

Effect of coenzyme Q10 supplementation on serum protein, mineral status, blood picture and immune status in broilers

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In poultry, coenzyme Q10 (CoQ10) is widely used as a feed additive to control mortality due to ascites in broilers. Apart from its use the treatment of a variety of disorders *viz.*, ischemic heart disease, diabetes mellitus, Parkinson's disease, muscle fatigue and muscle weakness, its supplementation has been reported to be beneficial for cardiovascular disease, chronic heart failure, cancer, migraine, asthma and hypertension. In this study, we investigated the effect of CoQ10 supplementation on serum protein, serum minerals, blood parameters, ascites susceptibility and humoral immune status in broilers fed with different energy levels which influence their productivity, biochemical profile and ascites incidences. The treatment had three levels of CoQ10, namely 0, 20 and 40 mg/kg at normal (NE), low (LE) and high (HE) energy levels in which 2X3 factorial design was followed. The haemoglobin and packed cell volume were not affected ($P > 0.05$) by either energy or CoQ10 levels but the erythrocyte osmotic fragility per cent (EOF %) and blood glucose levels were decreased by CoQ10 supplementation at both 20 and 40 mg/kg. The serum calcium level had significantly ($P < 0.01$) increased with CoQ10 at 40 mg/kg (12.70 *vs.* 11.58 and 11.98 mg/dL) in NE diet group over the unsupplemented and 20 mg/kg supplemented birds. Compared to the respective unsupplemented groups, CoQ10 @40 mg/kg reduced ($P < 0.01$) the serum total protein (4.69 *vs.* 5.23 g/dL) and serum albumin (2.46 *vs.* 2.78 g/dL) in NE group but increased ($P < 0.01$) (4.70 *vs.* 4.08 g/dL) and (2.59 *vs.* 2.04 g/dL), respectively in LE group. High energy birds showed significantly ($P < 0.01$) increased serum albumin (2.74 *vs.* 2.24 g/dL). The humoral immunity against Newcastle Disease (ND titre) was significantly ($P < 0.01$) higher in 21 days of growth period at both the levels of supplementation but on 42 days no significant difference among the groups were observed. It can be concluded that CoQ10 supplementation at 20 mg/kg decreased blood glucose level and increased erythrocytes osmotic stability and hence, could reduce bird's susceptibility to ascites.

Keywords: Ascites, CoQ10, Erythrocyte fragility, Newcastle Disease, Poultry feed, Ubiquinol

Coenzyme Q10 (CoQ10) is a vitamin like substance that acts as a coenzyme for the mitochondrial enzymes of oxidative phosphorylation system¹. Coenzyme Q10 (2-methyl-5,6-dimethoxy-1,4-benzoquinone) is ubiquitous and found in plant, humans and animals as an endogenous lipophilic antioxidant. It is lipid-soluble and involved in the mitochondrial adenosine triphosphate (ATP) synthesis bioenergetics². In addition to its role of electron transfer in oxidative phosphorylation, CoQ10 inhibits certain enzymes of free radicals formation and attenuates the oxidative stress^{3,4} and used as a food supplement for its antianaemic and antioxidant properties⁵. CoQ10 is used to treat a variety of disorders such as ischemic heart disease, diabetes mellitus, Parkinson's disease, muscle fatigue and muscle weakness⁶⁻⁹. Liu *et al.*¹⁰

reported that CoQ10 supplementation will be beneficial for cardiovascular disease, chronic heart failure, cancer, migraine, asthma and hypertension. CoQ10 is also beneficial in treatment of statin-induced reduction in plasma CoQ10^{9,11}. CoQ10 reduces the blood viscosity and improves blood flow to cardiac muscle in ischemic heart disease condition¹². In poultry feeding, CoQ10 is widely used as a feed additive for reducing the mortality due to ascites in broilers¹³.

Ubiquinol (CoQ10H₂), the reduced form of ubiquinone (CoQ10) exists mostly in blood and known to serve as an antioxidant to the erythrocytes. Low erythrocyte's osmotic fragility (EOF %) protects the erythrocyte membrane from osmotic damage in broilers¹⁴. Supplementation of CoQ4 (analogue of CoQ10 in mammals) helps to maintain hemoglobin and packed cell volume (PCV) levels normal in anemic monkeys⁵. Asadi *et al.*¹⁵ have observed that the PCV per cent is not influenced by CoQ10

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supplementation. Earlier researchers have reported improved haemopoietic activity on CoQ10 supplementation in deficient chicks, turkey pullets, monkeys and in children suffering from marasmus or kwashiorkor¹⁶⁻¹⁸.

CoQ10 acts as a non-specific host immune defence system stimulant and also as an immune modulator necessary to maintain the immune system at the mitochondrial level^{19,20}. CoQ10 supplementation not only prevents tumor growth and decreases the circulating levels of tumor necrosis factor- α (TNF- α) and interleukin-6 but also enhances the immunity against various viral challenges among different experimental animals^{21,22}. The immune suppression associated with aging and/or chronic diseases could be reversed by treatment with CoQ10 and with a significant increase in serum immunoglobulin G (IgG) levels¹⁹.

Under the present intensive system of poultry production especially in tropics, the stress on broilers due to environment, metabolic and management have become inevitable which resulted in low productivity and immune status especially humoral immunity (HI). In the present study, we have tried to investigate the effects of dietary supplementation of CoQ10 on serum protein, mineral, hematological pictures and immunity of broiler chickens fed with different levels of energy.

Materials and Methods

Birds and Treatment

The broiler trial carried out with 270 day-old Cobb 400 broiler chicks had nine treatments with three replicates each containing ten chicks. The treatment included three levels of CoQ10 namely 0, 20 and 40 mg/kg of diet and three energy levels, namely normal energy (NE) as specified by the breeder; low energy, LE (normal energy – 100 kcal); and high energy, HE (normal energy + 100 kcal). The treatments were labelled as NE without CoQ10, LE without CoQ10, HE without CoQ10 and 20 and 40 mg of CoQ10 supplemented to the above three levels of energy diet. The first three treatments represent the control group for respective energy levels. All diets contained same levels of calorie: protein, calorie: lysine and calorie: methionine ratio. Nine experimental broiler pre-starter, starter and finisher diets were formulated. The chicks were wing banded, weighed individually and assigned randomly to nine experimental groups with three replicates, each consisting of 10 chicks. Each replicate had even number of male and female chicks.

Completely randomized design was followed. The birds were immunized with F₁+IB on 7th day and IBD on 14th day of growth period. At the end of 21 and 42 days of growth period the blood samples were collected and serum was separated.

Blood analysis

The whole blood samples were collected with anticoagulant and analysed for its haemoglobin (Hb) content and packed cell volume (PCV) was determined by micro hematocrit centrifugation. The RBC, WBC count together with absolute count of heterophils, lymphocytes, monocytes, eosinophils and basophils as well as H/L ratio were calculated^{23,24}.

Erythrocyte osmotic fragility per cent (EOF %) was determined by Dacie's method²⁵ with following modification. Fresh heparinized blood (10 mL) was added to tubes containing 5 mL of 0.1, 0.5 and 0.9 % phosphate-buffered saline. The tubes were mixed and incubated at room temperature (24°C) for 30 min. After mixing, the suspension was centrifuged at 530 rpm for 5 min. The supernatant was measured at 540 nm with a spectrophotometer, using the blood in 0.9% saline as a blank.

The EOF% is expressed as:

$$\text{Haemolysis rate (\%)} = \left(\frac{\text{OD value at 0.5\% saline}}{\text{OD value at 0.1\% saline}} \right) \times 100.$$

The blood glucose level of the birds was estimated using the instant blood glucose monitoring system (One touch select simple, LifeScan Europe, Switzerland).

Serum mineral profiling

Serum minerals calcium, sodium and potassium levels were determined using flame photometer and whereas phosphorus was quantified as inorganic phosphorus and serum chloride was also estimated^{26,27}. The serological analysis of phosphorus and chloride were carried out using the Span® diagnostic kits.

Serum protein profiling

The serum total protein content was determined by the peptide bonds of protein which reacted with cupric ions at alkaline pH to form blue coloured chelate and the absorbance was measured at 578 nm. The serum albumin acts as a cation (pH 3.68) was bound to the anionic dye bromocresol green to form a green coloured complex whose absorbance was measured at 630 nm to determine serum albumin level and serum globulin levels were calculated using the following equation^{28,29}.

$$\text{Serum globulin} = \text{Serum total protein} - \text{serum albumin}.$$

Immune profiling

The serum samples were collected from all the treatments for estimating the immune status Newcastle Disease (ND) on 21st and 42nd day of the growth period. ELISA technique was used to measure the antibody titre against Newcastle Disease.

Statistical analysis

The data collected on various parameters were statistically analysed by factorial method using SPSS version 20 and the significance is expressed at 5% level ($P < 0.05$).

Results

Effects on blood parameters

The observations on the effect of dietary CoQ10 supplementation and different energy levels on the blood parameters are presented in Table 1. The haemoglobin (g/dL) and PCV (%) were not significantly altered by the CoQ10, energy levels, CoQ10 and energy interactions. However, numerically higher haemoglobin and PCV levels were observed in the CoQ10 supplementation at both 20 and 40 mg/kg. The osmotic fragility of erythrocytes were significantly ($P < 0.01$) reduced at CoQ10 20 mg/kg but not affected by energy levels. However, the interaction

effect between the CoQ10 and energy levels showed significant ($P < 0.01$) difference in the EOF% (Fig. 1). The CoQ10 at 20 mg/kg improved the membrane stability of RBCs at the all the three energy levels over unsupplemented groups in respective energy levels. However, at 40 mg/kg, the fragility was significantly reduced in LE than unsupplemented groups but in other two energy levels the EOF % is similar to the unsupplemented groups. The blood glucose levels (mg/dL) is significantly ($P < 0.01$) reduced by 40 mg/kg of CoQ10, followed by 20 mg/kg over the unsupplemented group. Interestingly,

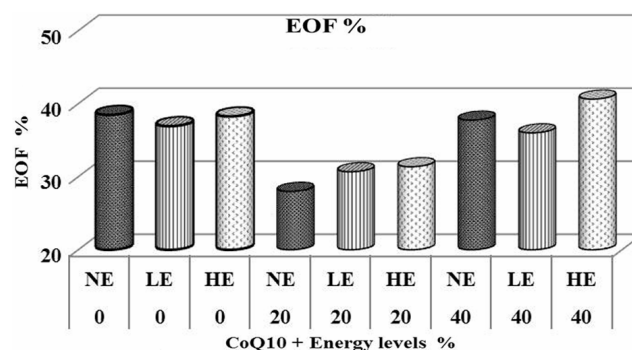


Fig. 1—Interactive effects of CoQ10 and energy levels on erythrocytes osmotic fragility (EOF %).

Table 1—Influence of Coenzyme Q10 and energy each at three levels on blood picture

CoQ10 (mg/kg)	Energy level	Haemoglobin (g %)	Packed cell volume (%)	Erythrocyte osmotic Fragility (%)	Blood Glucose (mg/dL)
0	NE*	10.0±0.5	30.1±1.4	38.46 ^a ±0.44	350 ^d ±10
0	LE	11.1±0.7	33.4±2.2	36.92 ^a ±0.55	314 ^{ab} ±10
0	HE	11.5±0.7	35.0±2.2	38.25 ^a ±0.49	314 ^{ab} ±08
20	NE	11.4±1.5	34.2±4.5	28.00 ^c ±0.60	341 ^{cd} ±07
20	LE	12.1±0.8	36.3±1.7	30.68 ^c ±0.34	320 ^{bc} ±07
20	HE	12.0±0.9	35.9±2.6	31.37 ^c ±0.45	323 ^{bc} ±09
40	NE	12.0±1.5	35.9±4.4	37.75 ^a ±0.62	304 ^{ab} ±09
40	LE	11.2±0.6	33.7±1.7	36.01 ^b ±0.53	291 ^a ±10
40	HE	12.2±0.6	36.5±1.8	40.63 ^a ±0.36	316 ^b ±09

GLM analysis of Coenzyme Q10 (mg/kg)

0	10.9±0.4	32.8 ±1.2	36.21 ^a ±0.37	327 ^b ±13
20	11.8±0.6	35.5±1.2	30.02 ^b ±0.38	328 ^b ±10
40	11.8±0.5	35.4±1.6	33.80 ^{ab} ±0.58	304 ^a ±07
P value	0.359	0.407	0.0004	0.0034

GLM analysis of Energy level (kcal/kg)

NE	11.1±0.7	33.4±2.1	33.07±0.75	332 ^b ±06
LE	11.5±0.4	34.5±1.2	35.54±0.73	308 ^a ±06
HE	11.9±0.4	35.8±1.2	34.42±1.08	318 ^{ab} ±05
P value	0.613	0.564	0.141	0.014

GLM analysis of CoQ10 X Energy level interaction

P value	0.84	0.83	0.0006	0.0004
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[NE, Normal Energy; LE, Low Energy; HE, High Energy. *NE-Normal Energy (3000, 3125, 3250 Kcal/kg for pre starter, starter and finisher diet respectively). Each value of interaction is a mean of nine observations. Each value of CoQ10 and energy levels is mean of twenty seven observations. Means with atleast one common superscript in a column do not differ significantly ($P > 0.05$)]

in HE fed group the blood glucose was not increased than NE and also similar to LE (Fig. 2).

However, there was no significant difference among the treatments in terms of CoQ10 levels, energy level nor the interaction effects on the haematological parameters, such as RBC count ranged $2.76-2.88 \times 10^6$ cells/ μ L; WBC count range from $24.79-24.94 \times 10^3$ cells/ μ L; Heterophil numbers $6.45-6.69 \times 10^3$ cells/ μ L; Lymphocytes $15.0-15.60 \times 10^3$ cells/ μ L; the H/L ratio ranged 0.44 -0.47; Monocytes counted $1.32-1.39 \times 10^3$ cells/ μ L;

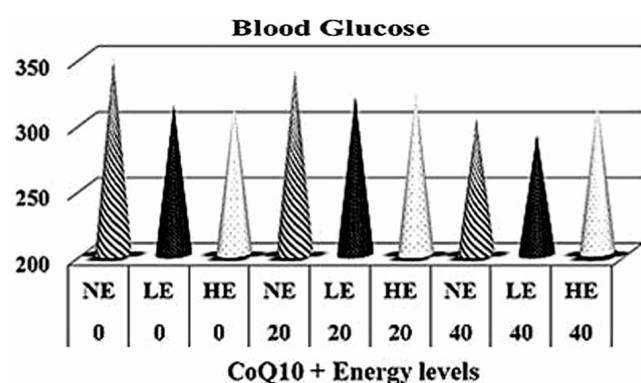


Fig. 2—Interactive effects of CoQ10 and energy levels on blood glucose levels (mg/dL).

Eosinophils $1.33-1.37 \times 10^3$ cells/ μ L; and Basophils $2.48-2.53 \times 10^3$ cells/ μ L.

Effects on serum minerals

The results of serum calcium, phosphorus, sodium, potassium and chloride levels are presented in Table 2. The serum calcium level was significantly ($P < 0.01$) reduced in low (LE) and high energy (HE) diet fed groups than the normal energy (NE) fed groups. The serum calcium level was significantly ($P < 0.01$) higher in HE with 40 mg/kg than the other two levels of CoQ10 within the energy level. Similarly, sodium levels were significantly higher in 20, 40 mg/kg CoQ10 and in HE groups. However, the serum phosphorus, potassium and chloride levels were not affected by CoQ10, energy and interaction effects.

Effects on serum total protein and immune response

The observations on the effects of CoQ10 and energy levels on serum protein profile and immune response are presented in Table 3. Serum total protein level and albumin were significantly ($P < 0.01$) lower in LE group than the other two energy (NE and HE) levels. On supplementation of CoQ10 at 40 mg/kg to LE group, the total protein and albumin levels were significantly ($P < 0.01$) increased to that of other

Table 2—Influence of Coenzyme Q10 and energy each at three levels on serum minerals

CoQ10 (mg/kg)	Energy level	Calcium (mg/dL)	Phosphorus (mg/dL)	Sodium (meq)	Potassium (meq)	Chloride (meq)
0	NE*	11.58 ^b ±0.56	6.88±0.20	152.28 ^{ab} ±7.05	10.16±2.20	109.3±2.40
0	LE	11.63 ^{bc} ±0.65	6.85±0.22	141.20 ^a ±13.14	13.58±4.16	108.56±1.16
0	HE	8.55 ^a ±0.13	6.86±0.13	171.36 ^{cd} ±0.77	7.74±0.58	107.47±3.78
20	NE	11.98 ^{bc} ±0.21	6.60±0.22	156.52 ^{abc} ±1.83	9.23±0.97	109.23±1.74
20	LE	11.28 ^b ±0.17	6.51±0.49	164.35 ^{bcd} ±2.43	10.72±1.94	108.97±2.33
20	HE	8.35 ^a ±0.21	6.83±0.37	161.30 ^{bc} ±5.76	13.42±3.11	111.24±3.64
40	NE	12.70 ^c ±0.37	6.74±0.38	158.04 ^{bc} ±1.36	9.01±0.84	109.72±3.11
40	LE	8.68 ^a ±0.49	6.88±0.29	168.80 ^{cd} ±2.48	8.76±1.38	107.84±2.45
40	HE	8.58 ^a ±0.18	6.83±0.36	177.50 ^d ±3.30	9.50±0.80	108.47±1.87
GLM analysis of Coenzyme Q10 (mg/kg)						
0		10.58±0.08	6.86±0.10	154.95 ^a ±5.42	10.49±1.59	108.51±2.40
20		10.53±0.07	6.65±0.21	160.72 ^{ab} ±2.18	11.12±1.26	107.00±3.78
40		09.98 ±0.09	6.82±0.19	168.12 ^b ±2.16	09.09±0.58	109.31±1.82
P value		0.51	0.66	0.04	0.49	0.64
GLM analysis of Energy level (kcal/kg)						
	NE	12.08 ^c ±0.05	6.74±0.15	155.6 ^a ±2.4	09.47±0.82	109.11±2.30
	LE	10.53 ^b ±0.08	6.75±0.20	158.1 ^a ±5.0	11.02±1.58	107.00±1.76
	HE	08.49 ^a ±0.02	6.84±0.17	170.1 ^b ±2.5	10.22±1.15	110.44±1.42
	P value	0.000	0.89	0.011	0.67	0.79
GLM analysis of CoQ10 X Energy level interaction						
	P value	0.000	0.99	0.001	0.50	0.84

[NE, Normal Energy; LE, Low Energy; HE, High Energy. *NE-Normal Energy (3000, 3125, 3250 Kcal/kg for pre starter, starter and finisher diet respectively). Each value of interaction is a mean of nine observations. Each value of CoQ10 and energy levels is mean of twenty seven observations. Means with atleast one common superscript in a column do not differ significantly ($P > 0.05$)]

Table 3—Influence of Coenzyme Q10 and energy each at three levels on serum protein profile

CoQ10 (mg/kg)	Energy level	Total Protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)	A/G ratio	ND titre value (log ₂)	
						21 st day	42 nd day
0	NE*	5.23 ^c ±0.25	2.78 ^c ±0.09	2.44±0.29	1.39±0.21	7.13 ^{de} ±0.02	6.20±0.02
0	LE	4.08 ^a ±0.14	2.04 ^a ±0.16	2.04±0.25	1.37±0.30	7.17 ^{de} ±0.03	6.18±0.03
0	HE	4.73 ^b ±0.04	2.24 ^{ab} ±0.18	2.49±0.18	1.01±0.15	6.87 ^b ±0.02	6.16±0.03
20	NE	4.91 ^{bc} ±0.2	2.85 ^c ±0.10	2.26±0.26	1.76±0.29	7.46 ^f ±0.04	6.30±0.04
20	LE	4.10 ^{ab} ±0.13	2.01 ^a ±0.14	2.09±0.14	1.04±0.13	7.40 ^f ±0.03	6.24±0.03
20	HE	4.72 ^b ±0.08	2.74 ^c ±0.20	1.98±0.20	1.72±0.32	7.20 ^e ±0.03	6.16±0.02
40	NE	4.69 ^b ±0.14	2.46 ^{bc} ±0.15	2.02±0.26	1.24±0.18	7.00 ^c ±0.04	6.10±0.02
40	LE	4.70 ^b ±0.13	2.59 ^{bc} ±0.11	2.11±0.23	1.51±0.25	7.10 ^d ±0.03	6.21±0.03
40	HE	4.78 ^{bc} ±0.18	2.25 ^{ab} ±0.16	2.54±0.32	1.07±0.13	6.70 ^a ±0.04	6.16±0.02
GLM analysis of Coenzyme Q10 (mg/kg)							
0		4.68±0.12	2.35±0.10	2.32±0.14	1.26±0.13	7.06 ^b ±0.03	6.30±0.03
20		4.58±0.10	2.53±0.11	2.05±0.12	1.51±0.16	7.35 ^c ±0.03	6.24±0.03
40		4.73±0.09	2.43±0.08	2.22±0.14	1.27±0.11	6.93 ^a ±0.04	6.16±0.04
P value		0.58	0.43	0.33	0.35	0.0001	0.94
GLM analysis of Energy level (kcal/kg)							
	NE	4.94 ^b ±0.12	2.70 ^b ±0.07	2.17±0.14	1.46±0.13	7.20 ^b ±0.04	6.20±0.02
	LE	4.29 ^a ±0.09	2.21 ^a ±0.07	2.08±0.12	1.31±0.14	7.22 ^b ±0.03	6.24±0.03
	HE	4.74 ^b ±0.07	2.41 ^a ±0.11	2.34±0.14	1.27±0.13	6.92 ^a ±0.04	6.16±0.03
	P value	0.000	0.001	0.39	0.56	0.0001	0.65
GLM analysis of CoQ10 X Energy level interaction							
P value		0.000	0.000	0.49	0.17	0.001	0.45

[NE, Normal Energy; LE, Low Energy; HE, High Energy. *NE-Normal Energy (3000, 3125, 3250 Kcal/kg for pre starter, starter and finisher diet respectively). Each value of interaction is a mean of nine observations. Each value of CoQ10 and energy levels is mean of twenty observations. Means with atleast one common superscript in a column do not differ significantly ($P > 0.05$)]

energy levels. However, the globulin and albumin: globulin ratio did not alter among the treatments. The ND titre value at 21st day of age was significantly ($P < 0.01$) low in HE diet fed group than the NE and LE levels. CoQ10 at 20 mg/kg increased ($P < 0.01$) the titre than the 0 and 40 mg/kg level of supplementation. In all the three energy levels, supplementation CoQ10 at 20 mg/kg increased the ND titre value than the other two levels. However, there was no significant ($P > 0.05$) difference in the titre value was observed on 42 days of age.

Discussion

The CoQ10 supplementation had no influence on the haemoglobin (Hb) and packed cell volume (PCV %) among the groups. Similar reports on haemoglobin and PCV % were observed by various authors with same level of supplementation^{2,13,15}. Supplementation of CoQ10¹⁷ or its analogue CoQ4¹⁸ improved the haemopoietic activity in CoQ deficient or anaemic conditions. In the present study, the birds were normal, hence the CoQ10 supplement did not have any significant effect. However, the erythrocyte osmotic fragility per cent (EOF%) was found to be significantly reduced at 20 mg of CoQ10/kg of diet

than the unsupplemented and 40 mg/kg groups^{2,25}. The EOF% reflects the degree of erythrocyte membrane extensibility and cell geometry¹⁴. A lower EOF% reflects the stability of the cellular structure and extensibility of erythrocyte membrane. The susceptibility of broilers to ascites will be more as the erythrocyte osmotic fragility percentage is high. The reduction in osmotic fragility or increase in membrane stability at 20 mg CoQ10/kg might be due to the reduced form of CoQ10 in blood which served as an antioxidant to the erythrocytes membranes². Supplementation of CoQ10 for a period of 29 days showed reduction in EOF% but thereafter fragility increased which credited as an age related effect in broilers². However, the increased fragility at 40 mg/kg might be due to auto-oxidation of CoQ10 which would have resulted in mitochondrial ROS production or might be due to the influence of age of the birds on the absorption of CoQ10^{30,31}. Hence, CoQ10 at 20 mg/kg could be considered as an option for the treatment for ascites mortality³².

The interaction effect of CoQ10 and energy suggested that the haemoglobin and PCV were not influenced, whereas, the EOF% was found to decrease ($P < 0.05$) in CoQ10 supplemented birds over the

respective energy unsupplemented bird groups. The energy density of diet did not influence the haemoglobin, PCV, EOF% levels.

The significantly low level of blood glucose at 40 mg of CoQ10/kg of diet (304 *vs.* 327 and 328 mg/dL) supplement coincides with the previous findings³³. The blood glucose lowering effect due to CoQ10 supplementation was credited to the improvement in the β -cell functions, enhanced insulin sensitivity and thereby it could reduce insulin requirement for diabetic patients³⁴. Low energy (LE) fed birds had significantly low blood glucose level when compared to normal energy (NE) fed birds³⁵. At 40 mg of CoQ10/kg of diet supplemented in NE and LE diet, the blood glucose level was found to be reduced than the unsupplemented group.

CoQ10 supplementation did not alter the serum calcium, phosphorus, potassium and chloride levels but at 40 mg/kg of diet CoQ10 supplementation had high sodium level than the unsupplemented groups as well as the 20 mg/kg supplementation (168.12 *vs.* 154.95 and 160.72 meq). The increase in sodium level due to CoQ10 supplementation might have been due to its protective role on the kidney mitochondria and might increase the kidney electrolyte retention³⁶. High energy (HE) diet fed birds had significantly ($P < 0.01$) low serum calcium and higher sodium level. The low serum calcium level (8.49 *vs.* 10.53 and 12.08 mg/dL) could be attributed to the interaction between the fatty acids with cations (calcium) which might have resulted in the formation of insoluble calcium soap complex and lead to reduced calcium absorption from the gut^{37,38}. In the current study, the level of fat inclusion in the final phase was higher (8.0 %) in HE diet than the NE (5.8 %) and LE (3.6 %) diets. However, the increased serum sodium level in HE diet group might be due to its increased demand for glucose absorption and assimilation which might have enhanced the tubular sodium reabsorption. The serum phosphorus, potassium and chloride levels were not altered due to energy levels. In HE diet, supplementation of CoQ10 did not improve the serum calcium level over the unsupplemented group which is in agreement with the previous findings³⁹. The interaction effect of CoQ10 and energy levels suggested that in NE fed birds, serum calcium level increased with CoQ10 at 40 mg/kg of diet (12.70 *vs.* 11.58 and 11.98 mg/dL) over the unsupplemented and 20 mg/kg supplemented fed birds. The increased serum calcium level following CoQ10

supplementation was also observed in humans, which is attributed to the role of CoQ10 in increasing the production of vitamin D₃ in the mitochondria of the proximal tubule. Supplementation of CoQ10 even at 20 mg/kg of diet improved serum sodium level in the LE diet but not in NE and HE diet⁴⁰.

Serum protein profile (total protein, albumin, globulin and A/G ratio) was not influenced by the levels of CoQ10 supplementation. In all the three energy levels, the intake of protein was comparable, namely 657, 658 and 655 g between 0 and 6 wk in NE, LE and HE diets, respectively. But LE diet fed birds had lower ($P < 0.01$) serum total protein content (4.29 *vs.* 4.94 and 4.74 g/dL) and NE fed birds had higher ($P < 0.01$) serum albumin than the other two energy levels. When energy allowance was reduced without change in the protein intake, the serum protein and albumin was found to rise. The levels of globulin and albumin-globulin ratio (A/G ratio) were not influenced by energy levels. The serum total protein was lower in CoQ10 supplemented at 40 mg/kg in NE fed birds, whereas the same level of CoQ10 supplementation in LE increased the serum total protein over the respective energy unsupplemented birds. In the HE diet, CoQ10 supplementation did not have any influence. The albumin level was not influenced by the CoQ10 supplementation in NE diet; however, it was increased in low energy diet group supplemented with CoQ10 at 40 mg/kg of diet. In the HE diet, CoQ10 at 20 mg/kg increased the albumin level but not in 40 mg/kg which was comparable to the unsupplemented groups. The serum globulin and A/G ratio were not affected due to interactive effect of energy and CoQ10⁴¹.

The HI titre value against Newcastle disease was significantly higher in birds fed with CoQ10 at 20 mg/kg of diet than 0 and 40 mg/kg supplemented group at 21st day of growth period. CoQ10 supplementation in human subjects reported a significant increase in IgG levels in blood⁴². Among the energy levels, low energy groups had higher titre value and high energy diet fed birds. The low energy and protein diet was beneficial to broilers as it increases the antioxidant status and immune system. The titre value was significantly higher among all the energy groups supplemented with 20 mg/kg over the respective 0 and 40 mg/kg supplemented groups. The higher ND titre value in CoQ10 supplemented group might be due to its antioxidant activity⁴³. However, at 40 mg/kg CoQ10 supplemented group the titre value

was lower than the unsupplemented group which might be due to the auto-oxidation of CoQ10 at higher level of 40 mg/kg for prolonged supplementation⁴⁴.

Conclusion

In summary, CoQ10 supplementation in the broiler diet decreases the blood glucose level and decreases the erythrocyte fragility at 20 mg/kg which indicates the membrane stability activity of the CoQ10 and provides protection against ascites in broilers.

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