



# Impact of Diet Supplemented by Coconut Milk on Corticosterone and Acute Phase Protein Level under High Stocking Density

Majid SHAKERI<sup>1\*</sup>, Ehsan OSKOUEIAN<sup>2</sup>, Pardis NAJAFI<sup>1</sup>

<sup>1</sup>Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia

<sup>2</sup>Agriculture Biotechnology Research Institute of Iran (ABRII)-East and North-East Branch, P.O.B. 91735/844 Mashhad, Iran

**\*Sorumlu Yazar /  
Corresponding Author:**

Majid SHAKERI  
e-mail: majidmarch@live.com.my

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## Abstract

The purpose of this study was to investigate effects of coconut milk supplementation on corticosterone and acute phase protein level under high stocking density. A total 300 Cobb 500 male chicks were placed in cages and stocked as 10 birds/cage (normal stocking density) and 15 birds/cage (high stocking density). The treatments were as (i) control diet and stocked at 10 and 15 birds/cage (ii) control diet + 3% coconut milk from 1-42 day and stocked at 10 and 15 birds/cage (iii) control diet + 5% coconut milk from 1-42 day and stocked at 10 and 15 birds/cage. On day 42, 20 birds per treatment were slaughtered to collect blood samples. The results showed higher level of corticosterone and acute phase protein level in control diet compare to other supplemented diets with coconut milk. In conclusion, coconut milk decreased the level of corticosterone and acute phase protein when chicks were subjected to high stocking density.

## Introduction

The poultry meat industry is one of the biggest meat industries around the world and the main purpose of this industry is based on quantity of products beside the quality. In order to increase the amount of meat products, producers attempt to increase the number of chicken per square meter. From the economic points of this industry, high stocking density has some negative impacts on immune system and welfare of chickens such as illness (Bessei, 2006; Feddes et al., 2002), heat stress (Imaeda, 2000) and increase of ammonia level and in follow up can affect the immunity (Cravener et al., 1992; Heckert et al., 2002). Therefore, supplementing diet with some additives such as coconut milk have been a purpose of many studies to eliminate the negative impacts of high stocking density on chicken performance and improve the immunity.

Coconut fruit (*Cocos nucifera*) is introduced as one alternative to improve immune system when birds are subjected to stress or uncomfortable housing conditions. Coconut consists of fatty acids that work as

source of energy and antimicrobial effects. Some fatty acids are available in large amount in coconut as lauric acid (50%) and capric acid (8%). Luaric acid that has antimicrobial and antiviral effects in animal body (Enig, 1998), might be useful in animal diet to improve immunity. It can be available in large amount in coconut milk (Mensink et al., 2003). Intahphuak et al. (2010) showed that specific percentage of coconut oil can improve the immune system of rats when it supplemented in diet.

Coconut milk is acquired through the grating of a brown coconut, mixing the resulting substance with a small amount of water to dissolve the fat present in the grated meat. The main portion of coconut milk consists of lauric acid that can increase the level of cholesterol by increasing lipoprotein cholesterol (Mensink et al., 2003). Coconut milk has many benefits on health such as medium chain fatty acids which is healthier than other saturated fat products and can metabolize in body easier, contains mineral and vitamin in high level and increase the response of the immune system in rat

(Intahphuak et al., 2010). Coconut milk consists of high percentage of medium-chain fatty acid which is useful to increase weight without increasing cholesterol levels (St-Onge and Jones, 2002). Moreover, it is rich for protein (Capulso et al., 1981) which can provide more essential amino acids for body (Mepba and Achinewhu, 2003). Therefore, the reason of using coconut milk instead of coconut oil was related to high levels of saturate fat in coconut oil which can increase the level of harmful LDL cholesterol in blood (Gomez et al., 2000).

The aim of this study was to investigate impact of coconut milk supplementation on corticosterone and acute phase protein level when chicks were subjected to high stocking density.

## Materials and Methods

### Coconut extract preparation

Coconuts selected from mature nuts (12 months age) which has hard and thick meat. The brown skin was removed. In order to decrease initial microbial load, the coconut meat was washed with water (contain 100 ppm H<sub>2</sub>O<sub>2</sub>) at 80 °C for 10 minutes (Arumughan et al., 1993). The obtained meat was washed and grated by machine (mix with water). The prepared milk was extracted from the comminuted endosperm. The grating mix with water was repeated twice to maximize the extraction.

### Birds, husbandry and housing

The project was carried out under Department of Agriculture at University Putra Malaysia. A total of 300 a-day-old *Cobb 500* male broiler chicks were placed in cages immediately after arrival. On day 1, the chickens were allocated to cages (1m × 1m) as 10 and 15 birds/cage as normal stocking density (40 birds each treatment) and high stocking density (60 birds per treatment) with 4 replicates. The temperature (26-35°C) and humidity (65-80%) were determined by using digital Thermohygrometer (Testo, Malaysia). The cages were equipped with movable walls to change the size of cages in case of mortality, and to keep the size of cages according to the densities. The light was provided continuously during the experiment.

### Experimental treatments

The project was designed as treatments with or without coconut milk supplementation and stocked at two different densities. The treatments were as: (i) control diet and stocked at 10 and 15 birds/cage (ii) control diet + 3% coconut milk from 1-42 days and stocked at 10 and 15 birds/cage (iii) control diet + 5% coconut milk from 1-42 days and stocked at 10 and 15 birds/cage. The prepared coconut milk was added to the

feed in liquid form. The feed was provided commercially ad-libitum: starter from 1-21d (3.113 kcal/kg, 21.8% crude protein) and finisher from 22-42 days (3.216 kcal/kg, 20.83% crude protein).

### Corticosterone (CRT)

20 birds per treatment were randomly selected to collect blood sample. The blood samples were centrifuged at 3000 rpm for 10 minutes. The obtained plasma were kept at -80 °C to measure CRT level. Elisa kit (Cayman Chemical, Michigan, USA) was used to measure this parameter.

### Acute phase protein (APP)

The APP concentration was determined by using two methods as Alpha-1-acid glycoprotein (AGP) and ceruloplasmin (CER). Commercial Elisa kit (Life Diagnostics Institute, West Chester, UK) was used to determine the AGP concentration level.

The CER concentration was measured according to Sunderman and Nomoto (1970) method.

### Statistical analysis

All data were subjected to ANOVA using the GLM procedure of SAS (SAS Institute Inc., 2003). All results were analysed based on different percentage of coconut milk in diet and stocking densities. When significant effects were found, comparisons among multiple means were modelled by Tukey's test.

## Results

### Corticosterone (CRT)

The results showed significant differences among the diets and densities. The results are presented in Table 1. The treatments had higher level of CRT when birds were subjected to high stocking density. Interestingly, the diets were supplemented with coconut milk in the both densities showed lower level of CRT compare to the control diet. Moreover, diets supplemented with 5% coconut milk showed lower level of CRT in the both densities in comparison with the diets supplemented by 3% coconut milk. In fact, there was a negative interaction between coconut milk supplementation and CRT level; by increasing the percentage of coconut milk in the diet, the level of CRT was decreased in the plasma.

### Acute phase protein (APP)

The results showed some differences among the diets and densities (Table 1). The chickens were subjected to high stocking density showed higher level of APP compare to normal stocking density in the same diet. Moreover the diets were supplemented with

coconut milk, showed lower level of APP compare to the control diet in the both densities. Furthermore, the diet were supplemented with 5% coconut milk showed lower level of APP in comparison with the diet were supplemented by 3% coconut milk in the both densities.

In total, there was a negative interaction between coconut milk percentage and APP level; by increasing the percentage of coconut milk, the APP level was decreased.

**Table 1.** Ceruloplasmin (CER),  $\alpha$ -1 acid glycoprotein (AGP) and corticosterone (CRT) concentrations by treatments.

	TREATMENTS						n
	Control		Coconut Milk 3%		Coconut Milk 5%		
	Normal <sup>1</sup>	High	Normal	High	Normal	High	
<b>CRT<sup>2</sup> (ng/ml)</b>	2.29±0.13 <sup>b</sup>	2.62±0.11 <sup>a</sup>	2.12±0.15 <sup>b</sup>	2.19±0.08 <sup>b</sup>	1.72±0.14 <sup>c</sup>	1.86±0.18 <sup>c</sup>	60
<b>CER (mg/ml)</b>	10.1±1.72 <sup>a</sup>	11.3±1.13 <sup>a</sup>	9.7±1.31 <sup>ab</sup>	9.8±1.63 <sup>ab</sup>	7.4±0.74 <sup>b</sup>	7.5±0.62 <sup>b</sup>	60
<b>AGP (<math>\mu</math>g/ml)</b>	982±65 <sup>bc</sup>	1093±68 <sup>a</sup>	847±63 <sup>de</sup>	1055±24 <sup>ab</sup>	776± 41 <sup>e</sup>	934±76 <sup>cd</sup>	60

<sup>a,b,c,d,e</sup>; within a row-subgroup with no common superscripts are different at  $P < 0.05$ ,

<sup>1</sup>Normal: Normal stocking density; High: High stocking density,

n: Number of samples,

<sup>2</sup>CRT: Corticosterone; CER: Ceruloplasmin; AGP: Alpha-1-acid glycoprotein

### Discussion

Coconut milk is the liquid extracted from the coconut meat and has positive impact on immune system. It contains high portions of saturated fatty acids and medium chain triglycerides which can boost immune system by antimicrobial and antiviral activities (Enig, 1998). It is mentioned that high stocking density is a major welfare issue (Bessei, 2006; Estevez, 2007) which can adverse performance and immune system. Suggested by Estevez (2007) that 0.07 m<sup>2</sup>/bird is minimum space for the chicks under hot conditions.

The present results about high stocking density and CRT level were similar with other studies (Dawkins, 2003; Lin et al., 2006; Sandilands et al., 2006; Türkyilmaz, 2008; Vachon and Moreau, 2001) that indicated unpleasant physiological activities such as compete for feed or high temperature can increase CRT level in response to stress. In contrary, Thaxton et al. (2006) indicated that stocking density had no effect on CRT level when chicks were reared in floor pens.

The difference between our findings with other results may caused by the housing system, age and climate condition. Increasing the number of chicks per square meter may cause heat stress (Morris, 1993). Moreover, telomere length is a biomarker for oxidative stress may elicit physiological stress (Beloor et al., 2010). Under unpleasant conditions, CRT will be synthesised by activation of the hypothalamus-pituitary-adrenal axis (Sapolsky et al., 2000). The obtained results about

coconut milk in connection with CRT level were in agreement with studies (Vigila and Baskaran, 2008; Winarsi et al., 2008) that showed coconut oil can improve immunity and increase both B-lymphocytes cells and T-lymphocytes. Moreover, coconut oil prepared from coconut milk has anti-inflammatory and antipyretic action by reducing granuloma formation and serum alkaline phosphatase activity (Intahphuak et al., 2010). Coconut milk is rich in lauric acid which can boost immune system by antibacterial (Oyi et al., 2010) and antiviral (Arora et al., 2011) effects. Increasing total serum cholesterol is one of the main functions of lauric acid and mostly attribute to high-density lipoprotein which is introduced as a good blood cholesterol and protective indicator in damaged tissues (Kwiterovich, 2000).

The present data showed higher level of APP in chicken were subjected to high stocking density. These results were confirmed with some studies (Buyse et al., 2007; Georgieva et al., 2010; Gruys et al., 2005; Murata et al., 2004; Pineiro et al., 2007) indicated that APP concentration can change in response to different external stressors or inflammations. Moreover, non-inflammatory conditions such as housing system may affect the APP level (Holt and Gast, 2002). Positive impacts of coconut milk on immune system (Oyi et al., 2010) can confirmed by the results of this study. The reason of this phenomenon can be caused by the antimicrobial and antiviral action of luraic acid in coconut which cosisits high-density lipoprotein to boost

the immune system (Intahphuak et al., 2010). In stressful conditions, cortisol produced from the adrenal cortex, carried to affected tissues and incorporated into high-density lipoprotein particles. It attracts and activates leukocyte near damaged tissues. High-density lipoprotein has protective actions in inflamed tissues (Kwiterovich, 2000).

### Conclusion

It can be concluded that coconut milk supplementation can improve immune system even when chicken were subjected to external stressors as high stocking density.

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