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Antioxidant Enzyme Activity and Malondialdehyde Concentration on Broiler Fed Contain Lauric Acid and *Areca vestiaria* Giseke

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ABSTRACT

The objective of the study was to determine antioxidant enzyme activity and concentration of malondialdehyde on broiler which consumed feed containing lauric acid and natural antioxidant from *Areca vestiaria* Giseke. The study used 240 day-old chicks of Lohmann MB 202 P strain placed randomly at 24 experimental cage units (10 chickens each) of the litter system. The average temperature of the cage at the study was 25.80 to 32.08^0 C with relative air humidity rate of 67.69 to 86.20%. The research method followed a Factorial Completely Randomized Design 2 x 4 with 3 replications. The first factor was the source of lauric acid in feed, i.e. conventional coconut oil (CO) and pure lauric acid (LA). The second factor was the source of antioxidant in the form of dosage of *Areca vestiaria* (AV) and vitamin E supplementation (TF) with four levels of AV, ie 0, 625 $mg.kg^{-1}$, 1250 $mg.kg^{-1}$, and TF at a dose of 200 mg $.kg^{-1}$ feed. Feed and drinking water were given ad libitum. Meat sampling to measure SOD, catalase and MDA was taken at the end of the study by first chickens were fasted for ± 8 hours (overnight). The sample used was right breast meat of experimental male chicken. The data were average from three experimental chickens. The data obtained were analyzed for the variant with the general linear model on MINITAB (version 16), then tested the differences between treatments using Tukey's honestly significant difference (HSD). The results showed that the source of laurate had a significant effect on SOD, catalase, and MDA, while antioxidant concentration significantly decreased SOD and MDA compared with control. The interaction between lauric sources and antioxidant concentrations affected catalase and MDA. The use of coconut oil as much as 3% and antioxidant supplementation of *Areca vestiaria* Giseke in feed at a dose of 1250 mg kg^{-1} could decrease lipid oxidation product of meat to produce a healthy food product.

Keywords: *Areca vestiaria* Giseke, Catalase, Lauric acid, Malondialdehyde, Superoxide dismutase

Introduction

The consumption of animal products increases with the improvement of the economic status of people in developing countries. But an increase in the incidence of cardiovascular disease and type 2 diabetes has traditionally been considered as a result of increased consumption of the animal products (Salter, 2013). The fact that total fat, saturated fatty acids (SFA) and monounsaturated fatty acids (MUFAs) are not associated with death by coronary heart disease (Skeaff and Miller, 2009), as well as unrelated SFA consumption with risk of coronary heart disease, stroke and cardiovascular disease (Siri-Torino *et al*., 2010). Increased research in the field of meat production needs to be done with the supplementation of feed ingredients that can produce healthy meat products.

The use of coconut oil in broiler feed is limited to its use as an energy source. Its function as the basic ingredients for the production of functional food in the form of chicken meat has not been done. Lauric acid in coconut oil can be utilized for the production of functional food. The results of research on coconut oil containing medium chain fatty acids (MCFA) including lauric acid have not been widely applied, whereas there is potency as a source of monolaurin which has antibacterial, antiviral and antiparasitic effects (Dayrit, 2003; Preuss *et al*., 2005). MCFA in

coconut oil of 28.98 - 41.86% is lauric acid (Gopala-Krisna *et al*., 2010).

Oxidation of food lipid components is known as oxidative rancidity initiated by the presence of oxygen free radicals or molecular oxygen reactions with organic free radicals from long-chain polyunsaturated fatty acids (polyunsaturated fatty acids, PUFAs). The oxidation can be prevented or delayed by the use of antioxidants. This compound can reduce the involvement of oxygen free radicals in the oxidation of fatty acids (Valenzuela and Nieto, 1996), as well as neutralize free radicals and prevent the damage caused (Nimse and Pal, 2015). Free radicals have implications for human diseases in different levels related to their production and metabolism (Surai, 2003). Exploration of natural ingredients which have biological activity become one of the researcher targets, after synthetic compounds which have biological activities such as synthetic antioxidant compounds (tertrabutylhydroxyquinone, TBHQ, butylated hydroxytoluene, BHT, and butylated hydroxyanisole, BHA) are prohibited because they are carcinogenic. Based on several studies that have been developed, the compounds that have potency as antioxidants are generally phenol compounds such as flavonoids. One of the plants which contain flavonoids is *Areca vestiaria* Giseke.

Usually, plant protects itself through the accumulation of secondary metabolites (Gonzales-Lamothe *et al*., 2009). Phytochemical tests show that the seeds of *Areca vestiaria* Giseke contain compounds of tannins, triterpenoids, flavonoids, and saponins that potential to contain bioactive compounds as secondary metabolites of plants which have antioxidant activity. The triterpenoids can be used as antibacterial, anticancer, and to treat wounds and inflammation (Simbala and Tallei, 2010). *Areca vestiaria* fresh seed sample has an antioxidant activity of 3.80375 mmol.100g^{-1} and flavonoid total contained in fresh *Areca vestiaria* Giseke seed extract is 7.573 mg.kg^{-1} (Samosir *et al*., 2012). The more flavonoids contained, the greater total antioxidant activity. Several studies that had been conducted on the activity of the three flavonoids showed that quercetin extracted with methanol from guava leaves had not demonstrated contraceptive antifertility activity in white rats (Ariani *et al*., 2008). Catechins from green tea given to mice can inhibit the growth of mammary tumors (Gunawijaya *et al*., 1999). The antioxidant activity of catechins and epicatechins was studied in the *Acacia catechu* tree as well as the leaves and stems of *Uncaria gambir* (*Pale catechu*) (Duangyod *et al*., 2014). Gambir antibacterial activity performed by Amos (2009) with the catechin content of 25-35% and tannin of 60-65%. The greatest inhibitory affinity of tannins is greater in protein than carbohydrates, because of the strength of the affinity of hydrogen binding to carboxyl oxygen in the peptide group. The use of tannins in the feed is limited to 2.6 g per kilogram of feed (Pour-Reza and Edriss, 1997).

The potency is shown by coconut oil as a source of laurate, and *Areca vestiaria* Giseke as a source of antioxidants combined to see its effect on antioxidant enzyme activity and lipid oxidation products in the broiler to produce functional chicken meat.

Materials and Methods

This study used 240 day-old chicks broiler of Lohmann MB 202 P strain with an initial average body weight of 48.40±0.12 g, and each experimental chicken was marked. Experimental chicken used was free pullorum. Vaccination programs including New Castle disease were given in day-old chicks. AV fruit was obtained from Tomohon region, North Sulawesi. AV flour preparation began with the separation of the seeds from the skin of the fresh fruit; then the seeds were dried in the sun. Seeds were separated from the seed shell, and dried again using a 40ºC oven until the moisture content below 10%. The dried seed were milled using milling machine JZ7114 type at 1400 rpm and 65 mesh. The antioxidant used as a comparator was d-α-tocopherol (caprimun-E). The coconut oil used was produced from conventional processes commonly used by traditional farmers. Pure lauric acid product of Sinarmas oleochemical, Lauric Acid Sinar-FA 1299 was used as a comparison. Feed and drinking water were provided *ad libitum* with nutrient content in accordance with the livestock period (Table 1).

The treatments tried in this study were the lauric source and antioxidant concentration in broiler feed. The first factor was the source of lauric acid in feed, i.e. conventional coconut oil (CO) and pure lauric acid (LA). The second factor was antioxidant source of AV dosage and vitamin E supplementation (TF) with four levels of 0 AV, 625 mg.kg^{-1} AV feed, 1250 mg.kg^{-1} AV feed, and TF at a dose of 200 ppm. There were 8 treatment combinations in this research, 1). Experimental chicken was fed based on corn-soybeans supplemented with 3% CO without antioxidants (AV or TF). 2). Experimental chicken was fed based on corn-soybeans supplemented with 3% CO and AV at doses of 625 mg.kg^{-1}. 3). Experimental chicken was fed based on corn-soybeans supplemented with 3% CO and AV at a dose of 1250 mg.kg^{-1}. 4). Experimental chicken was fed based on corn-soybeans supplemented with 3% CO and TF at a dose of 200 ppm. 5). Experimental chicken was fed based on corn-soybeans and supplemented with 13 mg.kg^{-1} LA without antioxidants (AV or TF). 6). Experimental chicken was fed based on corn-soybeans supplemented with 13 mg.kg^{-1} LA and AV at doses of 625 mg.kg^{-1}. 7). Experimental chicken was fed based on corn-soybeans supplemented with 13 mg.kg^{-1} LA and AV at a dose of 1250 mg.kg^{-1}. 8). Experimental chicken was fed based

Table 1. Ingredient composition and nutrient content of diets as fed basis

Items	Starter phase (d 1-21)		Grower phase (d 22-35)	
	CO	LA	CO	LA
Feed ingredients (%)				
Corn	53	54.4	53	56.2
Soy bean meal	27	27	24	22
Fish meal	8	8	7.5	8
Rice bran	0	0	4	4
Meat bone meal	9	8.5	7	7
Coconut oil	1.5	0	3	0
Lauric acid (99%)	0	0.65	0	1.3
$CaCO_3$	1	1	1	1
NaCl	0.35	0.35	0.35	0.35
DL-methionina (99%)	0.05	0.05	0.05	0.05
Vitamin-mineral premix[1]	0.1	0.1	0.1	0.1
Total	100	100	100	100
Nutrient content (%) dan energy[2]				
EM (kcal/kg)	3140	3102	3205	3131
Crude protein	22.23	22.25	20.39	20.09
Extract ether	5.84	5.02	7.57	6.06
Crude fiber	1.88	1.89	2.43	2.42
Calcium	1.68	1.64	1.48	1.51
Available phosphor	0.49	0.49	0.49	0.50
Lysin	1.64	1.63	1.48	1.45
Methionine	0.56	0.56	0.53	0.53
Methionine+cysteine	0.97	0.97	0.90	0.90
Linoleic acid	1.73	1.73	1.67	1.73
Sodium	0.26	0.26	0.25	0.25
Chloride	0.17	0.16	0.15	0.15

[1]Mixtrouwvit mineral & vitamin supplied the following per ton of diet: Iron, 40 mg; Copper, 26.16 mg; Zinc, 40 mg; Manganese, 44 mg; Selenium, 0.08 mg; Cobalt, 0.08 mg; Iodine, 0.52 mg; Vit A, 12500 IU; Vit D3, 35000 IU; Vit E, 25 IU; Vit K3, 4 mg; Vit B1, 4 mg; Vit B2, 8 mg; Vit B6, 20 mg; Vit B12, 50 mcg; Pantothenic acid, 15 mg; Niacin, 50 mg; Biotin, 125 mcg; Calcium D-pantothenate, 16.30 mg; Folic acid, 1 mg. [2]Calculation based on Leeson and Summers (2008) (Londok *et al*., 2017).

on corn-soybeans supplemented with 13 $mg.kg^{-1}$ LA and TF at a dose of 200 ppm.

Sampling for measurement of carcass characteristics was performed on 3 male chickens, slaughtered, and then horizontally hung for blood removal. Feather removal was preceded by immersion in hot water (60-65^{0}C) in containers for ± 3 minutes. The head and legs were separated using a sharp knife. Further removed the viscera and weighed carcass weight of each chicken that was sampled. After a rapidly weighed, the sample was then stored in the refrigerator at -20^{0}C until further analyzed. The right breast meat was used in the analysis of antioxidant enzyme activity (catalase and SOD) and product of lipid oxidation (MDA) of experimental chicken meat.

Result and Discussion

Antioxidant enzyme activity of broiler meat

The antioxidant activity of broiler meat measured in this study was superoxide dismutase (SOD) and catalase. The average value of antioxidant activity could be seen in Table 2. The results of statistical tests showed that the lauric source and antioxidant concentration had a significant effect (P<0.01) on SOD. There was no interaction between the lauric source and the antioxidant concentration of the parameters. The statistical results showed that SOD in experimental chicken consuming coconut oil supplemented was significantly higher (28.19% or 2.30 $U.g^{-1}$) compared to that supplemented by pure lauric acid. Increased concentrations of AV antioxidants and the use of TF in feed significantly (P<0.01) decreased SOD enzyme concentrations, compared to without the use of antioxidant. AV supplementation at a dose of 626 $mg.kg^{-1}$ feed decreased SOD enzyme concentration by 5.53% (0.58 $U.g^{-1}$) compared to without supplementation. AV supplementation at a dose of 1250 $mg.kg^{-1}$ feed decreased SOD enzyme concentration by 6.01% (0.63 $U.g^{-1}$) compared to without supplementation. Supplementation of TF at a dose of 200 $mg.kg^{-1}$ feed decreased SOD enzyme concentration by 27.26% (2.86 $U.g^{-1}$) compared to without supplementation. Increased AV dose from 625 $mg.kg^{-1}$ AV to 1250 $mg.kg^{-1}$ AV decreased SOD enzyme concentration by 5.15% (0.05 $U.g^{-1}$).

Catalase activity was significantly affected (P<0.05) by the laureate source but was not significantly affected by antioxidants. The interaction between the lauric source and the antioxidant concentration was significant (P<0.01) affecting the catalase activity. The catalase enzyme activity of experimental chicken meat which was supplemented by pure lauric acid was significantly higher (6.38% or 0.006 $U.g^{-1}$) than that supplemented by coconut oil. As the lauric source and antioxidant concentrations showed a significant interaction, the lauric source depended on the antioxidant concentration and vice versa. The sequence of chicken response to catalase enzyme concentrations from highest to lowest was found in treatment combination of LA feed supplemented by TF at a dose of 200 $mg.kg^{-1}$, CO without antioxidant supplementation, LA supplemented by AV at a dose of 625 $mg.kg^{-1}$, LA supplemented by AV at a dose of 1250 $mg.kg^{-1}$,

CO supplemented by AV at a dose of 1250 mg.kg^{-1}, LA without supplementation, a CO supplemented by AV at a dose of 625 mg.kg^{-1}, CO supplemented by TF at a dose of 200 mg. kg^{-1}.

Inactivation of free radicals in muscle tissue was enzymatically performed by SOD, catalase, and GsPx (glutathione peroxidase). SOD and catalase were antioxidant enzymes that directly react with free radicals (Delles *et al.,* 2014).

Lipids oxidation products of broiler meat

The lipid oxidation product in broiler meat was expressed through MDA measurement. The results of statistical tests showed that the lauric source and antioxidant concentration had a significant effect (P<0.01) on MDA. The average value of MDA could be seen in Table 2. The results of statistical tests showed that the lauric source and antioxidant concentration had a significant effect (P<0.01) on MDA. There was a significant interaction (P<0.05) between the lauric source and antioxidant concentration of the parameters.

The MDA content in broilers supplemented by pure lauric acid was significantly higher (152.38% or 0.32 µg.g^{-1}) compared to that was supplemented by coconut oil. The results of statistical tests showed an increase in the antioxidant concentration of AV and the use of TF in feeds was very significant (P<0.01) decreased MDA content of breast meat of chicken research, compared to without the use of antioxidants. Supplementation of AV at doses of 626 mg.kg^{-1} feed decreased MDA content of meat by 26.92% (0.14 µg.g^{-1}) compared to without supplementation. Supplementation of AV at doses of 1250 mg.kg^{-1} feed decreased MDA content of meat by 50% (0.26 µg.g^{-1}) compared to without supplementation. Supplementation of TF at a dose of 200 mg.kg^{-1} of feed decreased MDA content of meat by 38% (0.2 µg.g^{-1}) compared to without supplementation. Increased AV dose from 625 mg.kg^{-1} AV to 1250 mg.kg^{-1} AV decreased SOD enzyme concentration by 46.15% (0.12 µg.g^{-1}).

As the lauric source and antioxidant concentrations showed a significant interaction, the lauric source depended on the concentration of antioxidants, and vice versa. The sequence of chicken response to MDA content of meat from highest to lowest was found in treatment combination of LA feed without supplementation, LA supplemented by AV at a dose of 625 mg.kg^{-1}, LA supplemented by TF at dose of 200 mg.kg^{-1}, LA supplemented by AV at dose of 1250 mg.kg^{-1}, CO without antioxidant supplementation, CO supplemented by TF at dose of 200 mg.kg^{-1}, CO supplemented by AV at dose of 625 mg.kg^{-1}, CO supplemented by AV at dose of 1250 mg. kg^{-1}.

MDA is one of the indicators of lipid peroxidation in the body which is often used in association with oxidative stress (Aksu *et al*., 2011). The compound can cause damage to cell components, such as lipids, proteins, and nucleic acids. Exposure to high environmental temperatures has increased the production of free radicals, both exogenous free radicals (outside) and endogenous free radicals (byproducts of oxidative respiration occurring in cells) resulting in increased MDA content. The results of this study in a hot environment with an average temperature of 25.80-32.08^{0}C and relative humidity between 67.69 to 86.20% could reduce the content of MDA in meat. This was not in line with the research of Gu *et al.* (2008) which proved that the hot environment (temperature 33^{0}C with 80% relative humidity significantly increased MDA content in muscle tissue from 0.6 to 1.3 nmol/mg protein. This might be caused by the presence of antioxidant intervention in the feed that can reduce free radicals. Free radicals could cause metabolic disorders and DNA function disorders, which ultimately affect the activity of enzymes. Free radicals attacked Poly Unsaturated Fatty Acid (PUFA) on a cell membrane called lipid peroxidation attack, thereby increasing byproduct, i.e. MDA. The low concentration of MDA in meat indicated that the meat contained high antioxidants (Santi *et al.,* 2015) . The antioxidants played a role in suppressing free radical activity,

Table 2. Effect of dietary lauric acid, and natural antioxidant from *Areca vestiaria* Giseke in feed on concentration of antioxidant enzymes and lipid oxidation product of broiler[1]

Parameter	Source of lauric acid	Antioksidan				Mean
		0	625 mg AV	1250 mg AV	200 ppm TF	
MDA (µg.g^{-1})	CO	0.23±0.02^{bc}	0.21±0.02^{c}	0.18±0.05^{c}	0.21±0.07^{c}	0.21±0.01^{B}
	LA	0.81±0.15^{a}	0.54±0.07^{ab}	0.34±0.03^{bc}	0.43±0.03^{bc}	0.53±0.10^{A}
	Mean	0.52±0.29^{A}	0.38±0.17^{AB}	0.26±0.08^{B}	0.32±0.11^{B}	
SOD (U.g^{-1})	CO	11.77±0.45	11.36±0.72	11.10±0.15	8.32±0.20	10.64±0.78^{A}
	LA	9.21±0.69	8.46±0.23	8.62±0.68	6.93±0.31	8.30±0.49^{B}
	Mean	10.49±1.28^{A}	9.91±1.45^{A}	9.86±1.24^{A}	7.63±0.23^{B}	
Katalase (U.g^{-1})	CO	0.104±0.001^{AB}	0.092±0.001^{BC}	0.097±0.003^{ABC}	0.090±0.004^{C}	0.097±0.0004^{b}
	LA	0.095±0.004^{ABC}	0.103±0.001^{AB}	0.097±0.001^{ABC}	0.106±0.004^{A}	0.099±0.002^{a}
	Mean	0.100±0.004	0.098±0.005	0.094±0.003	0.098±0.008	

[1]Values are expressed as mean±SEM)
CO: coconut oil, LA: lauric acid, TF: tokoferol.
$^{A-B}$ Different superscripts within row shows highly significantly different (P<0.01)
$^{A-B}$ Different superscripts within column shows highly significantly different (p<0.01)
$^{a-c}$ Different superscripts within column shows significantly different (P<0.01)
$^{A-B}$Different superscripts within row and column shows highly significantly different (P<0.01).

so that MDA expressed in meat which was obtained by chickens supplemented by antioxidant was also low, *Areca vestiaria* Giseke contained secondary metabolites that had the potency to be bioactive. Antioxidant activity of AV in this study was 31.58 ppm, classified as a ingredient that had antioxidant properties. Molineux (2004) said that a substance had antioxidant properties if the IC_{50} value was less than 200 ppm. The increase of AV doses in this study could decrease MDA concentration.

Conclusion

The use of coconut oil as much as 3% and natural antioxidant supplementation of *Areca vestiaria* Giseke in ration at a dose of 1250 mg kg^{-1} could decrease lipid oxidation product of meat to produce a healthy food product.

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