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To cite this article: Sudibya and J Riyanto 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **250** 012079

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# Effects of supplementation of cellulase, carnitine and fish oil on lipids and fatty acid contents of Indonesian native chicken meats

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**Abstract.** The research was conducted to investigate the effect of supplementation of cellulase, carnitine and tuna fish oil on lipid and fatty acid contents of Indonesian native chicken meats. A total of 120 male native chickens was allocated randomly to 4 dietary treatments with 6 replicates of 5 birds. The basal diet, which was consisted of corn, rice bran and layer concentrate, was defined as T1. The remaining 3 dietary treatments were the basal diet + 0.1% cellulase enzyme (T2), basal diet + 0.1% cellulase enzyme + 30 ppm L-carnitine (T3), basal diet + 0.1% cellulase enzyme + 30 ppm L-carnitine + 4% lemuru fish oil (T4). The dietary treatments were fed for 90 days. Supplementation of cellulase enzyme did not influence the lipid, cholesterol and fatty acid contents in the meats. Supplementation of carnitine in the diet containing cellulase enzyme decreased cholesterol content ( $P<0.05$ ) without affecting lipid and fatty acid contents in the meats. Furthermore, supplementation of lemuru fish oil in the diet containing cellulase enzyme and carnitine enhanced the lipid, high-density lipoprotein and unsaturated fatty acid contents, including omega-3 and omega-6 fatty acids in native chicken meats ( $P<0.05$ ). Accordingly, supplementation of lemuru fish oil decreased the low-density lipoprotein, cholesterol and saturated fatty acid contents in the meats ( $P<0.05$ ). It is concluded that the supplementation of carnitine and fish oil in the diet produced native chicken meats with considerably low cholesterol and high unsaturated fatty acids contents.

## 1. Introduction

There are several types of native chicken in Indonesia which mainly raised in rural areas in natural environments [1]. Their productive performances and reproductive rates are low; vary depending on the rearing systems [1, 2]. In extensive rearing system, a hen produces daily gain approximately 10 g per day, while in semi-intensive and intensive rearing systems, a hen can gain 29 and 40 g per day, respectively [2].

Maize and rice bran are respectively still the principal feed ingredients to native chicken in Indonesia. However, rice bran contains the considerable amount of fiber which is not digested due to lack of fiber degrading enzyme. Enzymes can be used as nutritional strategies to increase the nutritional value of the diet by improving nutrient availability and feed efficiency [3]. The addition of carbohydrase enzymes improves the utilization of nutrient, particularly energy [4]. In this regard, cellulase belongs to carbohydrase enzymes that can be used to increase nutrient digestibility by catalyzing the conversion of cellulose to glucose which can be used as the potential energy source [3,



4] and reduce environmental impact due to undigested nutrient [5]. The addition of enzyme complex containing  $\beta$ -glucanase,  $\beta$ -xylanase, cellulase and phytase were able to enhance production and feed efficiency, indicating improved utilization of nutrients [3].

L-carnitine ( $\beta$ -OH- $\gamma$ -N-trimethyl aminobutyric acid) is a water-soluble quaternary amine which naturally occurs in animals, plants and microorganisms [6]. The important role of carnitine in lipid metabolism is facilitating the transport of long-chain fatty acids across the inner mitochondrial membrane for  $\beta$ -oxidation leads to reduce the availability of lipids for peroxidation and reduce the amount of long-chain fatty acids availability for esterification to triacylglycerols and storage in adipose tissue [7, 11]. Thus, supplementation of carnitine improves  $\beta$ -oxidation of fatty acids to produce energy and increased the utilization of energy [7].

Poultry feed ingredients derived from plants such as maize, soybean and other plant products are low in carnitine, while animal-derived feedstuffs commonly contain high carnitine. Thus, poultry diet which contains high percentages of cereals may lead to a deficiency of carnitine [8]. Furthermore, carnitine has antioxidative properties, thus supplementation of carnitine in the diet containing high fat or oil may have benefit to reduce or lowering the rancidity process [6]. Carnitine supplementation to poultry diet has been shown to improve poultry performance and modify chemical composition in poultry products [9, 10, 11].

Moreover, fatty acids are the major components of egg yolk lipids and constitute about 4 grams in egg [12]. The egg yolk lipid and fatty acid compositions are easily affected and vary depending on the fat and fatty acid compositions in the diet [13]. Supplementation with unsaturated fatty acids sources such as menhaden oil, tuna fish meal, tuna fish oil, linseed oil, and others to the diets is a strategic approach to enrich poultry products with unsaturated fatty acids. Unsaturated fatty acids, such as omega-3 and omega-6, are commonly found in marine fish and fish oil [14]. Lemuru fish oil contains omega-3 fatty acids especially eicosapentaenoic acid 33.6 to 44.85% and docosahexaenoic acid about 14.64% of total fatty acids, 6.0% fat and 8280 kcal/kg total digestible nutrient [14]. Several studies have shown the beneficial effect of the addition of unsaturated fatty acid sources in poultry [13, 10, 12]. However, there is a lack of information on the study concerning supplementation of cellulase enzyme, carnitine and fish oil on meats lipid and fatty acid compositions, particularly in Indonesian native chicken. Therefore, this study was aimed to investigate the effect of supplementation of cellulase, carnitine and lemuru fish oil on lipid, cholesterol and fatty acid contents of native chicken meats.

## 2. Material and methods

In total, 120 male native chickens at 4 weeks of age were allocated to four dietary treatments. Each dietary treatment was repeated 6 times consisted of 5 birds. The basal diet, which was consisted of corn, rice bran and layer concentrate, was defined as T1. The remaining 3 dietary treatments were the basal diet + 0.1% cellulase enzyme (T2), basal diet + 0.1% cellulase enzyme + 30 ppm L-carnitine (T3), basal diet + 0.1% cellulase enzyme + 30 ppm L-carnitine + 4% lemuru fish oil (T4). The composition and nutrient contents of the assay diets are presented in Table 1.

The birds were housed in battery cages and kept with a standard management practice. The diets were fed ad libitum in a mash form. The dietary treatments lasted for 90 days. Furthermore, during the treatments, the birds had free access to water. Samples of meat (10 g per replicate) were collected for lipid, cholesterol and fatty acids contents analyses. Lipid and fatty acid contents were analyzed according to the procedure of [15]. The low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol were analyzed according to the guideline of [16].

The data were subjected to analyses of variance. If the results of variance analysis showed a significant effect ( $\alpha = 0.05$ ), it was then continued by orthogonal contrast test [17]. The following set contrast were applied: T1 vs T2, T3, T4 (to see the difference between the diet without and with cellulase enzyme supplementation); T2 vs T3, T4 (to see the difference between the diet without and with L-carnitine supplementation); and T3 vs T4 (to see the difference between the diet without and with tuna fish oil supplementation).

**Table 1.** Composition and nutrient contents of the assay diets

Ingredients	T1	T2	T3	T4
Rice bran (%)	50	50	50	50
Yellow corn (%)	25	25	25	25
Layer concentrate (%)	25	25	25	25
Cellulase enzyme (%)	0	0.1	0.1	0.1
L-carnitine (%)	0	0	0.003	0.003
Tuna fish oil (%)	0	0	0	4
Nutrient contents				
Metabolizable energy (kcal/kg)	2763.75	2760.99	2760.91	2985.22
Crude protein (%)	17.23	17.21	17.21	16.55
Crude fat (%)	6.85	6.84	6.84	6.81
Crude fiber (%)	5.15	5.14	5.14	4.98
Calcium (%)	3.02	3.02	3.02	2.90
Phosphorus (%)	0.23	0.23	0.23	0.22

T1 = basal diet, T2 = basal diet + 0.1% cellulase enzyme, T3 = basal diet + 0.1% cellulase enzyme + 30 ppm L-carnitine, T4 = basal diet + 0.1% cellulase enzyme + 30 ppm L-carnitine + 4% lemuru fish oil.

### 3. Results and discussions

#### 3.1. Cholesterol contents

Supplementation of cellulase enzyme did not affect the LDL, HDL and cholesterol contents in the meats (Table 2 and 3). Exogenous enzymes, such as cellulase, did not decrease lipid and cholesterol contents since this enzyme is used mainly to improve the digestibility of nutrients, particularly when the diet contains high fibre [18, 3].

**Table 2.** LDL, HDL and cholesterol contents of native chicken meats

Treatment	LDL (mg/dl)	HDL (mg/dl)	Cholesterol (mg/dl)
T1	32.34 ± 0.98	67.66 ± 0.97	347.31 ± 0.02
T2	32.24 ± 1.01	67.76 ± 1.01	519.83 ± 0.02
T3	31.25 ± 0.95	68.75 ± 0.95	512.66 ± 0.02
T4	27.15 ± 0.93	72.85 ± 0.93	204.97 ± 0.01
P value	<0.05	<0.05	<0.05

T1 = basal diet, T2 = basal diet + 0.1% cellulase enzyme, T3 = basal diet + 0.1% cellulase enzyme + 30 ppm L-carnitine, T4 = basal diet + 0.1% cellulase enzyme + 30 ppm L-carnitine + 4% lemuru fish oil.

**Table 3.** Orthogonal contrast result of LDL, HDL and cholesterol contents of native chicken meats

Treatment	LDL	HDL	Cholesterol
T1 vs T2, T3, T4	NS	NS	NS
T2 vs T3, T4	NS	NS	*
T3 vs T4	*	*	*

$\alpha = 0.05$ ; NS = Non-Significant, \* = Significant

Carnitine has the potential to induce desirable modifications in poultry performance and products due to its role in lipid metabolism. In the present study, supplementation of carnitine to the diet containing cellulase enzyme decreased cholesterol content in the meats but did not affect HDL, and LDL contents. This response was in accordance with the role of carnitine in lipid metabolism, which facilitates the transport of long-chain fatty acids across the membrane of mitochondria for  $\beta$ -oxidation, led to decrease lipid and cholesterol deposition [19]. Consequently, carnitine supplementation in the

diets reduces the amount of lipid and cholesterol deposition [11] and [19]. This finding was in support with previous observations, in which carnitine supplementation lowered the cholesterol content in chicken and quail eggs [20] and [11]. Furthermore, previous observation showed that carnitine supplementation to laying hen diets reduced blood cholesterol content but did not affect cholesterol content in the egg [9] and [21]. In addition, [11] did not find any effect of carnitine addition to diets of quails on HDL, LDL and very low-density lipoprotein (VLDL) contents in blood.

Supplementation of lemuru fish oil to the diet containing cellulase enzyme and carnitine increased HDL but decreased cholesterol and LDL contents in native chicken meats, which was associated with the high unsaturated fatty acid contents in lemuru fish oil, particularly omega-3 fatty acid. Fish oil is an energy and unsaturated fatty acids source [22], thus its supplementation increased lipid contents in eggs [10]. In agreement with this result, supplementation of Hemp seed oils in the diet of laying hens stimulated lipid deposition in eggs [23]. Similarly, supplementation of polyunsaturated fatty acids in the diet enhanced lipid content in body tissue of rats [24].

The results of this experiment confirmed previous observation, in which the administration of fish oil decreased serum and egg yolk cholesterol contents [25] and increased HDL contents [26]. The high HDL content then facilitates transportation of lipid and cholesterol to be excreted [22]. Furthermore, supplementation of polyunsaturated fatty acids in the diet increased HDL content in the body tissue [27, 12]. An enhance in HDL content in chicken eggs due to supplementation of unsaturated fatty acid has been observed previously [14].

Omega-3 fatty acid decreases cholesterol and LDL contents by stimulating cholesterol transportation by the HDL to liver and stimulating cholesterol secretion from liver to intestine to be excreted [19]. Previous investigation also revealed that administration of omega-3 fatty acid in the diet decreased cholesterol content in dairy goat, cow milk and native chicken eggs [18, 28, 22 and 33]. Accordingly, supplementation of polyunsaturated fatty acids in the diet decreased LDL content in the body tissue of chickens [27].

### 3.2. Fatty acid contents

Supplementation of cellulase enzyme did not affect the fatty acid contents including unsaturated and saturated fatty acids in meats. Similarly, supplementation of carnitine in the diet containing cellulase enzyme did not affect the fatty acid contents in meats (Table 4 and 5). Supplementation of lemuru fish oil in the diet containing cellulase enzyme and carnitine increased unsaturated fatty acid contents and decreased saturated fatty acids contents in meats ( $P < 0.05$ ). Lemuru fish oil contains a high unsaturated fatty acids which can be transferred to the meat resulting an increase in unsaturated fatty acid contents. Unsaturated supplementation enhanced unsaturated fatty acids contents, including omega-3 and omega-6, in body tissue [24]. This finding was also in support with previous observations that supplementation of lemuru fish oil increased unsaturated fatty acids contents, omega-3 and omega-6 contents in native chicken eggs [14, 26] dairy cow milk [28] and dairy goat milk [18, 22].

**Table 4.** Unsaturated and saturated fatty acids content of native chicken meats

Treatment	Unsaturated fatty acids (%)	Saturated fatty acids (%)
P <sub>0</sub>	60.52 ± 2.22	39.48 ± 4.89
P <sub>1</sub>	62.56 ± 2.57	37.44 ± 4.16
P <sub>2</sub>	63.04 ± 2.16	36.96 ± 2.16
P <sub>3</sub>	69.76 ± 2.83	30.24 ± 1.78
P value	<0.05	<0.05

T1 = basal diet, T2 = basal diet + 0.1% cellulase enzyme, T3 = basal diet + 0.1% cellulase enzyme + 30 ppm L-carnitine, T4 = basal diet + 0.1% cellulase enzyme + 30 ppm L-carnitine + 4% lemuru fish oil.

**Table 5.** Orthogonal contrast result of fatty acids contents of native chicken meats

Treatment	Unsaturated fatty acids (%)	Saturated fatty acids (%)
T1 vs T2, T3, T4	NS	NS
T2 vs T3, T4	NS	NS
T3 vs T4	*	*

$\alpha = 0.05$ ; NS = Non Significant, \* = Significant

Fish oil is an energy and unsaturated fatty acids source, thus, an addition of fish oil may increase unsaturated fatty acid contents in the animal products [22]. An enhance in omega-3 content in the egg of laying hens fed diet supplemented with fish oil rather than fed other oil, which indicated that unsaturated fatty acids were absorbed in the intestine and deposited in the eggs [29]. Supplementation of lemuru fish oil increased the unsaturated in egg yolk of laying hens [30]. Similarly, other authors observed a higher concentration of unsaturated fatty acid in an egg of laying hens received fish oil supplementation than those received soybean oil supplementation [31]. In addition, the combination between fish oil and peanut oil increased the level of long-chain unsaturated fatty acid contents [32].

Furthermore, supplementation of lemuru fish oil in the diet containing cellulase enzyme and carnitine decreased saturated fatty acid contents in meats ( $P < 0.05$ ) which associated with high unsaturated fatty acid contents in fish oil. In agreement with this study, supplementation of lemuru fish oil decreased saturated fatty acids contents in native chicken eggs [33]. Moreover, supplementation of unsaturated fatty acids in the diet decreased saturated fatty acids contents in body tissue [24, 26, 34]. Accordingly, supplementation of fish oil containing a high unsaturated fatty acids contents alleviated the saturated fatty acids in dairy goat milk [18, 22] and dairy cow milk [28].

#### 4. Conclusions

Supplementation of lemuru fish oil in the diet containing cellulase enzyme and carnitine generated a higher HDL but decreased LDL and total cholesterol contents in native chicken meats. Supplementation of lemuru fish oil in the diet containing cellulase enzyme and carnitine generated a higher unsaturated fatty acid contents in native chicken meats. Accordingly, supplementation of lemuru fish oil lowered the saturated fatty acid contents in native chicken meats.

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