

Original Research Article

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Liver HMG Co a Reductase Enzyme Estimation in CoQ₁₀ Supplemented Broiler Chicken

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ABSTRACT

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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 Co Q /kg diet. The minimum and maximum temperature inside the poultry house were recorded daily by using dry and wet bulb thermometer. The data were used to assess the impact of temperature on performance broiler chicken fed CoQ₁₀. At the end of the experiment (42nd day), ten birds per treatment group were randomly selected and slaughtered by halal method. Liver HMG CO A Reductase enzyme activity was estimated as per the method of Liang *et al.*, 2015. The result of the mean liver HMG Co A reductase ($\mu\text{mol}/\text{min}/\text{mg}$ protein) were 6.96, 5.26, 5.39 and 5.36 for T2, T3, T4 and T5 respectively, compared to the control 6.94.

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. 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 (Ioana, *et al.*, 2009). Dietary CoQ₁₀ significantly reduced egg yolk cholesterol content and suppressed hepatic hydroxymethylglutaryl-CoA reductase (HMGR) activity (Honda *et al.*, 2013).

Materials and Methods

Liver HMG Co A reductase enzyme activity was estimated as per the method of Liang *et al.*, 2015.

Briefly, chicken liver samples were homogenized in three fold volume by using ice cold buffer A contains 100mM sucrose, 50mM KCL, 40mM potassium phosphate, 30 mM potassium EDTA with PH 7.2. The homogenate was centrifuged for 15 min at 12000g. The supernatant was removed and centrifuged again for 15 min at 12000g and the resultant supernatant was centrifuged for 60 min at 100000g in an ultracentrifuge (Optima XPN-100 Ultracentrifuge, Beckman Coulter, U.S.A).

The pellets were resuspended in buffer A and centrifuged at 100000g for 60 min. Then the pellets were resuspended in buffer B (buffer A plus 10mM dithiothreitol) and homogenized to prepare microsomal suspension. Those procedures were performed at 4°C and the final suspension was stored at -20 °C.

The microsomal suspension was thawed at 37 °C in a water bath. An equal volume of buffer B supplemented with 50% glycerol was added. The mixture was homogenized thoroughly and incubated at 37 °C for 60 min. The suspension was diluted three fold with buffer B and then centrifuged at 100000g for 60 min at 25 °C. The supernatant containing solubilised HMG Co A reductase was used for the assay. The supernatant protein concentration was determined using the Bradford dye method and the protein concentration was adjusted to 1mg/ml. The total reaction volume was 1ml.

Both the microsomal protein (total protein concentration 200µg/ml) and 100µM NADPH were added to buffer C (0.2M KCl, 0.16M

potassium phosphate, 0.0004M EDTA and 0.01M dithiothreitol). The reaction was initiated with 50µM HMG Co A and was allowed to proceed for 60 min. When the reaction was finished, the OD at 340nm was measured using spectrophotometer.

Results and Discussion

The result of the mean liver HMG Co A reductase (µmol/min/mg protein) were 6.96, 5.26, 5.39 and 5.36 for T2, T3, T4 and T5 respectively, compared to the control 6.94.

The result of present study is in agreement with the findings of Omkumar *et al.*, (1992) in rats, Honda *et al.*, (2013) in layers and Santos (2014) in rats. All these authors suggested that CoQ₁₀ in diet reduced the activity of HMG CoA gene not in the transcription level but influenced at the post transcription level. In rats supplemented with ubiquinone showed significantly decreased serum cholesterol, activity of HMG co A reductase was decreased in liver microsomes and increased ubiquinone concentration in liver and its microsomes. (Omkumar *et al.*, 1992).

In an experiment with layer chicks between 0-21 days of age supplementation of CoQ₁₀ at 0.02, 0.4 and 0.8 percent, Honda *et al.*, (2010) reported reduced hepatic total cholesterol, plasma cholesterol and VLDL cholesterol at 0.04 percent and above inclusion level. However the plasma HDL, LDL cholesterol and total bile acids were not significantly influenced by supplementation. The reduction in cholesterol level was due to decreased enzymatic activity of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) in the liver, whereas, it had no influence on the enzymatic activity of 3-hydroxy-3-methyl glutaryl coenzyme A synthase (HMGS).

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