82
Calcium (%)	1.05	0.9	0.85
Available Phosphorus (%)	0.52	0.45	0.42

[‡] Vitamin C (0 or 250 mg/kg) and Coenzyme Q10 (0, 20, or 40 mg/kg) were included on top of the basal diet.

[†] Vitamin premix supplied the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D₃, 215 IU; vitamin E, 18 IU; vitamin K₃, 2 mg; vitamin B₁, 18 mg; vitamin B₂, 6.6 mg; vitamin B₆, 3 mg; vitamin B₁₂, 0.015 mg; nicotinic acid, 10 mg; folic acid, 1 mg; and pantothenic acid, 12 mg.

[‡] Mineral premix supplied the following per kilogram of diet: choline chloride (60%), 500 mg; Mn, 100 mg; Zn, 84.7 mg; Cu, 10 mg; Se, 2 mg; I, 1 mg; and Fe, 50 mg.

Blood components

Blood samples were collected from two randomly selected broilers from each pen at d 42 of age after 5 hrs of fasting. The samples were collected as quickly as possible from the wing vein using a 3 mL syringe with a 25 gauge needle. From this, 0.5 mL was carefully moved into heparinized tubes for measuring white blood cells (WBC), red blood cells (RBC), heterophil to lymphocyte ratio (H/L), and hemoglobin (Hb), while 1.5 mL was moved into non-heparinized tubes to measure cholesterol, triglycerides, glucose, corticosterone, T3 and T4 concentrations.

An automatic blood analyzer (Boehringer Mannheim, Ingelheim am Rhein, Germany) was used to measure blood serum concentrations of cholesterol, triglycerides and glucose. An automated hematology analyzer (Hitachi 912, Sysmex K-1000, Sysmex Corp., Kobe, Japan) that was standardized for analyses of chicken blood was used to measure the concentrations (cells/ μ l) of WBC and RBC. Blood smears were prepared on slides and painted by Gimsa method. 100 leukocytes per sample were counted by heterophil to lymphocyte separation under an optical microscope, then H/L was determined. Serum concentrations of

corticosterone, T3 and T4 were measured by RIA method using commercial kits (Pars Azmoon Inc. Tehran, Iran).

Statistical analysis

All statistical analyses were conducted in SAS (2001) using the GLM procedures with the following models:

$$Y_{ijk} = \mu + C_i + V_j + (CV)_{ij} + \varepsilon_{ijk}$$

where Y: dependent variables, μ : overall mean, C_i: the effect of CoQ10 (i = 1, 2, 3), V_j: the effect of VC (j = 1, 2), (CV)_{ij}: the effect of interaction between CoQ10 and VC and ε_{ijk} : residual.

For analyses of data within the finisher phase, the BW of birds at 26th day of age (onset of finisher phase) was incorporated as a coverable factor in the statistical model. In this case, the statistical model was as under:

$$Y_{ijkl} = \mu + C_i + V_j + (CV)_{ij} + b(x_{ijk} - x) + \varepsilon_{ijkl}$$

where b(x_{ijk} - x): the effect of BW of birds at d 26 of age. The remaining elements were similar to the model above.

Tukey Multiple Range test was used to evaluate significant differences between the means. The linear and quadratic effects of dietary CoQ10 supplementation were evaluated. Probability values $P < 0.05$ were taken to indicate significance.

Results

The effects of dietary CoQ10 and VC supplementation on BWG, FI, and F:G during the starter, grower, finisher phases, and whole period of growth in broiler chickens are presented in Table 2. BWG, FI, and F:G were

unaffected by VC and CoQ10 during the starter phase. During the grower, finisher and whole periods, CoQ10 improved BWG and F:G ratio ($P < 0.05$). Dietary inclusion of VC increased ($P < 0.05$) the value of BWG during the grower and whole periods.

Table 2. Effects of Coenzyme Q10 (CoQ10) and vitamin C (VC) supplementation on performance parameters in broiler chickens under heat stress[‡]

Item	CoQ10 (mg/kg)				VC (mg/kg)			P-value †			
	0	20	40	SEM*	0	250	SEM*	L	Q	VC	CoQ10 × VC
FI (g/b/d) [§]											
Starter	17.16	17.34	17.77	0.21	17.22	17.63	0.18	-	-	0.09	0.92
Grower	66.17	66.44	66.57	0.10	66.19	66.59	0.08	0.04	0.192	0.003	0.17
Finisher	152.19	152.55	152.40	0.35	152.26	152.56	0.28	-	-	0.38	0.89
Whole period	92.13	92.42	92.51	0.16	92.15	92.56	0.13	-	-	0.047	0.85
BWG(g/b/d) [§]											
Starter	11.09	11.21	11.22	0.05	11.13	11.22	0.04	0.09	0.21	0.10	0.67
Grower	40.58	41.44	41.73	0.18	40.91	41.59	0.15	0.001	0.824	0.005	0.04
Finisher	88.04	88.57	89.32	0.27	88.37	88.91	0.22	0.01	0.28	0.10	0.60
Whole period	54.39	54.95	55.37	0.14	54.66	55.15	0.11	0.0003	0.782	0.006	0.19
F:G [§]											
Starter	1.55	1.54	1.58	0.02	1.55	1.57	0.02	-	-	0.33	0.97
Grower	1.63	1.60	1.59	0.008	1.62	1.60	0.007	0.01	0.27	0.050	0.15
Finisher	1.73	1.72	1.70	0.006	1.72	1.71	0.005	0.03	0.36	0.35	0.83
Whole period	1.69	1.68	1.67	0.005	1.68	1.67	0.004	0.01	0.33	0.11	0.42

[‡] FI = feed intake, BWG = body weight gain and F:G = feed to gain ratio.

[†] L = linear and Q = quadratic effects of CoQ10.

[§] Data represent the mean of 4 replicate pens of 10 broiler chickens per pen.

* SEM, standard error of means

Table 3. Effects of Coenzyme Q10 (CoQ10) and vitamin C (VC) supplementation on blood profile of broiler chickens under heat stress[‡]

Item	CoQ10 (mg/kg)				VC (mg/kg)			P-value†			
	0	20	40	SEM*	0	250	SEM*	L	Q	VC	CoQ10 × VC
Blood components [§]											
Cholesterol (mg/dL)	143.00	131.0	127.25	2.43	135.1	132.41	1.99	0.0006	0.975	0.35	0.90
Triglycerides (mg/dL)	70.87	68.37	65.25	1.50	69.91	66.41	1.23	0.050	0.581	0.06	0.80
Glucose (mg/dL)	207.12	197.87	190.50	2.45	201.16	195.83	2.10	0.0006	0.954	0.07	0.47
Blood Cells [§]											
RBC (10 ⁶ /μL)	2.68	2.59	2.53	0.05	2.54	2.67	0.04	-	-	0.04	0.92
WBC (10 ³ /μL)	21.87	22.51	22.76	0.15	22.25	22.51	0.12	0.002	0.682	0.15	0.86
Hb (g/dL)	11.13	10.65	10.45	0.21	10.60	10.88	0.17	-	-	0.27	0.80
Heterophil (%)	18.12	17.25	16.50	0.38	17.75	16.83	0.31	0.027	0.351	0.05	0.77
Lymphocyte (%)	81.88	82.75	83.50	0.38	82.25	83.17	0.31	0.021	0.483	0.05	0.77
H/L	0.22	0.21	0.19	0.005	0.21	0.20	0.005	0.036	0.594	0.05	0.79
Blood Hormones [§]											
Corticosterone (μg/dL)	1.94	1.79	1.60	0.06	1.82	1.73	0.04	0.002	0.247	0.24	0.29
T3 (ng/mL)	1.14	1.19	1.24	0.03	1.16	1.22	0.03	-	-	0.14	0.40
T4 (ng/mL)	9.67	10.23	10.81	0.26	10.06	10.40	0.21	0.021	0.335	0.27	0.77

[‡] WBC = white blood cells, red blood cells (RBC), Hb = hemoglobin and H/L = Heterophile to lymphocyte ratio.

[†] L = linear and Q = quadratic effects of CoQ10.

[§] Data represent the mean of 4 replicate pens of two broiler chicken per pen.

* SEM, standard error of means

A significant interaction between VC and CoQ10 was observed on BWG during the grower period ($P < 0.05$). In this case, the highest (41.86 ± 0.19 g/b/d) BWG was recorded for birds fed 40 mg of CoQ10/kg plus 250 mg VC/kg, and the lowest (39.82 ± 0.19 g/b/d) BWG was in birds from the control group (no feed additives) ($P < 0.05$). Birds from the other treatment groups (0 mg/kg CoQ10 plus 250 mg/kg VC; 20 mg/kg CoQ10 plus 0 mg/kg VC; 20 mg/kg CoQ10 plus 250 mg/kg VC; 40 mg/kg CoQ10 plus 250 mg/kg VC) had similar BWG during the grower phase.

Effects of CoQ10 and VC supplementation on blood profile are shown in Tables 3. CoQ10, but not VC, reduced blood serum concentrations of glucose, cholesterol, and triglycerides ($P < 0.05$). Dietary inclusion of both feed additives decreased H/L ratio ($P < 0.05$), though WBC increased from CoQ10 supplementation and RBC increased from VC supplementation ($P < 0.05$). CoQ10 reduced corticosterone but increased T4 in blood serum ($P < 0.05$), while VC did not affect corticosterone hormonal concentrations.

Discussion

Over the past decades, considerable attention has been focused on understanding the mechanisms underlying biological responses associated with heat stress and alleviating its negative impacts on productivity of modern poultry genotypes using dietary supplementation of vitamins, minerals, antioxidants and herbal plants (Balnave, 2004; Lara and Rostagno, 2013; Alba *et al.*, 2015; Rafiee *et al.*, 2016). The present study examined the possible impacts of VC and CoQ10 as feed additives on the performance and blood profiles of broiler chickens challenged with heat stress conditions for 8 hrs each day during the 26-42 days of their lives. Our observations demonstrated that dietary inclusion of VC and CoQ10 improved the performance of broilers particularly in the grower and finisher stages. These findings are similar to other studies investigating combinations of additives (such as VC and flavonoids (Peña *et al.*, 2008), and the antioxidants genistein and hesperidin (Kamboh *et al.*, 2013)) in broilers under heat stress condition. Such findings could be associated to the high ambient temperature (33-34°C) required for birds to grow during starter phase, in contrast to later stages (i.e. grower and finisher

stages) in which birds are highly sensitive to hot temperatures and thus need greater protection. Kutlu (2001) defined the onset of heat stress conditions in poultry as an ambient temperature above 27°C, as well as a decrease in FI and body weight gain.

An important finding of this study was that VC and CoQ10 supplementation elicited a decrease in H/L ratio ($P=0.05$). Zulkifli and Siegel (1995) stated that stress can decrease the number of lymphocytes and increase the number of heterophils in birds. Hence, the blood H/L ratio has been considered a good stress marker in birds (Vleck *et al.*, 2000). Likewise, other studies (Borges 1997; Borges *et al.*, 2004; Mashaly *et al.*, 2004; Cîrule *et al.*, 2012) demonstrated that heat stress increases the H/L ratio index by increasing heterophils count and decreasing lymphocyte count in broiler chickens. Our results of H/L ratio and counts of heterophils and lymphocytes indicate that dietary supplementation of VC and CoQ10 could potentially ameliorate the harmful effects of heat stress in broilers. Kamboh *et al.* (2013) had similar observations in broilers supplemented with the flavonoids genistein and hesperidin under persistent summer stress.

We found that dietary inclusion of VC did not affect plasma glucose, cholesterol, and triglycerides concentrations. These results are in line with those reported by Lee *et al.* (2003), Kırkpınar *et al.* (2011), and Alba *et al.* (2015). In contrast to our results, Sahin *et al.* (2002) and Gursu *et al.* (2004) found that dietary inclusion of VC decreased blood glucose and cholesterol concentrations in heat-stressed birds. VC may decrease blood glucose by decreasing the secretion and/or synthesis of glucocorticoid hormones (Gursu *et al.* 2004).

We also found that increasing dietary inclusion of CoQ10 reduced serum glucose, cholesterol, and triglycerides concentrations, which may be due to a CoQ10-induced decrease in the oxidative effects of heat stress (Feher *et al.* 2007). CoQ10 can also decrease serum levels of glucose and lipids in rats (Modi *et al.* 2006). We also found that CoQ10 reduced serum corticosterone concentration (as an important biomarker for heat stress), which contrasts previous findings (Cîrule *et al.* 2012; Ma *et al.* 2014). The heat stress responses in poultry are primarily induced by the activation of the hypothalamic-pituitary-adrenal axis from plasma corticosterone and changed metabolic

status elicited by decreased plasma levels of T3 and T4 (Lara and Rostagno, 2013).

Although serum T3 concentration was unaffected in this study, T4 concentrations increased from dietary inclusion of CoQ10. It is well known that the animal body temperature and metabolic activity are regulated by T3, T4, and their balance. Heat-stressed birds continuously exhibit lower T3 and T4 concentrations (Lara and Rostagno 2013; Ma *et al.* 2014). Therefore, the increase in T4 concentration that we observed after dietary inclusion of CoQ10 may demonstrate its ability to alleviate the negative effects of heat stress. Similarly, a positive linear correlation between thyroid hormone concentrations and growth performance parameters was observed in turkeys under high ambient temperature (Yahava, 1999).

Conclusion

Dietary inclusion of VC and CoQ10 can

compensate for the harmful impacts of high ambient temperature in broilers. Although both feed additives individually improved growth performance parameters of broilers, their combination led to a higher rate of weight gain, especially during the grower phase. This may be attributed to mutual (synergistic) interactions that warrant investigation in special models. CoQ10 and VC supplemented together is an approach that can offer benefits to poultry in hot regions to ameliorate the negative impacts of high ambient temperatures.

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