

# EFFECT OF RICE BRAN OIL ON THE LIPID PROFILE OF MILD-MODERATE HYPERCHOLESTEROLEMIC MALE AGE 19-55 YEARS OLD

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**Abstract—Introduction:** Adult individuals in Indonesia showed changes in diet and lack of physical activity, therefore increasing the risk of hypercholesterolemia and obesity. One of nutritional therapy for hypercholesterolemia is rice bran oil, which contains active substances that works synergistically in controlling lipid profile. The substances are gamma-oryzanol, phytosterols, and vitamin E isomers (tocotrienol and tocopherol).

**Methods:** A clinical trial was conducted for 4 weeks in City Hall of Jakarta on 20 males, age 19–55 years old, with mild-moderate hypercholesterolemia, to assess lipid profile improvement of the supplementation of rice bran oil 45 ml/days compared to 15 ml/days without changing eating patterns.

**Results:** After 4 weeks of intervention, there was significant difference in total cholesterol level between both groups ( $p = 0.049$ ). In the group that received 45 ml/days of rice bran oil, total cholesterol level decreased 14%, and in the group of 15 ml/days total cholesterol level decreased 7.8%. The reduction of LDL and triglycerides and the increase of HDL were not significantly different between both groups ( $p > 0,05$ ). There was no significant changes of body weight in both groups.

**Conclusion:** This study showed that consumption of 45 ml/days rice bran oil led to better improvements in lipid profiles compare to 15 ml/days. It has been demonstrated that gamma oryzanol and plant sterols in rice bran oil have the capability to remove cholesterol from bile salt micelles, thus decreasing cholesterol absorption in intestine.

**Keywords:** rice bran oil, gamma-oryzanol, hypercholesterolemia, lipid profile

## INTRODUCTION

Cardiovascular disease has a tendency to increase in developing countries as a result of lifestyle changes.<sup>1</sup> According to WHO data of non-communicable

diseases in year 2010 in Indonesia, the prevalence of elevated cholesterol level was 35.1% and was higher in women (37.2%) than in men (32.8%).<sup>2</sup> Male adults are at risk for suffering hypercholesterolemia and central obesity due to lifestyle changes.<sup>3,4</sup> This condition may lead to increased risk of metabolic syndrome, hypertension, diabetes mellitus, obesity and other chronic diseases.<sup>4-6</sup>

Nutritional therapy became the first pillar as part of lifestyle intervention in hypercholesterolemia. Phytosterol, which is one of functional food, has been recommended by the National Cholesterol Education Program (NCEP) for controlling lipid profiles.<sup>7</sup> Rice bran oil contains gamma-oryzanol, phytosterols, and vitamin E (tocotrienols and tocopherols), which are known as unsaponifiables fractions. Gamma-oryzanol consists of ferulic acid and phytosterol esters, which are structurally and functionally similar to cholesterol. Both substances can reduce the absorption of cholesterol in the intestine as competitive inhibitors. Unsaponifiable fractions of rice bran oil act synergistically in controlling blood lipid profile. The composition of fatty acids in rice bran oil are closest to the recommendation of the American Heart Association (AHA).<sup>8-13</sup>

There were some studies done by Raghuram<sup>14</sup>, Kuriyan<sup>15</sup>, Rajnarayana<sup>16</sup> and Eady<sup>13</sup> which showed rice bran oil effect in improving lipid profile in hypercholesterolemic patients. Rice bran oil products are currently widely recommended by clinicians because of their benefits of preventing cardiovascular diseases. Earlier studies mostly used 300 mg/day dose of gamma-oryzanol or 50 grams of rice bran oil, which were included in the daily diet or cooking oil.<sup>13,17</sup> However no studies regarding the effective amount or dose of rice bran oil in daily consumption without changing food intake on lipid profiles of hypercholesterolemic patients has been done yet. This study aimed to assess the lipid profile

improvement after intake of different amount of rice bran oil without changing the eating patterns.

## METHODS

### Subjects

A total of 26 males with hypercholesterolemia, age 19-55 years old, were recruited for the study. Inclusion criteria were cholesterol levels 200-300 mg/dl and body mass index 20-30 kg/m<sup>2</sup>. The exclusion criteria were any consumption of cholesterol-lowering drugs, steroids and other drugs that affect lipid metabolism; smoking more than 10 cigarettes/day; taking weight loss program or consuming appetite inhibitor drugs; taking supplements containing phytosterol; have gastrointestinal, thyroid, heart, liver and kidney diseases which are determined by medical records; in hormonal therapy that affects the metabolism of fat, and suffering from type 2 diabetes mellitus. Subjects agreed to sign the informed consent and they were assured that all information will be confidential. Medical Ethics Committee from University of Indonesia has approved the study protocol.

### Clinical Study

This study was a randomized, single-blind, parallel design conducted at City Hall of Jakarta in April through May 2015. Subjects who met the study criteria were interviewed to complete the demographic data, physical activity, food intake using semiquantitative FFQ, and anthropometric measurements. Before and after 4 weeks of intervention, subjects were asked to complete food intake data of an estimated 3 days of food intake record of their usual dietary pattern. They were asked to record their food intake of 3 non-consecutive days : 2 weekdays and 1 day off or non working day, prior to rice bran oil treatment. They were also given a leaflet to help their estimation of food portion sizes.

The subjects were randomly assigned to 1 of 2 groups. One group was assigned to consume 45 ml/day rice bran oil (n=13), while another was 15 ml/day rice bran oil (n=13). Rice bran oil used for this study was purchased from the market. The oil were manufactured by Pietro Coricelli S.p.a., Italia. The oil was measured and packed into smaller bottles, which were then given to the subjects.. The subjects were asked to consume two bottles of rice

bran oil everyday. Compliance was assessed by facilitated researchers. The oil could be consumed with lunch and dinner or be drunk directly. Subjects were asked to not changing their daily diet.. Each subject was asked to consume the rice bran oil once a week until the end of treatment and also be interviewed to determine any complaints while taking the oil.

### Laboratory analysis

Subjects were asked to fast for at least 10 hours prior to blood sampling. Laboratory tests for lipid profile were performed twice, before and after 4 weeks of intervention.. During blood sampling, 3.5 ml of blood was drawn for the test. Fasting blood samples were collected into tubes and were placed for 30–40 min. Afterwards, blood samples were centrifuged at 1300–3000g for 20 min, and then the blood serum were taken for subsequent analysis in laboratory. Total cholesterol and triglycerides serum level were measured by enzymatic methods in an automated biochemistry analyzer. HDL-C values were measured by precipitation and direct methods. LDL was estimated with the Friedewald equations.<sup>18</sup> Lipid assays were standardized by the Center for Disease Control and Prevention.<sup>19</sup>

### Statistical analysis

Data were analyzed using SPSS version 20 for Windows. Unpaired t-test or Mann-Whitney test was used to compare the data from before and after the study intervention,  $p < 0.05$  indicated a statistical difference data from the food intake analyzed with Nutrisurvey 2007 for windows.

## RESULTS

From 26 who initially agreed to participate, 20 subjects completed the 4-week study.. One subject developed illness unrelated to the study and 5 refused to continue the study protocol. Rice bran oil consumption compliance was reported to be greater than 90% as assessed by self-completed check sheets. Baseline characteristics of the subjects are shown in Table 1.

The mean age of the 45 ml/day group and 15 ml/day group were  $48.90 \pm 5.26$  and  $45.70 \pm 6.82$  years old, respectively. Most of the subjects in this

**Table 1. Baseline Characteristic of The Subjects (n=20)**

Variables	45 ml/day RBO group (n=10)	15 ml/hari RBO group(n=10)
Age	48,90±5,26	45,70±6,82
Height,cm	164,70±6,18	164,40±6,13
Weight,kg	75,06±12,90	73,07±11,54
BMI,kg/m <sup>2</sup>	27,63±4,20	26,97±3,65
Energy,kkal	2205,25±193,53	2071,07±254,28
Carbohydrate,g	281,2±28,02	266,92±37,24
Protein,g	91,86±13,21	97,22±16,96
Fat,g	78,78±11,01	68,73±13,67
Fiber,g	11,06±0,96	12,25±3,18
Total cholesterol,mg/dL	242,30±33,66	228,80±16,96
LDL cholesterol, mg/dL	173,70±46,34	157,10±25,41
HDL cholesterol, mg/dL	40,70±7,02	45,70±10,06
Triglyceride, mg/dL	176,50 (84-308)	161,00 (97-427)

Values are expressed as mean±SD, median(min-max)

study were classified as in both groups. Physical activity of 45 ml/day group were mostly mild to moderate, and 15 ml/day RBO group were mostly moderate. Most subjects did not have family history of hypercholesterolemia. Also, most of the subjects were non-smokers. The two groups had similar characteristics in terms of age, education level, nutritional status, physical activity, family history of hypercholesterolemia, and smoking history (data not shown).

There was no significant difference in food intake between both groups before treatment. Composition of food intake in 45 ml/day RBO group was 51% carbohydrate, 17% protein and 32% fat. Composition of food intake in a 15 ml/day RBO group was 52% carbohydrate, 18% protein and 30% fat.

Average calorie intake after 4 weeks of intervention in 45 ml/day RBO group was 2387 kcal/day with composition of 46% carbohydrates, 16% protein and 43% fat. Whereas in 15 ml/day RBO group, calorie intake was 2224 kcal/day, with the composition of 48% carbohydrates, 18% protein and 37% fat. There was significant difference in total fat intake after intervention ( $p=0,014$ ). However, there was no significant difference in energy intake, carbohydrate, protein and fiber after intervention

between the two groups. Change of total fat intake ( $p=0,007$ ) and percentage of fat intake ( $p=0,003$ ) before and after treatment were also significantly different. Food intake analysis and changes are shown in Table 2.

The decline in serum cholesterol after 4 weeks of intervention was significantly different between both groups ( $p=0,049$ ). The average changes of cholesterol levels in 45ml/day RBO group were -37,50 (-53,00--9,00) and in 15ml/ day RBO group was -10,50 (-74,00-4,00). Decrease of LDL and triglycerides level were greater in 45 ml/day RBO group compared to the other group. Levels of HDL increased greater in 45 ml/day RBO group compared to the other group. However, LDL, HDL and triglyceride levels after treatment and changes during treatment were not statistically significant difference between both groups. Lipid profile examination results are shown in Table 3. Body weight was measured before and after treatment to ensure the safety of RBO intervention for 4 weeks. There was no difference of body weight before and after treatment in both groups.

## DISCUSSION

To our knowledge, this was the first study in Indonesia which compared intervention of rice bran

Table 2. Average Daily Intake of Energy, Protein, Fat, Carbohydrate and Fiber at Baseline, after Intervention and The Changes during Intervention

Variables	45 ml/day RBO group (n=10)			15 ml/day RBO group (n=10)		
	Baseline	Week 4	Change ( $\Delta$ )	Baseline	Week 4	Change ( $\Delta$ )
Energy,kkal	2205,25 $\pm$ 193,53	2205,25 $\pm$ 193,53	182,73 $\pm$ 79,78	2071,07 $\pm$ 254,28	2224,86 $\pm$ 317,38	153,79 $\pm$ 130,30
Carbohydrate,g	7,35 $\pm$ 6,24	275,76 $\pm$ 20,89	-5,44 $\pm$ 16,98	266,92 $\pm$ 37,24	267,45 $\pm$ 32,69	0,53 $\pm$ 23,94
Protein,g	91,86 $\pm$ 13,21	94,18 $\pm$ 14,15	2,31 $\pm$ 7,81	97,22 $\pm$ 16,96	103,09 $\pm$ 18,53	4,80(0,20–21,50)
Fat,g	78,78 $\pm$ 11,01	115,16 $\pm$ 12,05*	36,38 $\pm$ 5,8*	68,73 $\pm$ 13,67	91,56 $\pm$ 23,44	22,82 $\pm$ 12,03
Fiber,g	11,06 $\pm$ 0,96	11,48 $\pm$ 0,73	-0,42 $\pm$ 0,84	12,25 $\pm$ 3,18	12,32 $\pm$ 3,08	0,40(-2,30–1,40)

Values are expressed as mean  $\pm$  SD, and median (min-max). Values with asterisk (\*) are significantly different from 15ml/day RBO group,  $p < 0,05$ .

oil with different doses without any change in daily diet to determine the effect of unsaponifiable fractions in both groups. Most of the study subjects were obese with mild-to-moderate physical activity. Lack of physical activity stimulates escalation of lipid profiles and blood glucose which independently

15 mL/day group, however, the fat intake difference between both groups was only 14 gram or 5%. This may happened because the subjects who consumed oil 45 ml/day felt full and satisfied faster and longer, which then reduce the consumption of carbohydrates and fat.

Tabel 3. Lipid Profile of Research Subjects (n=20)

Variables	45 ml/day RBO group (n=10)			15 ml/day RBO group (n=10)		
	Baseline	Week 4	Change ( $\Delta$ )	Baseline	Week 4	Change ( $\Delta$ )
TC (mg/dL)	242,30 $\pm$ 33,66	207,90 $\pm$ 29,07	-37,50 (-53,00–-9,00)*	228,80 $\pm$ 16,96	210,70 $\pm$ 26,75	-10,50 (-74,00–4,0)
LCL-C (mg/dL)	173,70 $\pm$ 46,34	157,10 $\pm$ 38,58	-16,6 $\pm$ 11,18	157,10 $\pm$ 25,41	151,60 $\pm$ 24,59	-5,5 $\pm$ 19,36
HDL-C (mg/dL)	40,70 $\pm$ 7,02	45,90 $\pm$ 4,77	5,20 $\pm$ 4,52	45,70 $\pm$ 10,06	47,50 $\pm$ 12,17	1,80 $\pm$ 4,39
TG (mg/dL)	176,50 (84-308)	148,50 (104-294)	-37,70 $\pm$ 37,65	161,00 (97-427)	158,00 (105-367)	-20,5 $\pm$ 35,43

Values are expressed as mean  $\pm$  SD, and median (min-max). Values with asterisk (\*) are significantly different from 15ml/day RBO group,  $p < 0,05$ . TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDLC, high density lipoprotein cholesterol; TG, triglyceride.

affect central obesity.<sup>20</sup> Higher fat and lower fiber intake compared to recommendations can cause hypercholesterolemia and weight gain. Nutritional recommendation for dyslipidemia suggested that fat intake should be around 20-30% of total calories and fiber around 30 g/day.<sup>4,7</sup> Consistent to that, food intake data of the subjects in this study showed that unbalanced nutritional intake contributes to hypercholesterolemia and obesity.

In this study, intervention with additional rice bran oil (RBO) to the daily diet that contains high fat would affect the food intake proportion of the subjects. There was significant difference in fat intake ( $p = 0,014$ ) between the two groups. Change of fat intake in 45 ml/day RBO group was 15% and in 15 ml/day RBO group was 10%. This result shows eventhough the 45 mL/day RBO group consumed as much as three times the amount of RBO compared to

This study results showed improvement in lipid profiles, decrease in total cholesterol, LDL and triglycerides as well as increase of HDL level, which are consistent with the previous studies. In several studies previously, gamma oryzanol, phytosterols and vitamin E in rice bran oil have been reported in some previous studies to have hypocholesterolemic properties.<sup>14,15,21</sup> Effective dose of gamma-oryzanol itself is still unclear until now. Most earlier studies used doses of gamma-oryzanol 300 mg/day or, in the form of rice bran oil, 50 grams/day which was included in the daily diet or cooking oil.<sup>13,17</sup>

Research by Eady et al.<sup>13</sup> used 20 grams of RBO spread (118 mg of phytosterols and 30 mg of gamma-oryzanol) and showed significant improvements in lipid profiles. It showed that administration of small quantities of gamma-oryzanol still gave positive results on the lipid

profile. A study by Berger et al,<sup>22</sup> which compare the intervention of gamma-oryzanol 50 mg/day and 800 mg/day in the same amount of RBO (50 grams), showed decreased levels of lipid profile, however the differences were not statistically significant. Adding 18 g/day of rice bran oil (gamma-oryzanol 40 mg/dl) combined with diet for 5 weeks in diabetic significantly lowers total cholesterol and tends to decrease LDL level.<sup>23</sup> Earlier studies showed that a dose of 0.8-1 g/day of phytosterols can lower LDL level by 5%.<sup>13</sup> Related to the doses of RBO, this study found that administration 45 ml/day RBO led to greater improvements in lipid profiles. It is likely influenced by the amount of unsaponifiable fractions greater in administration of 45 ml/day. According to the data from the RBO manufacturer, there is around 33 mg gamma oryzanol in 15 ml RBO. Therefore, in this study, subjects consumed approximately 33 mg gamma oryzanol in 15 ml/day RBO group and 100 mg gamma oryzanol in 45 mg/day RBO group, which both still led to improvement in lipid profile.

Gamma-oryzanol inhibits HMG-CoA reductase enzyme which then lower the level of cholesterol in the liver, therefore, increasing hepatic LDL receptors and the uptake of LDL in the blood. The decrease of LDL level was influenced gamma-oryzanol and phytosterol contained in the rice bran oil. The two substances help to increase the expression of hepatic LDL receptors which facilitate reduction in LDL cholesterol, and increase the expression of CYP7A1 which facilitate the catabolism of cholesterol. Phytosterols and gamma-oryzanol have also been shown to inhibit the absorption of cholesterol in the body, thus increasing the fecal excretion of cholesterol.<sup>17,24,25</sup>

The percentage of increase of HDL level in 45 ml/day RBO group was 14%, indicating that there was clinically significant increase of positive effect eventhough not statistically significant. Many previous studies regarding administration of RBO in humans had not shown changes in HDL levels. The result of this study is different compared to Berger et al,<sup>22</sup> which showed no significant difference in HDL levels between the group who received low dose compare to high dose of gamma oryzanol. In this study, greater improvement of other lipid profiles indirectly lead to improvements in HDL value, although not significantly. For safety reason, subjects' body weight were measured before and after treatment. The result showed there was no

significant body weight difference in both groups after 4 weeks intervention.

There were some limitations in this study, such as possible errors in recording food intake. Furthermore, the analysis and composition of rice bran oil, factor storage, packaging, and environmental factors, which may influence the effective nutrients in rice bran oil, were not included in the data. This study was only done in male subjects in one community, thus the results of this study generally only could be used in individuals with similar characteristics.

In conclusion, this study showed that consumption of rice bran oil 45 ml/day led to better improvements in lipid profiles compared to 15 ml/day. Unsaponifiables fractions of rice bran oil had a role in controlling lipid profiles in hypercholesterolemic subjects. Rice bran oil in small dose (15 ml/day) still improved lipid profiles in hypercholesterolemic males. This study is a preliminary study with a short duration of time, therefore larger sample size and a longer intervention time to observe changes in body composition and lipid profile in the hypercholesterolemic patients is needed. Moreover, to assess the RBO effect in more diverse subject is necessary.

#### Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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