

THE EFFECT OF ACUTE CONSUMPTION OF HYDROLYZED OILS ON SWIMMING CAPACITY ENDURANCE OF MICE (*MUS MUSCULUS*)

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ABSTRACT

Objective: The aim of this study was to investigate the effect of hydrolyzed oils of different fatty acids composition on swimming capacity of mice.

Methods: The oils used were Virgin Coconut oil (VCO) and Palm Kernel oil (PKO) as medium chain triglyceride (MCT) oils, Palm oil and Corn oil as long chain triglyceride (LCT) oils. Hydrolysis of the oil was performed with *Rhizomucor miehei* lipase enzyme. The mice were acclimatized and tested motoric to select the mice and divided into twenty groups, each group consisted of 5 mice. Each oil in the experiment were 0.1 ml, 0.2 ml, 0.4 ml/ 20 g BW, positive control was caffeine 13 mg/kg bw. Then, the mice were placed in a glass aquarium. Then swimming capacity endurance was measured.

Results: Non-hydrolyzed oils with dose of 0.1ml significantly induced swimming capacity of mice compared with negative control (distilled water) from 184.8 increased to 248.0 seconds by MCT oil, to 239.8 seconds by unsaturated LCT oil and to 214.8 seconds by saturated LCT oil. The hydrolyzed oils increased swimming capacity \pm 65% by hydrolyzed MCT oils, \pm 54% by hydrolyzed unsaturated LCT oil and 21% by hydrolyzed saturated LCT oil. The hydrolyzed corn oil at high dose (0.4ml) was more effective to increase swimming capacity than saturated MCT (VCO and PKO) and LCT oil (palm oil).

Conclusion: The results suggest that hydrolyzed MCT and LCT oils are more effective to increase swimming capacity than non-hydrolyzed.

Keywords: Coconut oil, palm kernel oil, palm oil, corn oil, hydrolysis, swimming capacity.

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INTRODUCTION

Chemically, fat and oil are composed of various triglyceride molecules in which three fatty acids esterified in a glycerol molecule called triacylglycerol. The fatty acid is a monocarboxylic acid containing even number of carbon atom from 4,6,8 up to 22 atoms and usually classified into short (C:4–8), medium (C:10–12), and long (C:14–22) chain fatty acids. Based on the fatty acids composition, oils are classified into medium chain triglyceride (MCT) and long-chain triglyceride (LCT) oils. Virgin coconut oil (VCO) and palm kernel oil (PKO) belong to MCT oils with lauric acid (C:12) as a major component, and the other oils belong to LCT oils. Fatty acid composition and distribution (sn-1, sn-2, and sn-3) in the triglyceride molecules of oils will determine the chemical, physical, and biochemical properties of the oil [1,2]. VCO and PKO composed of medium chain fatty acids (MCFA) dominated by lauric acid, while corn oil (CO) and palm oil (PO) are dominated by long chain fatty acids (LCFAs) and classified as LCT oils. CO is an unsaturated oil contains linoleic acid and linolenic acid while PO is a saturated oil dominated by palmitic acid (C:16) [2,3]. Coconut oil is extracted from copra by the heating process, while VCO is prepared from the milk of fresh and mature coconut meat of coconut fruit and processed at low temperature without refining, bleaching, and deodorising process. Coconut oil is used as frying or cooking oil, while VCO is already an edible oil [4]. VCO also contains more phenolic compounds and antioxidants capacity than copra or commercial coconut oil [5].

MCT oils are metabolized differently from the LCT oils VCO and PKO (MCT oils) are hydrolyzed in mouth and stomach by lingual and gastric lipases and the hydrolyzed products generated from each triglyceride molecule are one 2-monoglyceride and two free fatty acids, due to lipase in the gastrointestinal tract specifically active only on sn-1,3 positions. The hydrolyzed products directly transported into the liver through the portal vein and quickly metabolized to produce energy

resulting in thermogenesis, and increase metabolic rate which may cause weight loss. MCT oils will not (or very small amount) enter the circulation so that they do not increase blood triglyceride level. Due to this metabolic pathway, MCT oils may increase stamina, insulin production and sensitivity, not affecting blood lipid level, while LCT oils may cause otherwise VCO has been used in health promotion, disease prevention, and medication [1,5,6].

MCT is immediately hydrolyzed into monoacylglycerol and directly absorbed and delivered to the liver through the portal vein and can pass through the mitochondrial membranes without carnitine so that it is more easily decomposed via β -oxidation and provides energy in a short time and stimulates the metabolism to maintain stamina. Lingual lipase and gastric lipase do not hydrolyze LCT in mouth and stomach, therefore LCT enter small intestine and emulsified by bile salts and hydrolyzed by pancreatic lipase to produce free fatty acid and 2-monoglyceride which then converted back (resynthesize) into triglycerides and bind to proteins and cholesterol to form chylomicron, then enter the blood circulation through the lymph system and hence may increase blood triglyceride level, and LCT oil is slowly metabolized in to energy. Due to this different metabolism pathway, there will be the different physiological effect of MCT including stamina compared with LCT [2,6,7]. The effect on the stamina of coconut oil and PO without hydrolysis has been investigated and reported that coconut oil was more effective to increase swimming capacity endurance of rats compared to PO [8,9]. The objective of this study was to study the effect of hydrolyzed oils of different fatty acid composition on swimming capacity of mice.

METHODS

Equipment

Equipment used in this study were magnetic stirrer, hotplate, oven, measuring cup, separating funnel, burette, glass aquarium, oral syringe,

pumpkin shaft, stopwatch, animal box, 1 ml syringe, funnel, dropper pipette, analytical balance, spatula, thermometer, air pump, hairdryer, ruler, and necessary glasswares.

MCT oils were VCO, PKO, LCT oils included PO (BIMOLI®), CO (MAZOLA®), lipase enzyme (*Rhizomucor miehei*), CaCl₂ 0.063M, Tris HCl buffer 1M pH 8, neutral ethanol 96 %, phenolphthalein, KOH 0.1 N, potassium biftalate, n-hexane, distilled water, and caffeine BPF1 (Indonesian Pharmacopeia Grade) 13 mg/10 ml in distilled water.

This experiment consisted of four stages; the first stage was enzymatic hydrolysis of coconut oil, CO, and PO. The second stage was an animal acclimatization test. The third stage was animal motoric test and the final stage was swimming endurance test.

Hydrolysis of oils

About 30 g oil was transferred into an Erlenmeyer to which 30 ml of distilled water, 12.5 ml CaCl₂ 0.063M, 25 ml Tris HCl buffer 1M pH 8, and 10% lipase enzyme (*Rhizomucor miehei*) were added, shaken using magnetic stirrer for 10 min and incubated at 50°C for 12 h. Then, it was transferred in to separating funnel, extracted with 50 ml n-hexane which resulted in two layers. The upper layer (n-hexane fraction) was separated (filtrate 1). The bottom layer was extracted again as above and obtained filtrate-2. Both filtrates were combined and to which anhydrous Na₂SO₄ added and allowed to stand for 15 min. It was then evaporated on water bath to dryness to obtain hydrolyzed oil. The acid value was determined by transferring 10 g hydrolyzed oil into an Erlenmeyer of 200 ml. Added 50 ml neutral alcohol of 95%, then warmed for 10 min on water bath while stirred. Then, it was titrated with KOH of 0.1 N using phenolphthalein of 1% in alcohol as an indicator. The endpoint of titration was when the pink color appeared; then the acid value was calculated [10,11].

$$\text{Acid value} = \frac{A \times N \times 56.1}{G}$$

A: Total volume of ml KOH used for titration

N: Normality of KOH solution

G: Weight of hydrolyzed oil (g).

Acclimatization of experimental animals

Acclimatization was performed on experimental animals by placing in standard cages with the same condition, diet, and under controlled condition. Acclimatization of experimental animals was conducted for 7 days to achieve adaption to experimental conditions. A good adaptation of mice was identified by a constant change in BWs [8,12].

Motoric test on mice

After the acclimatization, the motoric test (swimming capacity test) was carried out to select the mice those found to have relatively the same capacity to swim. Mice were placed in the aquarium for 10 min, and mice that cannot swim properly were not used in the experiment. From this test, male mice weighing 20–40 g about 2–3 months of age were used in this study [8,12].

Assay of the effect of hydrolyzed oils on swimming capacity of mice

Before treatment, mice were fasted for 12 h. Mice were divided into 20 groups, each consisted of 5 mice. Hydrolyzed oils were administered orally once to the mice (acute consumption). Group I (negative control) was given distilled water at 1% body weight (BW) (0.2 ml/20 g BW); Groups II, III, and IV were given PKO at 0.1 ml/20 g BW, 0.2 ml/20 g BW, and 0.4 ml/20 g BW, respectively. Groups V, VI, and VII were treated with CO as above; Groups VIII, IX, and X were treated with PO as above. Groups XI, XII, and XIII were treated with the same doses as above with hydrolyzed VCO, Groups XIV, XV, and XVI were treated with hydrolyzed CO, and Groups XVII, XVIII, and XIX were treated with hydrolyzed PO with the same dose as above. Group XX was given caffeine (positive control) with 13 mg/kg BW (0.2 ml caffeine solution/20 g BW). After feeding, experimental animals were allowed to rest for 30 min, then

the mice were placed in an aquarium containing water and with an air pump. The effect of each treatment on swimming capacity endurance determined by measures the time spent by mice to swim until stop swimming. Total periods of swimming to fatigue were measured and used as swimming capacity index [8,12,13].

RESULTS AND DISCUSSION

Acid value of hydrolyzed oils

Hydrolysis of oils was carried out by enzyme active only to hydrolyze fatty acids esterified on Sn-1,3 positions in triglyceride molecule, and hence it was partial hydrolysis. The levels of free fatty acids in hydrolyzed oils determined as acid value shown in Table 1.

Table 1 shows that the acid value of hydrolyzed oil is to indicate free fatty acids released from hydrolysis of oil and found to be lower than saponification value but higher than acid value of an oil. Acid value in this study was to indicate amount of free fatty acids present in oil after partial hydrolysis, while saponification value is to measure fatty acids present as triglyceride molecules measured after total hydrolysis with KOH through saponification reaction, while acid value of an oil is the amount of mg KOH required to neutralize free fatty acids contained in 1 g of oil [14]. In this study, enzymatic hydrolysis of oil with lipase produced two free fatty acids and one 2-monoglyceride molecule generated from one triglyceride molecule; since the lipase in *Rhizomucor miehei* active specifically on the sn-1 and sn-3 positions in the triglyceride molecule, therefore acid value in this study would be two-third (±65%) of saponification value of each oil. The similar acid values of partially hydrolyzed oil in this study also reported previously [8].

Acclimatization test of experimental animals

Test animal acclimatization was done to select appropriate experimental animals those being able to adapted environmental condition and the test conducted on day 0 to day 7. The data obtained presented in Table 2.

Table 1: Acid value of hydrolyzed oils

No.	Hydrolyzed oils	Acid value (mg KOH/g oil) (n=3)
1	VCO	170.71±8.644
2	PO	131.35±1.389
3	CO	130.11±2.655

VCO: virgin coconut oil, PO: Palm oil, CO: Corn oil

Table 2: Changes in BW of mice during acclimatization

Groups	BW (g)(n=5)		
	Day 0	Day 3	Day 7
I	30.74±2.56	34.72±2.50	33.00±1.88
II	21.68±4.28	26.62±3.90	27.72±3.37
III	22.40±3.78	26.14±4.26	27.08±2.93
IV	28.00±4.23	30.90±4.77	31.48±4.68
V	24.82±6.34	28.74±5.62	29.18±3.90
VI	18.40±4.67	23.54±3.67	27.84±1.72
VII	22.44±5.80	26.68±6.35	27.30±5.32
VIII	20.08±6.48	24.76±5.79	26.38±4.96
IX	23.36±5.20	27.14±5.25	28.68±4.05
X	22.04±8.38	25.54±7.81	27.12±6.00
XI	32.28±1.69	33.28±2.25	33.38±2.51
XII	32.74±2.13	32.44±1.83	28.76±1.99
XIII	31.88±2.45	31.22±2.79	31.32±2.94
XIV	33.36±0.80	33.10±0.88	33.58±1.17
XV	32.10±0.98	32.80±3.18	31.50±2.29
XVI	32.21±2.59	32.08±4.40	31.48±3.98
XVII	33.64±1.98	32.44±1.80	31.78±1.28
XVIII	30.00±2.21	32.64±3.12	32.20±2.85
XIX	30.28±2.77	32.12±2.70	31.84±2.93
XX	32.28±1.99	33.42±2.25	33.62±1.94

BW: Body weight

From Table 2 can be seen that there was a change of BW on the 3rd and 7th days. On the 7 days compared with 3 days, the weight of the animals changed or different within the value of 1.1 g in weight and not much different, indicating that the tested animals were able to adapt the experimental condition.

Motoric test and swimming capacity of mice

The swimming capacity of animals by the motoric test was evaluated before treatment. The motoric test data are presented in Table 3.

As shown in Table 3, it can be seen that the average swimming time for all mice was about 186.89 s. There is no significant difference in the swimming endurance of one group with other groups. Determination of the effects of acute consumption of tested oils given orally, then animals were allowed to stand for 30 min before placing in an aquarium. After that, the swimming capacity of mice was measured and data obtained shown in Table 4 and Fig. 1.

Table 3: Swimming capacity of mice in motoric test before treatment

Group	Swim capacity (s) n=5	Group	Swim capacity (s) n=5
I	188.80±6.099	XI	177.40±8.502
II	196.00±3.937	XII	191.60±12.259
III	193.00±10.848	XIII	184.80±9.203
IV	175.60±6.348	XIV	193.80±10.329
V	193.40±6.949	XV	191.20±6.942
VI	194.00±7.211	XVI	187.20±4.086
VII	178.80±7.049	XVII	180.40±9.659
VIII	192.20±6.534	XVIII	185.00±7.245
IX	180.80±8.467	XIX	175.80±9.576
X	190.60±7.300	XX	187.40±8.561

From Table 4 and Fig. 1, it is shown that the swimming capacity of mice increased with increasing doses. The mice given with dosage of 0.4 ml/20 BW with non-hydrolyzed PKO oil have a swimming capacity of 420.4 s, and non-hydrolyzed CO is 408.4 s, while non-hydrolyzed PO at the same dosage of 0.4 ml/20 g BW is the lowest (307 s). This is to indicate that non-hydrolyzed MCT oils are more effective than non-hydrolyzed unsaturated and saturated LCT oils. Hydrolyzed oils are more effective than non-hydrolyzed oils to increase swimming capacity. The mice given with dosage of 0.4 ml/20 BW with hydrolyzed MCT (coconut) oil have a swimming capacity of 502.8 s, and hydrolyzed CO is 533.2 s, while the swimming capacity by hydrolyzed PO at the same dosage of 0.4 ml/20 g BW is the lowest (398 s). This indicates that hydrolyzed unsaturated LCT (corn) oil has the highest effect to increase stamina compared to all given doses of oils, but it is still significantly lower than the positive control (caffeine 13 mg/kg BW). The mice treated with caffeine found to have the highest swimming ability (627.6 s) which is about three times higher than untreated mice or fed with distilled water (184.8 s).

Similar finding was also reported that the effect of coconut (MCT) oil was more effective to increase stamina compared to PO one of LCT oils [8]. In this study, as mentioned above that hydrolyzed oils found to increase swimming capacity more effective compared to non-hydrolyzed oils. This is attributed to the different metabolism, since MCT is hydrolyzed in stomach, quickly absorbed and enter liver through the portal vein and directly oxidized to produce energy. On the other hand, LCT oil such as PO is hydrolyzed in small intestine by pancreatic lipase and converted back into triglycerides and transported as chylomicron through lymphatic system into blood circulation and partly deposited [8].

The fatty acid composition of Virgin coconut and palm kernel (MCT) oils dominated by MCFA are more easily interacting with water (polar),

Table 4: The effect of oil and doses on mice swimming capacity

Treatments with increasing doses of oils	Swimming capacity (s) (n=5)					
	MCT oils		LCT oils			
	PKO without hydrolysis	Hydrolyzed VCO	CO (unsaturated)		PO (saturated)	
			Without hydrolysis	With hydrolysis	Without hydrolysis	With hydrolysis
0.1 ml/20g BW	248.00±10.488	313.20±17.612	239.80±19.677	288.00±18.220	214.80±8.288	239.80±5.718
0.2 ml/20 g BW	366.20±15.546	449.80±14.096	341.60±8.561	386.40±17.169	241.40±17.444	329.60±15.388
0.4 ml/20 g BW	420.40±9.502	502.80±15.303	408.40±16.486	533.20±15.530	307.60±19.204	398.20±13.217
Caffeine 13 mg/kg BW (positive control)					627.60±19.932	
Distilled water 1% BW (negative control)					184.80±8.012	

PO: Palm oil, CO: Corn oil, BW: Body weight, MCT: Medium chain triglyceride, LCT: Long-chain triglyceride

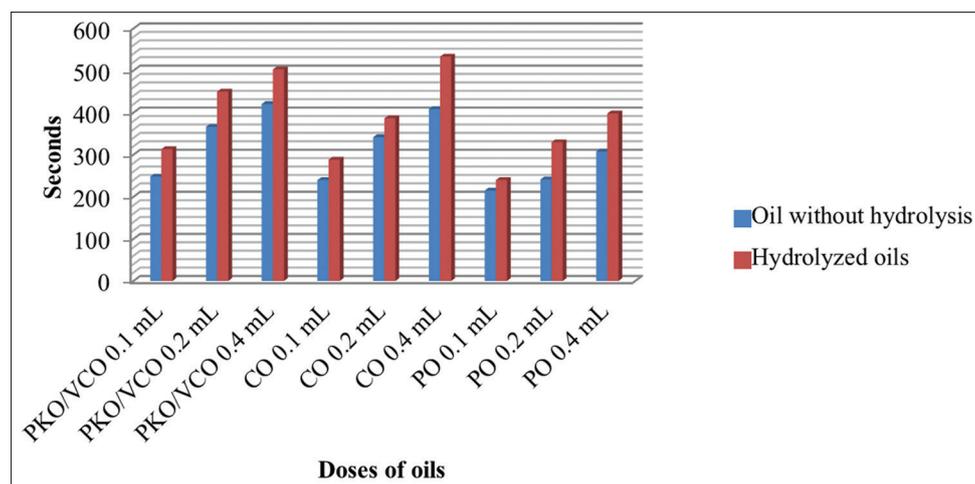


Fig. 1: Effect of oil feeding on the swimming capacity of mice. PKO: Palm kernel oil, VCO: Virgin coconut oil, CO: Corn oil, PO: Palm oil

rapidly absorbed into the portal vein directly transported into the liver and enter the mitochondria to be oxidized to produce energy so that MCT is not accumulated in the tissues adipose. MCT may decrease fatty deposits in adipose tissue and can increase ketone bodies in the blood. High concentration of ketone bodies in the blood indicate that the high oxidation of fatty acids, and increased fatty acid oxidation is proportional to the increase in endurance capacity (stamina) of mice [8,9,15].

In the present study, the effect of hydrolysis of oil is more effective to increase swimming capacity of mice than those without hydrolysis, because hydrolyzed oil directly absorbed without the need of lipase enzyme in the digestive system. However, unexpected results obtained suggest that the effect of hydrolyzed CO which belongs to unsaturated LCT oil found to be more effective than hydrolyzed MCT (PKO and VCO) oils [1,8].

CONCLUSION

Unhydrolyzed and hydrolyzed oils will increase the swimming capacity of the mice with increasing doses. The results show that hydrolyzed oils will increase higher swimming capacity of mice than oil without hydrolysis. The results suggest that non-hydrolyzed saturated MCT oils are more active to increase swimming capacity than saturated LCT (palm) oil and unsaturated LCT (corn) oil. However, hydrolyzed unsaturated LCT (Corn) oil found to have the highest effect among the hydrolyzed saturated MCT and saturated LCT oil.

AUTHORS' CONTRIBUTION

Jansen Silalahi conceived the study and was in charge of overall direction and planning and supervised the Works. Wina A Barus and Dwi R Anggraini were involved in planning and performed the experiments. Aminah Dalimunthe and Yosy C E Silalahi contributed in the experimental design, collected and analyzed the data, and wrote the manuscript with input from all authors.

CONFLICTS OF INTERESTS

The authors declare that they have no conflicts of interest.

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