

Original Research Article

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Liver Total Glycogen, Mitochondrial Protein and CoQ10 Analysis in CoQ10 Supplemented Broiler Chicken

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ABSTRACT

A Pilot study was conducted to find out the level of CoQ₁₀ inclusion in broiler diet to bring about better performance and conducted during the period from November to December. Fifty numbers of day old broiler straight run chicks were wing banded, weighed and randomly allotted to five groups of ten chicks each based on the body weight. The treatments include, 20mg, 40mg, 60mg, 80mg, 100mg of CoQ₁₀/kg diet. Based on the results of FCR in pilot study experiment II was conducted with Two hundred and forty (240) numbers of day old broiler straight run chicks were wing banded, weighed and randomly allotted to 5 groups 6 replicates of eight chicks each based on the body weight. The treatments were, Basal diet without CoQ₁₀ supplementation, Low energy diet without CoQ₁₀ supplementation, Low energy diet with 20mg of CoQ₁₀/kg diet, Low energy diet with 40mg of CoQ₁₀/kg diet, Low energy diet with 60mg of CoQ₁₀ /kg diet. Glycogen content of the liver was estimated as per the method of Seifter *et al.*, (1949). Hepatic mitochondria were obtained by differential centrifugation as outlined by Cawthon *et al.*, (1999). Mitochondrial protein concentration was estimated as per the method of Lowry *et al.*, (1971). The CoQ₁₀ content of liver was extracted as per the method of (Barros *et al.*, 2011) and liver CoQ₁₀ level was estimated as per the method of (Ioana *et al.*, 2009). The mean liver glycogen ($\mu\text{g/g}$) was 152.66, 167.55, 169.14, 171.13 in the treatment groups T2 to T5 as compared with 158.45 in the control group of broiler chickens. The mean liver glycogen was significantly ($P<0.05$) highest in the Co Q₁₀ supplemented group of birds (T3, T4 and T5) in comparison to control and T2 group. There was no significant difference in mean liver glycogen among the treatment groups T4 and T5. The mean liver glycogen was significantly ($P<0.05$) lowest in the control and T2 group of birds. The mean liver mitochondrial protein concentration ($\mu\text{g/g}$) was 15.90, 24.70, 22.00 and 23.20 in the treatment groups T2 to T5 as compared with 19.70 in the control group of broiler chickens. The mean liver mitochondrial protein concentration was significantly ($P<0.05$) highest in the CoQ₁₀ supplemented group of birds (T3, T4 and T5) in comparison to control and T2 groups. The mean liver CoQ₁₀ (mg/kg) was 49.26, 54.53, 55.75 and 55.74 in the treatment groups as compared with 53.83 in the control group of chicken.

Keywords

CoQ₁₀,
Mitochondrial
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Introduction

India has vast resource of livestock and poultry, which play a vital role in improving the socio-economic conditions of the rural masses. Among different countries in the World, India owns considerable proportion of livestock and poultry wealth. As per 2012 livestock census, India possessed 512.06 million livestock and 729.21 million poultry. However, the annual growth rate of livestock population in India during 2007 to 2012 implied that the total livestock population had marginal negative trend at 0.68 per cent and the total poultry population had annual compound growth rate of 2.36 per cent. The scenario of livestock population in Tamil Nadu over the last two census periods implied that the total livestock and poultry population had negative growth rate of 5.88 per cent and 1.74 per cent, respectively. Poultry industry occupies a major position in the livestock sector of agricultural production because birds reproduce much quicker to produce meats and eggs for human consumption within the shortest possible time (Sanni and Ogundipe, 2005). An increasing in per capita consumption by one egg and 50 grams of poultry meat can create employment for about 26,000 persons per year (Kazi, 2003). The present per capita availability of poultry meat is 1.8 kg against the requirement of 11 kg, as per the National Committee on Human Nutrition in India (www.indiastat.com, 2006).

In India, the production of broilers increased from 1.89 lakh tones in 1989-90 to 23.13 lakh tones in 2009-10, at a compound annual growth rate of 13.21 per cent. In broiler production, India stands 5th in the world with 2.31 million tones of broiler meat, contributing Rs 9000 crore to the national economy. Coenzyme Q₁₀ (CoQ₁₀) is a naturally occurring compound with a ubiquitous distribution in nature. Based on an isoprenoid moiety, the presence of various CoQ homologs has been confirmed. CoQ₁₀,

which has a polyisoprene chain containing 10 isoprene units, was predominant in humans and birds, whereas CoQ₉ was predominant in rats and mice (Ibano *et al.*, 2002). Hagavan *et al.*, (2006) stated that CoQ₁₀ acts as an electron carrier in the mitochondrial respiratory chain and as a lipid-soluble antioxidant. Kamisoyama (2010) found that dietary CoQ₁₀ significantly reduced the levels of cholesterol in the egg yolks of laying hens, but the mechanisms underlying this reduction in egg-yolk cholesterol have not been identified.

In chickens, dietary CoQ₁₀ supplementation reduced broiler chickens susceptibility to ascites, perhaps as a result of improved hepatic mitochondrial function, respiratory chain-related enzyme activities, and the mitochondrial antioxidant activity of CoQ₁₀. Nakamura (1996) stated that fed broiler chicks diets supplemented with coenzyme Q₉, an analogue of CoQ₁₀, at 40 mg/kg and showed that dietary coenzyme Q₉ supplementation was beneficial in reducing ascites incidence in broiler chicks.

Geng *et al.*, (2010) reported that the mortality of broilers due to ascites was reduced by L-carnitine and CoQ₁₀ supplementation alone and in combination the reason may be partially associated with the antioxidative effects of these substances.

In broiler chicken higher body weight gain and better feed efficiency with less feed cost per kilogram weight gain was observed in high energy group supplemented with 20 mg of CoQ₁₀/kg diet and the dressing percentages, weight of giblet, liver, spleen, abdominal fat, intestinal length were not significantly altered by CoQ₁₀ supplementation but the heart weight, gizzard weight and ascites heart weight (AHI) were significantly decreased due to CoQ₁₀ supplementation (gopi *et al.*, 2014).

Materials and Methods

Experiment I (Pilot study)

Pilot study was conducted to find out the level of CoQ₁₀ inclusion in broiler diet to bring about better performance. Pilot study was conducted during the period from November to December-2015. Fifty numbers of day old broiler straight run chicks were wing banded, weighed and randomly allotted to five groups of ten chicks each based on the body weight.

The treatments include, 20mg, 40mg, 60mg, 80mg, 100mg of CoQ₁₀/kg diet.

Experiment II

Two hundred and forty (240) numbers of day old broiler straight run chicks were wing banded, weighed and randomly allotted to 5 groups 6 replicates of eight chicks each based on the body weight. The treatments were (T₁–T₅), Basal diet without CoQ₁₀ supplementation, Low energy diet without CoQ₁₀ supplementation, Low energy diet with 20mg of CoQ₁₀/kg diet, Low energy diet with 40mg of CoQ₁₀ /kg diet, Low energy diet with 60mg of CoQ₁₀ /kg diet.

Total glycogen

Glycogen content of the liver was estimated as per the method of Seifter *et al.*, (1949). Briefly, immediately after slaughter 1g of liver is dropped into a previously weighed test tube containing 3ml of 30% KOH. After delivery of the sample, the test tube and its contents were reweighed and weight of sample determined by difference. The tissue was digested by heating the tube for 20 min in a boiling water bath and then the digest was cooled. 5ml of aliquot was taken from this digest and transferred to the 50ml volumetric flask. The contents were diluted to the mark with double distilled water. Again 5ml of

aliquot was taken from this contents and diluted in another 50ml volumetric flask.. Five ml aliquots of the final dilution were pipette into a glass tube. While submerged in cold water the test tube received 10 ml of the anthrone reagent. The tubes were vortexed and absorbance was taken at 620nm in UV spectrophotometer.

Mitochondrial protein concentration

Preparation of mitochondria

Hepatic mitochondria were obtained by differential centrifugation as outlined by Cawthon *et al.*, (1999). Approximately 2 g of liver tissue was suspended in 5ml of isolation media (PH7.4) containing 220mM d-mannitol, 70mM sucrose, 2mM HEPES, 0.5 mg/ml BSA and 1mM EGTA. The tissue was homogenized with a hand driven glass-teflon homogenizer. Aliquots were transferred into centrifuge tubes & centrifuged twice for 10min at 600g. The pellets containing nuclei and cell debris were discarded and the supernatant was centrifuged for 15 min (7750g).

The mitochondrial pellets were resuspended in an isolation buffer (PH 7.0) containing 220mM d-mannitol, 70mM sucrose, 2mM HEPES and 0.5mg/ml BSA and were washed twice. Mitochondria were resuspended in incubation media (210mM d-mannitol, 70mM sucrose, 2mM HEPES and 10mM succinate) and placed on ice.

Estimation of mitochondrial protein concentration

Mitochondrial protein concentration was estimated as per the method of Lowry *et al.*, 1971. Briefly, standard curve was prepared by using BSA as standard at different concentration. 200µL of sample was added with 2 mL of alkaline copper sulphate

solution and then 0.2mL of Folin Ciocaltaeau was added in the test tube. The reagents were incubated for 30min. The absorbance was recorded by using spectrophotometer at 660nm.

Liver CoQ₁₀

The CoQ₁₀ content of liver was extracted as per the method of (Barros *et al.*, 2011) and liver CoQ₁₀ level was estimated as per the method of (Ioana *et al.*, 2009).

Results and Discussion

Based on the body weight and feed conversion ratio of the birds in experiment 1 concluded that the mean body weight was not affected by CoQ₁₀ supplementation at graded levels. Supplementation of broiler chickens with normal diet without CoQ₁₀ supplementation (T1) and low energy diet (100kcal less) with CoQ₁₀ supplementation (20mg/kg diet) (T3) resulted in increased body weight gain as compared to other treatment groups T2, T4 and T5. CoQ₁₀ supplementation did not show any significant difference in the mean body weight gain. But it could show significant difference in the mean feed conversion ratio. The group supplemented with 20mg of CoQ₁₀/kg diet could show significantly lowest mean FCR in comparison to control group and other CoQ₁₀ supplemented groups (Table 1).

Effect on liver glycogen

The mean liver glycogen ($\mu\text{g/g}$) was 152.66, 167.55, 169.14, 171.13 in the treatment groups T2 to T5 as compared with 158.45 in the control group of broiler chickens. The mean liver glycogen was significantly ($P < 0.05$) highest in the CoQ₁₀ supplemented group of birds (T3, T4 and T5) in comparison to control and T2 group. There was no significant difference in mean liver glycogen

among the treatment groups T4 and T5. The mean liver glycogen was significantly ($P < 0.05$) lowest in the control and T2 group of birds.

The result of the present study is agreement with the findings of Oztay (2007) in Swiss black mice.

Effect on liver mitochondrial protein concentration

The mean liver mitochondrial protein concentration ($\mu\text{g/g}$) was 15.90, 24.70, 22.00 and 23.20 in the treatment groups T2 to T5 as compared with 19.70 in the control group of broiler chickens. The mean liver mitochondrial protein concentration was significantly ($P < 0.05$) highest in the CoQ₁₀ supplemented group of birds (T3, T4 and T5) in comparison to control and T2 groups.

There was no significant difference in the mean liver mitochondrial protein concentration of T3, T4 and T5 groups. The mean liver mitochondrial protein concentration was significantly ($P < 0.05$) lowest in the control and T2 group of birds.

The result of the present study is in agreement with the findings of Huang *et al.*, (2011) in broilers, Geng *et al.*, (2006) in broilers and Kwong *et al.*, (2002) in rats.

Effect on liver coenzyme Q

The mean liver CoQ₁₀ (mg/kg) was 49.26, 54.53, 55.75 and 55.74 in the treatment groups as compared with 53.83 in the control group of chicken. The mean liver CoQ₁₀ was ($P < 0.05$) significantly highest in the T3, T4 and T5 group in comparison to control and T2 group. There was no significant difference in the mean liver Co Q₁₀ level among the treatment groups T3, T4 and T5 (Table 2).

Table.1 Mean (\pm S.E) cumulative feed conversion ratio of broiler chickens as influenced by dietary inclusion of Co Q10 at graded level

Treatment groups	Body weight	FCR
Control	1850.6 ^a \pm 36.81	1.93 ^c \pm 0.02
T1(20mg)	1922.3 ^c \pm 40.12	1.58 ^a \pm 0.06
T2(40mg)	1892.5 ^{ab} \pm 42.52	1.60 ^a \pm 0.02
T3(60mg)	1899.8 ^b \pm 39.27	1.58 ^a \pm 0.06
T4(80mg)	1891.2 ^{ab} \pm 37.68	1.68 ^{ab} \pm 0.04
T5(100mg)	1889.8 ^{ab} \pm 41.73	1.64 ^{ab} \pm 0.02
Means within the same column bearing different superscripts differ significantly (P<0.05)		

Table.2

Treatment	Mitochondrial protein (μ g/g)	Liver COQ(mg/kg)	Total glycogen (μ g/g)
T1 (control)	19.70 ^b \pm 0.84	53.83 ^b \pm 0.72	158.45 ^c \pm 0.64
T2	15.90 ^c \pm 0.57	49.26 ^a \pm 0.35	152.66 ^d \pm 0.64
T3	24.70 ^a \pm 1.50	54.53 ^b \pm 0.43	167.55 ^a \pm 0.25
T4	22.00 ^a \pm 1.03	55.75 ^b \pm 0.78	169.14 ^b \pm 0.76
T5	23.20 ^a \pm 0.73	55.74 ^b \pm 0.69	171.13 ^b \pm 0.62

Means bearing same superscript do not differ significantly (P<0.05)

The results of weight gain and feed conversion ratio are in accordance with that of Geng *et al.*, (2004), Geng *et al.*, (2010), Huang *et al.*, (2011), Gopi *et al.*, (2014), and Honda *et al.*, (2013). However the result of the present study does not agree with findings of Gopi *et al.*, (2014) during pre-starter and starter periods. It is inferred that CoQ₁₀ supplementation would improve FCR when less energy diet (100kcal) is practised. Body weight of broilers was increased by supplementation with CoQ₁₀ at 20mg/kg diet, but not at 40 and 60mg/kg diet. This implied that CoQ₁₀ might have an interaction with age because the positive effect was more pronounced in later period than earlier period. Another possible reason for not producing better performance in birds fed higher CoQ₁₀ in diet might be due to the process of auto-oxidation of CoQ₁₀ in the body (Turrens *et al.*, 1985). The other reasons might be that long term supplementation with CoQ₁₀ at 40mg/kg feed might weaken the beneficial effects of CoQ₁₀ (Huang *et al.*, 2011). The higher feed intake in low energy diet (T2) might be due to the bird's trying to meet its energy requirement. Gopi *et al.*, (2014). The reason for the accumulation of higher glycogen may be due to positive effect of CoQ₁₀ on glycogen synthesis. Mitochondrial protein content in CoQ₁₀ fed birds were significantly increased as compared to control. The increase (13-21%) in the mitochondrial protein may be due to the positive effect of CoQ₁₀ on mitochondrial enzymes and their activities. Geng *et al.*, (2006) also found enhanced cytochrome oxidase and H⁺-ATPase activity when CoQ₁₀ fed to broilers and observed the susceptibility of broiler chicken to ascites. Similarly, Matthews *et al.*, (1998) recorded higher brain mitochondrial enzymes in rats fed CoQ₁₀. Addition of CoQ₁₀ at different level in the broiler diet did not accumulate CoQ₁₀ in liver tissues of birds T3, T4 and T5 groups. The level was almost similar to control. However,

the group T2 level was low compared to all the groups. The possible explanation for no change in the liver CoQ₁₀ content of treated birds may be due to unknown mechanism of regulating liver CoQ₁₀ level (Krizman *et al.*, 2012). It has been observed that transfer of CoQ₁₀ to various organs was probably linked to the longer periods of feeding (Krizman *et al.*, 2012).

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