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Muscle Total Lipid, Total Protein, Total Antioxidants, Co Q and Mitochondrial Concentration Analysis in Coq_{10} Supplemented Broiler Chicken

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A B S T R A C T

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 CoQ_{10} supplementation, Low energy diet without CoQ_{10} supplementation, Low energy diet with 20mg of CoQ_{10} /kg diet, Low energy diet with 40mg of CoQ_{10} /kg diet, Low energy diet with 60mg of CoQ_{10} /kg diet. Muscle total lipid was estimated gravimetrically by using Folch method of lipid extraction. The antioxidant ability of muscle was determined by the method described by Benzie and Strain. One gram of muscle tissue was taken and CoQ_{10} was extracted by using solvents methanol:hexane. Protein content of meat was estimated by kjeldhal method according to procedure described in AOAC. 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. There was a significant difference in protein content of the breast muscle was observed between treatment groups and control. The protein accretion on the muscle ranged from 7 to 9%. The result of the present study agrees with the average value of 22% in breast muscle of broiler chicken.The effect of CoQ_{10} on muscle lipid did not exhibit any variation. It is understood from the result that CoQ_{10} on lipid accretion in muscle is negligible. The results of the study clearly proved that antioxidants level was influenced by the level of CoQ_{10} in the diet. Further the present study also shown that antioxidants status was lower in birds fed less energy in the diet. Our findings agrees with many earlier observations (Littarru *et al.,* 2007), Mates *et al.,* (1999), Kapoor and Kapoor (2013) and Fathi (2015) on antioxidants status due to supplementation of $CoQ_{10.}$ There was no significant difference existed in the mean breast muscle CoQ_{10} for T3 and T4 group of birds. The mean breast muscle CoQ_{10} was significantly ($P<0.05$) lower in low energy diet without CoQ_{10} supplemented group (T2). The mean muscle mitochondrial protein concentration was significantly ($P<0.05$) higher in the CoQ_{10} supplemented group of birds (T3, T4 and T5) in comparison to T2 and groups. There was no significant difference in the mean mitochondrial protein concentration of T3, T4 and T5 groups of chicken.

Introduction

Coenzyme Q_{10} (CoQ_{10}) 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 (lbano *et al.*, 2002). Kamisoyama (2010) found that dietary CoQ_{10} 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 CoQ_{10} 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 CoQ_{10}. Nakamura (1996) stated that fed broiler chicks diets supplemented with coenzyme Q9, an analogue of CoQ_{10}, 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 CoQ_{10} 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 CoQ_{10}/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 CoQ_{10} supplementation (gopi *et al.,* 2014).

Reactive oxygen species (ROS) are mainly mitochondrial derived and directly affects the vascular remodeling and also causes pulmonary hypertension and growth rate (Bautista-Ortega *et al.,* 2010). Broilers fed to an energy dense diet were more susceptible to oxidative stress (Cardoso *et al.,* 2010), whereas restricted feeding decreases oxidative damage (Ozkan *et al.,* 2010). However, an early feed restriction has severely affected the growth performance and lipid metabolism in broilers (Zhan *et al.,* 2007; Saber *et al.,* 2011).

Materials and Methods

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 (T_1 –T_5), Basal diet without CoQ_{10} supplementation, Low energy diet without CoQ_{10} supplementation, Low energy diet with 20mg of CoQ_{10} /kg diet, Low energy diet with 40mg of CoQ_{10} /kg diet, Low energy diet with 60mg of CoQ_{10} /kg diet.

Muscle total lipid

Muscle total lipid was estimated gravimetrically by using Folch method of lipid extraction. One gram muscle tissue was homogenized with 10 ml of Folch solution ($CHCl_3$:CH_3OH). The chloroform layer containing lipid was taken in preweighed petridishes. The contents were dried in a hot air oven until the consequent weights were uniform. The results were expressed in %.

Estimation of muscle protein

Protein content of meat was estimated by kjeldhal method according to procedure described in AOAC (1995).

Estimation of muscle mitochondrial protein

Approximately 2g of muscle tissue was taken and mitochondrial protein concentration was estimated.

Preparation of mitochondria

Hepatic mitochondria were obtained by differential centrifugation as outlined by Cawthon *et al.,* (1999). Approximately 2 g of muscle tissue was suspended in 5ml of isolation media (PH 7.4) containing 220mM d-mannitol, 70mM sucrose, 2mM HEPES, 0.5 mg/ml BSA and 1mM Ethylene glycol-bis beta amino ethyl ether NNN'N' tetra acetic acid (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 at 7750g for 15 min.

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

Mitochondrial protein concentration was estimated as per the method of Lowry *et al*., (1951). 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 a test tube. The reagents were incubated for 30 min. The absorbance was recorded by using spectrophotometer at 660nm.

Estimation of muscle total antioxidants

The antioxidant ability of muscle was determined by the method described by Benzie and Strain (1996).

FRAP reagent:

0.3M Sodium acetate buffer (PH 3.6)-25ml

0.01N 2,4,6-tripyridyl-S-triazine (TPTZ) in 0.04M HCl-2.5ml

0.02M ferric chloride ($FeCl_3.6H_2O$) -2.5ml

0.5g of muscle tissue was homogenized with 5ml of phosphate buffer saline and centrifuged at 3000rpm for 15 min.The final muscle extract was used for total antioxidant assay. Briefly, 3ml of FRAP reagent was prewarmed to 50ºC and mixed with 100µL of muscle extract. The absorbance of the blue Fe-II-complex at 593nm was recorded using spectrophotometer after 5min incubation at 37 ºC. Total antioxidant in muscle extract was expressed as µmol/g of muscle tissue.

Estimation of muscle Co Q level

One gram of muscle tissue was taken and CoQ_{10} was extracted by using solvents methanol:hexane.. 100µL of muscle extract, 100µL of Tween 20, 1.5ml of methanol and 1.5 ml of hexane were added to a glass tube. The samples were subjected to mechanical shaking for one min and centrifuged at 1752.8g for 10 min at 10ºC. The residues were evaporated without heating under a flow of nitrogen for 20 min. Then the residues were resuspended in 3ml of methanol:hexane (2:1). The samples were again homogenized under vortex mechanical shaking for 15 sec and rotary shaking for 15 min. The final solution was used for CoQ_{10} assay. The absorbance was taken at 340nm in UV spectrophotometer.

Results and Discussion

Muscle lipid and protein

The mean breast muscle protein (%) was 15.96, 16.88, 16.63 and 17.31 for T2, T3, T4 and T5 respectively compared to 15.77 (%) in the control group (Table 1). Also, the highest mean muscle protein noticed in the group T5 but no significant difference was observed between T3 and T4 group of birds. The reason for this may be due to higher CoQ_{10} in the muscle but the higher protein content did not reflect upon the body weight. This needs further investigation. The Present study also revealed that lower mean muscle protein in control and low energy diet without CoQ_{10} supplemented group of birds (T2)

Overall, the protein content was lower in the breast muscle when compared to normal muscle protein of 20-22%. The higher THI recorded throughout the study might be responsible for the reduction in the protein content of the muscle. (Table 1)

The mean breast muscle lipid (%) was 1.11, 1.09, 1.10 and 1.08 for T2, T3 and T5 respectively as compared with 1.13 (%) in the control group of broiler chicken. The effect of CoQ_{10} on muscle lipid did not exhibit any variation. It is understood from the result that CoQ_{10} on lipid accretion in muscle is negligible. The observations of our study is in accordance with the earlier workers (Chartrin *et al*., 2004) and (Mane *et al*., 2014). The above authors recorded 0.75-1.5%of fat on an average in breast muscle of broiler chicken.

However, there was a significant difference in protein content of the breast muscle was observed between treatment groups and control. The protein accretion on the muscle ranged from 7 to 9%. The result of the present study agrees with the average value of 22% in breast muscle of broiler chicken.

Antioxidants

Table 1 shows, the mean breast muscle antioxidants (µmol/g) were 3.25, 5.53, 5.54 and 6.25 for T2, T3, T4 and T5 respectively as compared with 4.08 (µmol/g) in the control group of broiler chicken.

The results of the study clearly proved that antioxidants level was influenced by the level of CoQ_{10} in the diet. Further the present study also shown that antioxidants status was lower in birds fed less energy in the diet. Our findings agrees with many earlier observations (Littarru *et al*., 2007), Mates *et al.*, (1999), Kapoor and Kapoor, (2013) and Fathi (2015) on antioxidants status due to supplementation of CoQ_{10}. Many earlier researchers found that CoQ_{10} supplementation improved antioxidants capacity of broiler chicken and in other animals.

Coenzyme Q

The mean muscle CoQ_{10} (mg/kg) were 7.57, 8.61, 8.91 and 9.17 in the treatment groups T2 to T5 as compared with 8.78 (mg/kg) in the control group of broiler chickens (Table 1).

The mean breast muscle CoQ_{10} content was significantly ($P<0.05$) high in the T5 than T3 and T4. There was no significant difference existed in the mean breast muscle CoQ_{10} for T3 and T4 group of birds. The mean breast muscle CoQ_{10} was significantly ($P<0.05$) lower in low energy diet without CoQ_{10} supplemented group (T2).

The supplementation of CoQ_{10} did not improve CoQ_{10} content of the breast muscle except in T5. This finding of our study concurs with the observation of low CoQ_{10} level in breast muscle compared to leg muscle (Krizman *et al*., 2012). The present study lacks the information on leg muscle CoQ_{10} level to compare breast muscle.

Table.1 Mean (±S.E) muscle lipid, protein, COQ, Antioxidants, mitochondrial protein concentration of breast muscle fed CoQ_{10} at graded levels

Treatment	Lipid (%)	Protein (%)	CoQ_{10} (mg/kg)	Antioxidants (µmol/g)	Mitochondrial protein (µg/g)
Control	1.13±0.24	^{a}15.77 ±0.44	^{c}8.78 ±0.44	^{b}4.08 ±0.18	^{b}5.72 ±0.30
T2	1.11±0.30	^{a}15.96 ±0.31	^{a}7.57 ±0.62	^{a}3.25 ±0.13	^{b}5.27 ±0.27
T3	1.09±0.21	^{b}16.88 ±0.38	^{b}8.61 ±0.27	^{c}5.53 ±0.28	^{a}8.90 ±0.28
T4	1.10±0.29	^{b}16.63 ±0.32	^{c}8.91 ±0.48	^{c}5.54 ±0.34	^{a}8.27 ±0.43
T5	1.08±0.24	^{c}17.31 ±0.43	^{d}9.17 ±0.36	^{d}6.25 ±0.29	^{a}8.54 ±0.27

Means within the same column bearing different superscripts differ significantly (P<0.05).

Table.2 Temperature and humidity of poultry house

PERIODS	TEMPERATURE(°C)			RH (%)			THI (°C)		
	MOR (6 -8A.M)	A.N (1-2P.M)	EVE (5-7P.M)	MOR (6 -8A.M)	A.N (1-2P.M)	EVE (5-7P.M)	MOR (6 -8A.M)	A.N (1-2P.M)	EVE (5-7P.M)
PRESTARTER 2WKS	27.07 ±0.54	34.57 ±0.27	31.36 ±0.50	60.86 ±0.48	34.36 ±0.36	43.29 ±0.39	26.40 ±0.44	32.80 ±0.23	30.00 ±0.43
STARTER 2-4WKS	25.79 ±0.48	35.36 ±0.28	30.71 ±0.49	65.50 ±0.56	31.43 ±0.81	45.00 ±0.58	25.83 ±0.52	33.14 ±0.59	29.72 ±0.43
FINISHER 4-6WKS	27.53 ±0.50	35.80 ±0.56	28.93 ±0.25	59.73 ±0.52	29.40 ±0.56	55.00 ±0.51	26.11 ±0.42	33.35 ±0.67	29.14 ±0.46

Further investigation in large samples are required to explain the variations between muscles.

Mitochondrial protein

The mean muscle mitochondrial protein concentration (mg/g) was 5.27, 8.90, 8.27 and 8.54 in the treatment groups T2 to T5 as compared with 5.72 in the control group of broiler chickens.

The mean muscle mitochondrial protein concentration was significantly ($P<0.05$) higher in the CoQ_{10} supplemented group of birds (T3, T4 and T5) in comparison to T2 and groups. There was no significant difference in the mean mitochondrial protein concentration of T3, T4 and T5 groups of chicken. On the other hand, the mean mitochondrial protein concentration was significantly ($P<0.05$) lower in the T2 and control group of birds.

The result of the present study agrees with the average value of 22% in breast muscle of broiler chicken. The literature on muscle lipid and protein level due to supplementation of CoQ_{10} in broiler chickens was scarce. Hence, a detailed discussion was not attempted.

The results of the study clearly proved that antioxidants level was influenced by the level of CoQ_{10} in the diet. Further the present study also shown that antioxidants status was lower in birds fed less energy in the diet. Our findings agrees with many earlier observations (Littarru *et al*., 2007), Mates *et al.,* (1999), Kapoor and Kapoor (2013) and Fathi (2015) on antioxidants status due to supplementation of CoQ_{10}. Many earlier researchers found that CoQ_{10} supplementation improved antioxidants capacity of broiler chicken and in other animals.

The supplementation of CoQ_{10} did not improve CoQ_{10} content of the breast muscle except in T5. This finding of our study concurs with the observation of low CoQ_{10} level in breast muscle compared to leg muscle (Krizman *et al*., 2012). The present study lacks the information on leg muscle CoQ_{10} level to compare breast muscle. Further investigation in large samples are required to explain the variations between muscles.

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

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