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Virgin Coconut Oil Increase High Density Lipoprotein (LDL), Lower Triglyceride And Fatty Acids Profile (C6-C18) In Blood Serum of *Mus musculus.*

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ABSTRACT

The Virgin Coconut Oil (VCO) products have different qualities and controversy effects of lipids metabolism. This research used VCO from Padang Pariaman West Sumatra Indonesia, rich in Lauric acids. The procesing of VCO by naturally fermentation method, without adding chemicals. It has high amount of lauric acid (C12) up to 58 %, caprilic acid (C8) 8.9 %, capric acid (C10) 7.8 % included, vitamins A, D, E, K. This research aim to investigate some debate of using VCO in related to image that using Coconut Oil is bad for lipid pofile. This eperiment using dietary VCO as food supplement to determined lipid profile using 40 mice of *Mus musculus*, which divided into 4 groups. Feeding on egg yolk to Group I (negative control), Group II (positive control). The others were Group III fed with (egg yolk and VCO 2 %) and group IV (egg yolk and VCO 4 %). It was determined in plasma lipid profile, the total of cholesterol, High Density Lipoprotein (HDL). The duration time was 10, 20 and 30 days after treated analysed by using the enzymatic methods. The dietary of VCO 2 to 4 % resulted in significant increases in HDL levels from 32 % to 69 %. The dietary of VCO 4 % for four weeks did not toxic to mice metabolism. Triglycerides level decreased from 177 mg/dL to 85 mg/dL, and similar resulted to cholesterol ratio. Feeding on VCO for 4 weeks, the (C10) SCFA and (C12) MCT not detected in plasma serum of mice. The LCFA (C16) palmitate is decreased from 0.96 mg/mL to 0.1 mg/mL.

Keywords: virgin coconut oil, HDL, triglyceride, MCT metabolism, *Mus musculus*

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INTRODUCTION

Coconut plant (*Cocos nucifera L.: Arecaceae*): is a tropical plant, that has a long history of ethnopharmacological use in west Sumatra people [1]. It is very resilient and can withstand any type of tropical wheather. It bears fruit all year round. Studies show that every coconut cultivar has its own unique charateristic that may explain for the variances in the percent composition of fatty acids like the lauric fatty acid in the oil. This is atttibuted to seceral factors such as location and genetic varietal difference. Our location of coconut plants is 100 m from beach also in equatorial line. Virgin coconut oil extracted directly from fresh coconut meat. Recently, small coconut home industry, has produce Virgin Coconut Oil (VCO) made by fermentation [2] , or without adding chemical.

VCO is extracted from fresh coconut fruit flesh at low temperature and without the use of chemicals. The physical methods for producing VCO includes the process of pressing, washing with water, settling, filtering and centrifugation. However, the natural way to produce VCO is through fermentation process in which it occurs through naturally-occurring microorganisms. The most important qualitly of VCO must be free from water, to avoid rancidity , high antioxsidant, vitamins and lauric acids contents. Coconut oil, is the most stable oil is highly saturated, and less than 10 % unsaturated fatty acids [3]. People of west Sumatra, have been consume VCO as food supplement and maintain positif effect of several deseases [4],and increase HDL in rat, so called good cholesterol. Althought studies may take year to probe the pharmalogical effects of VCO is growing interest worldwide such as lower the cholesterol level, cardiovascular, diabetic, cancer, skin burn, alzheimer, and many more deseases, people called VCO is miracle oil for total health. Antioxidant enzyme in rats showed reduce lipid peroxide content, and VCO polyphenol also capable of preventing in vitro lipid peroxidation [5].

The fatty acids molecules found in coconut oil are unique and possess properties that are different from other fats. They are called medium-chain fatty acids (MCFA), also known as medium-chain triglycerides (MCT) consis of (C12) lauric acids. Several reports, explain the miricle of saturated Lauric acids in coconut oil. Lauric acids will differently metabolized in our body without helping pancreatic lipase enzymes [6]. The length of the carbon chain is extremely important to metabolise depending on its size of fatty acids carbon. Therefore, the physiological effects of MCFA from coconut oil are significantly different from those of the Long Chain Fatty Acids (LCFA). Several epidemiological studies showed that the risk of Coronary Heart Disease (CHD) rises progresissively with high concentration of of Low Density Lipoprotein (LDL) cholesterol [7]. Since the effect of plasma lipid in lowering the TG and improve HDL has been controversial, this paper explain VCO is more beneficial for lipid metabolism. This paper also the first report to analyse the profil of fatty acids (C6-C16) , in plasma *Mus musculus*.

METHODS

Production of Virgin Coconut Oil (VCO)

Virgin Coconut Oil (VCO) fermentation have been use in this research. The fermentation is naturally for 24 h, room temperature, VCO is extracted and centrifugation at 60 min 10.000 x g, at room temperature and without the use of any chemicals.

Determination of Total Cholesterol (TC), Triglyceride (TG), High-Density Lipoprotein (HDL) Cholesterol and Low-Density Lipoprotein (LDL) Cholesterol Levels

The reagents for determined the total cholesterol, triglyceride, HDL and LDL used in the whole experiment were procured from DIASYS® kit which consists of enzymes that hydrolyze and oxidize total cholesterol, triglyceride and HDL into a colored compound kinonimin (red violet). Absorbance of this colored compound will be measured by *Genesys*$^{Tm\ 20}$ Spectrophotometer at 500 nm.

Animals Experiment

Forty two-month-old and 20-30 g body weight male mice from the Swiss strain were chosen for the study. All mice were divided into four groups: 4 mice in the negative control group were administered. The other 36 mice were divided into 3 groups. The positive control group were treated by feeding yolk for induced

serum total cholesterol and triglyceride. The experimental groups were administered with yolk and VCO 2% (0,4 mL/20g BW) and VCO 4% (0,8 mL/20 g BW) respectively. Each groups were consist of four mice for plasma total cholesterol, triglyceride and HDL measurement was conducted on day 10^{th}, 20^{th} and 30^{th}.

Measurement of Total Cholesterol, Triglycerides, HDL and LDL Levels

Measurement of total cholesterol and triglycerides was carried in the blood plasma, which blood was taken by cutting the blood vessels in the neck, the blood was collected in a test tube, and then left for 15 minutes. Later on the blood was centrifuged for 10 min at 3000 rpm, so the plasma was separated from the hemoglobin and thrombocyte.

For the measurement of total cholesterol and triglycerides, 10 μL of serum was pipetted, respectively. Then it was put in a test tube 1000 μL of cholesterol and triglycerides reagent solution was added. After that, the solution was mixed well by using a vortex. Then it was left for 20 minutes at room temperature and the absorbance was measured at 500 nm compared with the blank.

Measurement of absorption standard was performed in the same way to the absorption measurement of total cholesterol For the determination of HDL cholesterol level, 0.02 mL of serum total was pipetted, put in a centrifuge tube, then added a solution of 0.5 mL precipitator. The solution was mixed by using a vortex, then left for 10 minutes at room temperature and then centrifuged for 10 minutes at 4500 rpm. 0.1 mL of supernatant was taken and put into the test tube. Then 1 mL of cholesterol reagent solution was added, the solution was mixed using a vortex and left for 10 minutes at room temperature and absorbance was measured at 500 nm. LDL level was determined using the formula:

$$\text{LDL} = \text{Total cholesterol} - \frac{\text{Triglyceride}}{5} - \text{HDL}$$

Statistical analysis; were determined using ANOVA, Differences of $P<0.05$, were considered to be significant. Data are reported as mean SD.

GC Analysis of fatty acids

The fatty acid composition of lipid profile in plasma were analysed by gas chromatography using a system Hewlett- Packard GmbH, Waldbronn, Germany.Helium was used as carrier gas at flow rate of 5.5 mL/min.

RESULT AND DISCUSSION

Based on the **Table 1**, concentration of triglycerides of VCO treated and VCO untreated were significantly different. The triglycerides level were decreased significantly in VCO 2% and VCO 4%, subsequently after 30 days. Highest triglyceride levels were observed in positive control group (group II) compared to other treatment. Lowest triglyceride levels were obeserved in VCO concentration 4% (group IV) compared the other treatment.

The decreasing of triglyceride levels of VCO treated may be due to difference in transport and catabolism of medium chain fatty acids. Coconut oil contains mostly short and medium chain fatty acids such as, capric acid (7.46%), caprylic acid (7.69%) and lauric acid (58.4%), which are mostly absorbed through the hepatic portal vein and rapidly oxidized by both mitochondrial and peroximal pathways [8]. Lauric acid is the main medium chain fatty acid present in VCO. Small and medium chain fatty acids are easily absorbed through small intestine without enzymatic process. These fatty acids are carried to liver blood flow to be metabolized and transported to the mitochondria without camitine to produce energy quickly and efficiently, so they are not deposite as fat in tissue [9]. In other hand, beneficial effect of VCO is mainly due to the presence of unsaponifiable components, namely polyphenols and vitamin E. Studies revealed that the unsaponifiable components present in dietary oil have a role in regulating hepatic lipid metabolism. There are reports that polyphenols could inhibit hepatic lipogenesis, promoted hepatic lipid clearance and decreased serum and hepatic lipid accumulation [10].

According to Leontowicz et al [11] diet supplemented with polyphenolic compounds improved the lipid metabolism and increased the plasma antioxidant potential especially in rats fed with added cholesterol. Total polyphenol content of VCO were estimated at 84 mg/100 g oil[8]. Furthemore, another study revealed that phenolic fraction of VCO contain higher levels of caffein acid, p-coumaric acid, ferulic acid, and catechin acid than coconut oil[12]. The unsaponifiable fraction of VCO contains high amounts of antioxidant, namely Vitamin E (33.12µg/100g oil) and β-carotene (196µg/100g oil)[8,13]. Increased amount of unsaponifiable components in VCO may be partly be responsible for the decreased lipogenesis dan lipid accumulation[13].

Table 2 showed the increasing of HDL was significantly almost 2 times both in VCO 2% and 4% for 30 days of treatment. This similar results its true that effect of VCO will not cause an increase cholesterol desease[8]. The cholesterol ratio in **Table 3**, shows that treated VCO 2% in days 30 (2.222) and increase the VCO concentration 4% the cholesterol ratio become lower (1.502). This results possibly good for treated high cholesterol, because of the quality of VCO high in antioxidant and polyfenol[14] **.Table 4** showed an interesting saturated lipid profile (C6-C16), while in treated VCO (2-4%), almost long saturated Fatty acid was very low, (0.01 mg/mL). This is true that VCO rich in C12,C10 and C8 (MCT & SCFA), quicly burned and dissipated as energy unlike LCT which goes to the circulatory system and need lipase enzyme in lipid metabolism and deposits in the tissue.

Table 1. Triglyceride profile in blood serum of *Mus musculus*

Treatment	Concentration of Triglycerides (mg/dL)		
	10	20	30
Group I	59.07^{j}	59.07^{j}	59.07^{j}
Group II	136.67^{b}	135.22^{c}	177.30^{a}
Group III	89.89^{d}	79.16^{f}	73.66^{g}
Group IV	83.47^{e}	68.47^{h}	64.76^{i}

Superscript with different letter in line an column showed significantly different (P < 0.05)

Group I : Negative control
Group II : Positive control
Group III: VCO 2%
Group IV : VCO 4%

Table 2. Percentage of increasing HDL

Treatment	Duration (Days)		
	10	20	30
VCO 2%	5.25%	32.02%	69.01%
VCO 4%	2.24 %	42,99 %	74.08 %

P<0.05

Table 3. The Cholesterol Ratio

Treatment	Duration (days)			Average
	10	20	30	
Control (-)	1.326	1.326	1.326	1.326
Control (+)	7.252	6.069	4.427	5.582
VCO 2 %	4.098	1.416	1.154	2.222
VCO 4 %	1.000	1.893	1.613	1.502

Table 4. Plasma lipid profile (mg/mL) of saturated fatty acids (C6-C16)

No	Saturated Fatty Acid Carbon	Control positive	VCO 2%	VCO 4%
1	C6	---	---	---
2	C8	---	---	---
3	C10	---	---	---
4	C12	---	---	---
5	C14	1.5	---	---
6	C16	1.0	0.01	0.01

CONCLUSIONS

In this paper preclinic determination of lipid profile from Virgin Coconut Oil seems to have good supplement for lower the cholesterol levels, TG, and increase HDL. The interesting of Long Chain Fatty Acids was decrease in blood plasma of *Mus musculus*.

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