



## Hypocholesterolemic Effect of Analogue Rice with the Addition of Rice Bran

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### Abstract

The objective of this study was to evaluate the hypocholesterolemic effect of analogue rice added with rice bran from three different rice varieties (white, red and black). The Sprague Dawley rats were fed *ad libitum* with six different chow formulas, i.e. standard diet equal to AIN-93G (C-), high-cholesterol diet (C+), high cholesterol diet plus analogue rice containing 15% coconut dregs flour (AR1), high cholesterol diet plus 10% rice bran from Ciherang white rice (AR2), Cere red rice (AR3), and Campoireng black rice (AR4). The diet intervention was conducted for 28 days. The total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and triglyceride (TG) of rat plasma as well as total fat of rat liver were analyzed. The results showed that rats fed with AR1, AR2, AR3 and AR4 exhibited significant decreases of TC, LDL-C, AI and liver fat, but increased of HDL-C in comparison to those of a high-cholesterol group (C+). This hypocholesterolemic effect is associated with the significant role of dietary fiber and/or  $\gamma$ -oryzanol. Among groups with diet containing rice bran, AR2 demonstrated the highest hypocholesterolemic effect followed by AR4 and AR3. The AR1 group also gave a significant hypocholesterolemic effect ( $p < 0.05$ ) due to the role of the dietary fiber. As a conclusion, this study indicates that analogue rice added with rice bran and/or coconut dregs flour is a potential functional diet that is beneficial to lower the CHD risk.



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### Keywords

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Cholesterol;  
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Rice Bran.

### Introduction


Rice bran is the outer layer of rice seed, representing 5.0-8.0% of the grain,<sup>1</sup> which is usually removed during de-husking and hulling process of paddy. The rice bran is a source of carbohydrate (34-62%),

lipids (15-20%), protein (11-15%), dietary fiber (7-11%) and ash (7-10%).<sup>2</sup> Rice bran is also a good source of essential fatty acids, such as palmitate (21-26%), linoleate (31-33%) and oleate (37-42%).<sup>3</sup> There are a lot of rice varieties in Indonesia, which

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exhibit different nutrient composition and bioactive compound of their rice brans.<sup>4,5</sup> The rice pigment concentrates on its bran because of the presence of phenolic compounds and flavonoid.<sup>6-7</sup> Rice bran also contains phenolic compounds, such as tocopherols and tocotrienols,<sup>8,9</sup> oryzanols,<sup>10,11</sup> and phytosterols.<sup>11-14</sup>

Hypercholesterolemia is the presence of high level cholesterol in the blood as indicated by the elevated concentration of lipid and lipoprotein in blood.<sup>15</sup> Hypercholesterolemia has emerged as a strong risk factor for cardiovascular heart disease (CHD).<sup>16</sup> Several studies showed anti-hypercholesterolemia or so-called hypocholesterolemic effect of rice bran. Rice bran lowered blood cholesterol of experimental animals<sup>17,18</sup> and humans.<sup>13</sup> Its supplementation in the rat diet reduced body weight, total cholesterol, triglycerides and LDL-C, and increased HDL-C, without changing its blood glucose concentration.<sup>19</sup> The hypocholesterolemic effects of rice bran occurred through a decrease in liver cholesterol synthesis, i.e. decreasing the activities of acetyl-CoA acetyltransferase 2 (ACAT-2), 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA), and sterol-regulatory element-binding protein 2 (SREBP-2), and by increasing liver cholesterol degradation through human cholesterol 7  $\alpha$ -hydroxylase (CYP7a1) and human cholesterol 12  $\alpha$ -hydroxylase (CYP8b1).<sup>20</sup>

The phytochemical components available in rice bran, such as  $\gamma$ -oryzanol, significantly contributed to hypocholesterolemia.<sup>13</sup> The  $\gamma$ -oryzanol is a phenolic compound composed of ferulic acid esters and triterpene alcohols, which allow lowering total cholesterol and plasma LDL-C in experimental animals.<sup>18</sup> This is allied to the ability of  $\gamma$ -oryzanol to suppress lipogenesis in the liver and increase fecal fat excretion.<sup>21</sup>

The previous researches also showed the role of dietary fiber to reduce total cholesterol and LDL-C in blood, causing a decrease risk of CHD.<sup>22-24</sup> The dietary fiber is able to bind bile acids in the small intestine and then excreting them with feces. As a result, the endogenous cholesterol as a constituent of bile acids are breakdown, hence the cholesterol level decrease. In addition, the fermentation of soluble dietary fiber in colon produces short chain

fatty acids (SCFA) such as propionic acid which suppresses the cholesterol formation.<sup>24</sup>

The previous study provides information of rice bran potency in food processing as a functional ingredient that demonstrates hypocholesterolemic effect. One of the potential food product supplemented by rice bran is analogue rice. Analogue rice has been developed by applying an extrusion technology, which yielded artificial rice with acceptable physical and sensory quality.<sup>25-29</sup> Our previous study developed a cassava flour and sago starch-based analogue rice with the addition of coconut dregs flour.<sup>28</sup> In another study, analogue rice was formulated by the addition of rice bran with the aim of increasing nutrition content and health functional benefit.<sup>30</sup> The analogue rice supplemented by rice bran potentially provide a hypocholesterolemic effect, which is beneficial for people to lessen the risk of CHD attack. To provide scientific evidence, the study on hypocholesterolemic effect of analogue rice added by rice bran is required.

This study was aimed at evaluating the hypocholesterolemic effect of cassava flour, sago and coconut dregs flour mixture in analogue rice with the addition of rice bran. This research utilized three rice brans as by products of rice milling process, i.e. white, red and black rice. The study used experimental rats, and the hypocholesterolemic effect was measured based on plasma profile and total fat of liver rats after diet intervention.

## Materials and Method

### Materials

The materials used in analogue rice processing were cassava flour, sago flour, rice bran, coconut dregs flour and glycerol monostearate. Cassava flour was made from fresh cassava involving steps of washing, peeling, slicing into very thin shape, drying overnight in an oven and milling with a disc mill.<sup>27</sup> Sago flour was purchased from a local supplier in Bogor, Indonesia. Coconut dregs flour as a byproduct of coconut milk processing was obtained from a local manufacturer. Rice brans of three local rice varieties in Indonesia, i.e. Ciharang (white rice), Cere (red rice) and Campoireng (black rice), were obtained as a by product of rice milling process. Rice grain was milled using a rice miller (Satake) and the brown rice yield was further hulled using

a Satake grain huller. The rice bran was initially stabilized by heat treatment using a twin-screw extruder at a barrel temperature of 120°C and a screw speed of 900 rpm in order to inactivate lipase and lipoxygenase enzymes.<sup>30-31</sup> Glycerol monostearate (GMS) was purchased from Lautan Luas Ltd, Indonesia. Sprague Dawley rats for animal study was obtained from the School of Veterinary Medicine, Bogor Agricultural University. The  $\gamma$ -oryzanol standard was purchased from Wako Chemical Industries Co. Ltd., Japan. All chemicals used for chemical and biochemical analyses were of analytical grade.

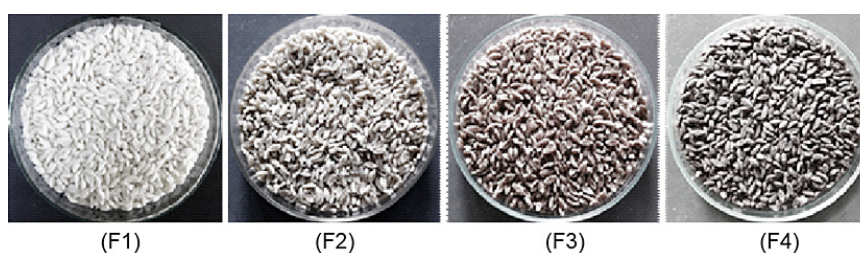
### Analogue Rice Formulation and Processing

The standardized ingredients and method of analogue rice processing referred to Patent

#PID201811079.<sup>32</sup> The ingredients (i.e. cassava flour, sago, coconut dregs flour and rice bran) were mixed gently using a dry mixer. The composition of dry mixture is presented in Table 1. Glycerol monostearate (GMS)(2%) and water (50%) were added into the mixture to form dough (the percentages of GMS and water were calculated as a total amount of dry mixture). The dough was fed into a twin-screw extruder (Berto BEX-DS-2256, Indonesia) at a barrel temperature of 85°C, screw speed of 96.8 rpm, and cutter speed of 71.2 rpm.<sup>27</sup> The analogue rice had a rice-like shape after passing out the die. The analogue rice was then dried in an oven at 85°C for 1.5 hours (Figure 1). Analogue rice samples were analyzed in terms of chemical composition,  $\gamma$ -oryzanol content, and animal study.

**Table 1: Formulation of analogue rice added with coconut dregs flour and rice bran**

Formula	Cassava flour (%)	Sago starch (%)	Coconut dregs flour (%)	Rice bran (%)		
				Ciherang	Cere	Campoireng
F1	44.0	41.0	15.0	-	-	-
F2	44.0	41.0	5.0	10.0	-	-
F3	44.0	41.0	5.0	-	10.0	-
F4	44.0	41.0	5.0	-	-	10.0



**Fig. 1: Analogue rice. Code of analogue rice formulation refers to Table 1**

### Animal Study

The animal study followed strictly the Guidelines for Health Research Ethics and was approved by the Ethical Approval Commission of the Health Research and Development Agency, Ministry of Health, Indonesia (LB.02.01/5.2/KE.288/2015). Sprague-Dawley rats (48 males, 40-60 g each) were housed individually in clean ventilated cages (28-30°C, relative humidity of 75%, and a photo cycle of 12 hours light/12 hours dark). Each rat was handled well to minimize suffering during the experiment.

Adaptation period was performed for a week by feeding the rats with a standard rat chow (AIAN 93G) and water available ad libitum. Rats were divided randomly into six groups (8 rats per group) for diet intervention (Table 2). Group 1 (negative control or C-) was fed by a standard feed. Group 2 (positive control or C+) was fed by standard feed plus cholesterol and sodium cholate in order to make hypercholesterolemic condition of rats.<sup>33</sup> Groups 3 (AR1) were fed same as C+ plus 15% coconut dregs flour, and Group 4 to 6 were fed by chow same as

C+ plus 5% coconut dregs flour and 10% analogue rice (AR2, AR3 and AR4, respectively).

Diet intervention was carried out every day at 9.00 AM for 28 days. Rat diets (20 g each) and water were provided ad libitum to each group. Diet consumption

was measured every day by weighing the remaining chow and subtracting this weight from the total diet weight given in the previous day. The rats were weighed every two days. At the end of experiment, body weight gain, total and average food intake were calculated.

**Table 2: Chow composition for each experimental rat group (in g/100 g)**

Components	Group					
	+Control – (C-)ª	Control + (C+)	AR1	AR2	AR3	AR4
Protein (casein)	20.00	20.00	18.95	18.32	18.22	17.92
Fat (palm oil)	7.00	7.00	3.76	4.74	4.75	4.08
Cellulose	5.00	5.00	0.45	2.43	2.26	1.50
Mineral mix	3.50	3.50	3.21	2.74	2.69	2.64
Vitamin mix	1.00	1.00	1.00	1.00	1.00	1.00
Water 10.00	10.00	5.43	4.09	3.13	3.61	
Sucrose	10.00	10.00	10.00	10.00	10.00	10.00
Carbohydrate (corn starch)	43.50	43.50	-	-	-	-
Sodium cholate	-	0.125	0.125	0.125	0.125	0.125
Cholesterol	-	0.50	0.50	0.50	0.50	0.50
Analogue rice <sup>b</sup>	-	-	57.21	56.94	57.96	59.25

<sup>a</sup>Standardized -AIN 93G<sup>34</sup>

<sup>b</sup>Analogue rice formulation in Groups F1 to F4 refers to Table 1

After fasting for twelve hours, the treated rats were anaesthetized using diethyl ether, and their blood (approximately 3 mL) was taken through the liver with a direct cardiac puncture. The blood was stored in a refrigerator of 4-6°C before analysis. Rats were then sacrificed and the organs (liver, kidney and adipose tissue) were collected. The organs were cleaned and washed with physiological saline solution, dried with dry tissue paper, and weighed. The organ weight is expressed as relative weight, which is weight of the organ per average body weight of rat. The liver part was analyzed for its total fat.

### Proximate Analysis

Analyses of moisture (gravimetric method), ash (gravimetric method), fat (soxhlet method), and protein (Kjeldahl method) followed the AOAC procedures.<sup>35</sup> Total dietary fiber (TDF), soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) were analyzed by an enzymatic-gravimetric method.<sup>35</sup> The total carbohydrate was determined by difference, i.e. 100 – (moisture + ash + fat + protein).

The chemical composition was expressed in the percentage of wet basis.

### γ-Oryzanol Analysis<sup>36</sup>

Sample (1 g) was reconstituted with 5 mL of distilled water and then added 0.2 g ascorbic acid. The solution was shaken and incubated at 60°C for 30 minutes. Five mL of isopropanol and hexane mixture (50: 50) was added to test tubes and shaken gently for 30 seconds to provide homogenous solution. The solution was centrifuged at 3000 rpm for 15 minutes, and organic layer was collected. The extraction process was repeated for the remaining residue and the organic layer obtained was mixed with the previous layer. The organic layer was washed with 5 mL aquabidest and blasted with nitrogen gas. Rice bran oil extract was diluted in the mobile phase (methanol: acetonitrile: dichloromethane : acetic acid; 50: 44: 3: 3, v/v) and filtered with PTFE membrane (0.45 µm). The sample solution (20µL) was injected into the C-18 HPLC column (Biorad Aminex HPX-87H, USA) with a flow rate of 1.0 mL/

min. The  $\gamma$ -oryzanol component was detected at a wavelength of 330 nm and quantification was performed using a standard curve of  $\gamma$ -oryzanol solution standard.

#### Total Fat Analysis of Liver<sup>37</sup>

Rat liver (1 g) was added with 20 mL of chloroform-methanol mixture (2:1). The liver extract was filtered with a free-fat filter paper. A total of 10 mL of liver extract was mixed with 2 mL of 0.9% NaCl solution in a centrifuge tube, and then centrifuged (2400 rpm, 10 minutes) to obtain a clear organic layer. The organic layer was washed twice using 1.5 mL of chloroform: methanol: water (8:4:3), and inserted into the vial. Extract was blasted with nitrogen gas and weighed. The total fat content was expressed as g fat per g liver.

#### Plasma Lipid Profile Analysis

Blood samples of experimental rats were incorporated into EDTA tubes and centrifuged at 3000 rpm for 10 minutes.<sup>38</sup> Transparent layer on the top was the plasma which was used for lipid profile analysis (TC, LDL-C, HDL-C, TG and atherogenic index (AI).

TC was analyzed using the cholesterol oxidase-p-aminophenozone (CHOD-PAP) method.<sup>39</sup> The plasma sample (10 $\mu$ L) was mixed with 1 mL reagent kit (containing cholesterol esterase, cholesterol oxidase, phenol, 4-aminoantipyrine, peroxidase and buffer), inserted into a tube and mixed until homogeneous. The mixture was incubated at 37°C

for 5 minutes, and the absorbance was read using a UV-Vis spectrophotometer at a wavelength of 546 nm and the total cholesterol concentration was determined using a cholesterol standard solution.

HDL-C was analyzed by applying a precipitation method.<sup>40</sup> The plasma sample (200  $\mu$ L) was mixed with 500  $\mu$ L precipitation reagents (phosphotungstic acid and MgCl<sub>2</sub>), then incubated at a room temperature for 10 minutes. The sample was centrifuged (4000 rpm, 10 minutes). After centrifugation, HDL-C fraction remained in the supernatant. A total of 100  $\mu$ L supernatant was taken and incubated at a room temperature for 10 minutes, and mixed with 1000  $\mu$ L CHOD-PAP reagent solution. The mixture was incubated at 37°C for 5 minutes and the concentration of HDL-C was measured using a UV-Vis spectrophotometer at a wavelength of 546 nm using a cholesterol standard solution.

TG analysis followed the glycerol-3-phosphate oxidase phenol aminophenazone (GPO-PAP) method.<sup>41</sup> The plasma sample (100  $\mu$ L) was initially hydrolyzed by lipase enzyme. The sample was then mixed with 1 mL of reagent kit, and incubated at 37°C for 5 minutes. The concentration of TG was measured using a UV-Vis spectrophotometer at a wavelength of 546 nm.

LDL-C and AI was calculated using the following formula:<sup>16,42</sup> LDL-C = TC - (HDL-C + TG/5) and AI = (TC - HDL-C)/HDL-C.

**Table 3: Chemical composition of rice brans of three rice varieties**

Component (%wb)	Rice varieties		
	Ciherang	Cere	Campoireng
Moisture (%wb)	13.76 $\pm$ 0.09 <sup>c</sup>	13.19 $\pm$ 0.00 <sup>b</sup>	11.59 $\pm$ 0.05 <sup>a</sup>
Ash (%wb)	8.02 $\pm$ 0.04 <sup>c</sup>	6.39 $\pm$ 0.11 <sup>a</sup>	8.21 $\pm$ 0.11 <sup>b</sup>
Protein (%wb)	12.69 $\pm$ 0.08 <sup>a</sup>	13.14 $\pm$ 0.08 <sup>b</sup>	14.48 $\pm$ 0.01 <sup>c</sup>
Fat (%wb)	14.31 $\pm$ 0.06 <sup>c</sup>	10.47 $\pm$ 0.05 <sup>a</sup>	12.39 $\pm$ 0.05 <sup>b</sup>
Total carbohydrate (%wb)	50.62 $\pm$ 0.24 <sup>a</sup>	52.81 $\pm$ 0.63 <sup>c</sup>	49.80 $\pm$ 0.75 <sup>b</sup>
TDF (%wb)	26.67 $\pm$ 0.04 <sup>b</sup>	22.58 $\pm$ 0.53 <sup>a</sup>	26.90 $\pm$ 0.74 <sup>b</sup>
SDF (%wb)	7.14 $\pm$ 0.15 <sup>c</sup>	6.01 $\pm$ 0.01 <sup>b</sup>	4.08 $\pm$ 0.29 <sup>a</sup>
IDF (%wb)	19.53 $\pm$ 0.19 <sup>b</sup>	16.57 $\pm$ 0.51 <sup>a</sup>	22.83 $\pm$ 0.45 <sup>c</sup>
$\gamma$ -oryzanol ( $\mu$ g/g)	1407.73 $\pm$ 50.68 <sup>a</sup>	1355.97 $\pm$ 21.95 <sup>a</sup>	1602.51 $\pm$ 24.16 <sup>b</sup>

Number followed by different letter presented at the same row shows significant differences at p<0.05

### Statistical Analysis

One-way analysis of variance (ANOVA) was performed using SPSS Statistics 20.00 software (SPSS Inc., Chicago IL, USA), which assessed the level of significant differences among data (cut

off of  $p < 0.05$ ). The data was presented in average followed by a standard deviation or error bar. The Pearson correlation analysis among parameters used SPSS 20.00 at a cut off of  $p < 0.05$ .

**Table 4: Chemical composition of analogue rice**

Components	F1	F2	F3	F4
Moisture (%wb)	7.41±0.09 <sup>a</sup>	9.40±0.06 <sup>b</sup>	10.60±0.18 <sup>d</sup>	9.74±0.20 <sup>c</sup>
Ash (%wb)	0.47±0.01 <sup>a</sup>	1.21±0.02 <sup>b</sup>	1.25±0.00 <sup>c</sup>	1.32±0.01 <sup>d</sup>
Protein (%wb)	1.69±0.04 <sup>a</sup>	2.66±0.09 <sup>b</sup>	2.74±0.03 <sup>b</sup>	3.17±0.08 <sup>c</sup>
Fat (%wb)	5.25±0.25 <sup>d</sup>	4.02±0.17 <sup>b</sup>	3.47±0.07 <sup>a</sup>	4.44±0.24 <sup>c</sup>
Total carbohydrate (%wb)	85.18±0.43 <sup>c</sup>	82.70±0.25 <sup>b</sup>	81.93±0.09 <sup>a</sup>	81.33±0.51 <sup>a</sup>
TDF (%wb)	6.11±0.82 <sup>a</sup>	6.76±0.26 <sup>a</sup>	6.90±0.62 <sup>ab</sup>	7.31±0.35 <sup>b</sup>
SDF (%wb)	2.03±0.65 <sup>a</sup>	2.15±0.14 <sup>a</sup>	1.70±0.34 <sup>a</sup>	1.92±0.03 <sup>a</sup>
IDF (%wb)	4.08±0.16 <sup>ab</sup>	4.61±0.12 <sup>a</sup>	5.27±0.75 <sup>b</sup>	5.39±0.32 <sup>b</sup>
$\gamma$ -oryzanol ( $\mu\text{g/g}$ )	n.d.	214.72±1.26 <sup>b</sup>	197.19±6.73 <sup>a</sup>	226.20±3.89 <sup>b</sup>

Number followed by different letter presented at the same row shows significant differences at  $p < 0.05$ ; n.d. not detected; Code of analogue rice refers to Table 1

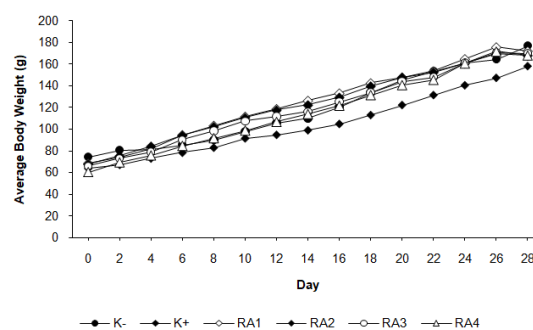
## Results

### Characteristics of Analogue Rice

The chemical composition of rice brans from Ciherang, Cere, and Campoireng varieties used in analogue rice formulation is presented in Table 3. Each rice bran had different contents of ash (6.39-8.21%), protein 14.71-16.38%, fat (10.47-14.31%) and total carbohydrates (49.80-52.81%). The total dietary fiber (TDF), which is composed of SDF and IDF, made up 42.8% to 54.0% of total carbohydrates. The rice brans also contained relatively high  $\gamma$ -oryzanol (1355.97-1602.51  $\mu\text{g/g}$ ). Rice bran from Cere varieties had the lowest content of TDF and  $\gamma$ -oryzanol in comparison to rice bran from Ciherang and Campoireng varieties. The TDF and oryzanol contents were comparable to the previous work.<sup>14,30,43</sup>

Analogue rice added with 10% rice bran (F2, F3, F4) had higher mineral and protein contents, and lower fat and total carbohydrate contents than those of analogue rice without rice bran (F1) (Table 4). Although the total carbohydrate decreased, the SDF and IDF increased in comparison to F1. Likewise, analogue rice (F2-F4) also contained a relatively high  $\gamma$ -oryzanol (197.19-226.20  $\mu\text{g/g}$ ). The analogue rice added with Cere rice bran showed the lowest  $\gamma$ -oryzanol content compared with the two others, which corresponded to its rice bran. The F1 did not

contain  $\gamma$ -oryzanol but it had TDF which derived from the mixture of coconut dregs flour, cassava flour, sago and cellulose.



**Fig. 2: Average body weight of rats during diet intervention. Code of rat groups refer to Table 2**

### Performance of Experimental Rats

Figure 2 presents average body weights for the six groups of rats over the 28 days of the experiment, which ranged from 60.2 to 74.2 g per each. There was no significant different ( $p > 0.05$ ) in the initial body weight among rat groups. The average of chow consumption by each rat ranged from 12.7-14.0 g per day or 66.5-70% of daily diet. It means that the different diet formulation did not significantly affect the proximate feed intake in experimental

groups (Table 5). The rat weight increased linearly during dietary treatments and the average weight gain of each rat was 83.6-107.8 g at the end of diet intervention. The body weight of rats in C- group tended to increase more slowly than that of the C+ and groups of AR1 to AR4. The average weight

gain of C- at the end of chow intervention was also lower than that of other groups. The addition of cholesterol and sodium cholate into the chow increase the absorption and accumulation of fat and cholesterol,<sup>33</sup> which was responsible to the increase of rat body weight.

**Table 5: Body weight and food intake of experimental rats**

Group	Total diet consumption (g)	Average food intake (g/day)	Initial body weight (g)	Final body weight (g)	Weight gain (g)
C-	383.48±16.62 <sup>bc</sup>	13.7±0.59 <sup>a</sup>	74.2±8.31 <sup>a</sup>	157.80±12.99 <sup>a</sup>	83.6±2.60 <sup>a</sup>
C+	386.72±12.72 <sup>bc</sup>	13.8±0.45 <sup>a</sup>	63.6±5.40 <sup>a</sup>	176.60±16.95 <sup>a</sup>	113.0±3.39 <sup>b</sup>
AR1	392.48±10.24 <sup>c</sup>	14.0±0.37 <sup>b</sup>	68.4±4.45 <sup>a</sup>	172.60±9.13 <sup>a</sup>	104.2±1.83 <sup>b</sup>
AR2	354.26±14.62 <sup>a</sup>	12.7±0.52 <sup>a</sup>	67.4±6.20 <sup>a</sup>	167.80±9.47 <sup>a</sup>	100.4±1.89 <sup>b</sup>
AR3	372.50±26.81 <sup>a</sup>	13.3±0.96 <sup>a</sup>	66.0±5.32 <sup>a</sup>	170.00±15.28 <sup>a</sup>	104.0±3.06 <sup>b</sup>
AR4	366.00±21.13 <sup>a</sup>	13.7±0.75 <sup>a</sup>	60.2±6.15 <sup>a</sup>	168.00±18.87 <sup>a</sup>	107.8±3.77 <sup>c</sup>

Number followed by different letter presented at the same column shows significant differences at  $p < 0.05$  ( $n = 5$ ). Code of rat groups refer to Table 2

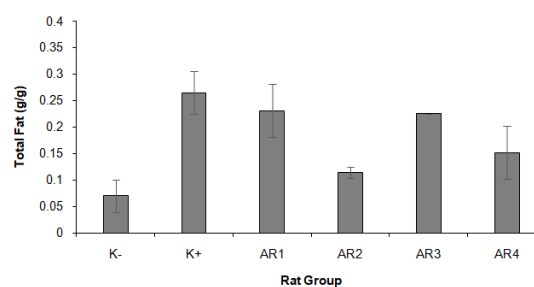
The relative weight of organs (liver, kidney and adipose tissue) of experimental rats is presented in Table 6. There was no noticeable difference ( $p > 0.05$ ) in the weight of kidney and adipose tissue in all groups under study. It indicates the different diet intervention did not affect the weight of kidney and adipose tissue. On the contrary, the liver weight significantly increased in C+ group and those rat groups fed with analogue rice (AR1, AR2, AR3, and AR4) compared with the C- group. These increases were attributed to the accumulation of cholesterol in

the liver.<sup>33</sup> The highest increase in the liver weight was observed for C+ group (Table 4), which is similar to earlier report.<sup>16,44</sup> The AR1 and AR3 groups did not change the liver weight in comparison to C+ group. However, the liver weight of rats in AR2 and AR4 groups decreased significantly by 13.55% and 14.80% respectively in comparison to that of C+ group. The result indicates that the consumption of analogue rice containing rice bran, especially AR2 and AR4, had a synergic effect to reduce the accumulation of cholesterol in rat liver.

**Table 6: Relative weight of liver, kidney and adipose of experimental rats at the end of diet intervention (g/g body weight)**

Group	Liver	Kidney	Adipose
C-	3.18±0.18 <sup>a</sup>	0.69±0.03 <sup>a</sup>	0.83±0.18 <sup>a</sup>
C+	5.05±0.25 <sup>c</sup>	0.71±0.02 <sup>a</sup>	0.95±0.27 <sup>a</sup>
AR1	4.82±0.50 <sup>bc</sup>	0.74±0.05 <sup>a</sup>	0.90±0.15 <sup>a</sup>
AR2	4.53±0.17 <sup>b</sup>	0.72±0.02 <sup>a</sup>	0.96±0.24 <sup>a</sup>
AR3	4.80±0.37 <sup>bc</sup>	0.69±0.01 <sup>a</sup>	0.93±0.10 <sup>a</sup>
AR4	4.50±0.25 <sup>b</sup>	0.73±0.06 <sup>a</sup>	0.88±0.15 <sup>a</sup>

Number followed by different letter presented at the same column shows significant differences at  $p < 0.05$  ( $n = 5$ ). Code of rat groups refer to Table 2



**Fig. 3: Total fat content in liver of experimental rats. Code of rat groups refer to Table 2**

Figure 3 shows the TF content in liver of experimental rats. The provision of high cholesterol chow in C+ group gave a higher TF of the liver compared to C-. The provision of analogue rice in the AR2,

AR3 and AR4 groups resulted in the decrease of TF accumulation in comparison to C+ group. The AR2 group gave the lowest TF, followed by the AR4 and AR3, while the AR1 group exhibited the lowest decrease of TF among AR group. The TF was positively correlated with liver organ weights ( $r=0.745$ ), representing the higher the TF of the liver resulted in the higher weight of the liver because of fat accumulation.<sup>38</sup>

### Plasma Lipid Profile

Rats fed with a normal diet (C-) showed a normal TC, LDL-C, HDL-C and TG concentrations (Table 7), indicating they were in a normal condition. The diet intervention with a high-cholesterol diet (C+) experienced a significant increase of TC and LDL-C and significant decrease of HDL-C and LDL-C ( $p<0.05$ ), meaning that the rats were in a hypercholesterolemic condition. The TC concentration in intervention groups was well below 200 mg/dL (normal range) in exception for AR3 (215.0 mg/dL). However the TC concentration in

AR3 group was significantly lower than that of C+ group ( $p<0.05$ ). AR2 group experienced the highest TC-lowering effect (65.45%) compared with those of C+ group, followed by AR4 group (57.15%), AR1 group (50.62%) and AR3 group (29.78%).

Intake of a high-cholesterol chow in C+ group caused a significant decrease of plasma HDL-C in comparison to C- (84.4 mg/dL). The provision of analogue rice in AR1, AR2, AR3 and AR4 increased significantly the plasma HDL-C in comparison to C+ group (Figure 3).

Rats at C- group showed a higher level of TG concentration (76.0 mg/dL) than that of C+ group (67.6 mg/dL). AR4 group had the highest TG concentration (140.2 mg/dL), but this concentration was below the normal limit (150.00 mg/dL). The AR1 group also showed a decrease in TG level (59.8 mg/dL), which was attributed to dietary fiber available in coconut dregs flour.

**Table 7: TC, LDL-C, HDL-C, TG concentrations, and AI in rats fed with experimental diets for 28 days**

	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)	AI
C-	90+11.1 <sup>a</sup>	84.4+11.0 <sup>c</sup>	40.8+4.1 <sup>a</sup>	67.6+12.6 <sup>a</sup>	0.07+0.04 <sup>a</sup>
C+	343+41.4 <sup>c</sup>	41.6+9.0 <sup>a</sup>	160.0+11.5 <sup>c</sup>	76.0+15.9 <sup>ab</sup>	8.14+0.84 <sup>d</sup>
AR1	151.2+49.7 <sup>ab</sup>	52.8+12.9 <sup>ab</sup>	104.0+41.7 <sup>b</sup>	59.8+8.8 <sup>a</sup>	2.27+1.49 <sup>bc</sup>
AR2	105.8+26.0 <sup>a</sup>	52.8+2.9 <sup>ab</sup>	91.2+28.4 <sup>b</sup>	100.0+43.5 <sup>c</sup>	1.00+0.42 <sup>b</sup>
AR3	215.0+64.7 <sup>b</sup>	49.2+3.9 <sup>ab</sup>	159.4+21.7 <sup>ab</sup>	85.0+21.4 <sup>b</sup>	3.34+1.13 <sup>c</sup>
AR4	131.2+49.8 <sup>a</sup>	58.0+12.1 <sup>b</sup>	116.4+31.3 <sup>b</sup>	140.2+14.0 <sup>d</sup>	1.19+0.45 <sup>b</sup>

Number followed by different letter presented at the same column shows significant differences at  $p<0.05$ . Code of rat groups refer to Table 2

AI indicates a major risk factor for atherosclerotic plaque formation that affect the incidence of CHD.<sup>38</sup> The higher AI indicates the higher risk of CHD. AI decreased significantly in all AR groups compared to that of C+ group, i.e. 72.1% (AR1), 87.7% (AR2), 59.0% (AR3) and 85.4% (AR4).

### Discussion

The three types of rice bran (Ciherang, Cere and Campoireng) had different TDF content (6.11-7.31%wb), which was composed of IDF and SDF. Rice bran from Cere rice contained the lowest TDF compared to that of two others. The different TDF

content of rice bran affected the composition of the TDF in analogue rice (Table 4). The SDF correlated significantly with TC, LDL-C, AI and TF, whereas IDF only correlated significantly ( $p<0.05$ ) with LDL-C (Table 8). Neither SDF nor IDF correlated significantly with HDL-C. The result indicates that SDF contributed significantly to the decrease in TC, LDL-C, AI and TF, while IDF contributed significantly ( $p<0.05$ ) to the decrease of LDL-C. SDF is able to absorb cholesterol and bile acids, which are then removed from the body through the stool.<sup>45</sup> Moreover, the fermentation of SDF in the large intestine stimulates the production of short chain



fatty acids, such as propionic acid which inhibits the production of cholesterol.<sup>22</sup>

**Table 8: Coefficient correlation between TC, LDL-C, HDL-C, TG, AI, and TF with  $\gamma$ -oryzanol, SDF and IDF**

	$\gamma$ -oryzanol	SDF	IDF
TC	-0.890*	-0.943*	-0.624
LDL-C	-0.712*	-0.972*	-0.770*
HDL-C	0.975*	0.302	0.379
TG	-0.930*	-0.076	-0.235
AI	-0.888*	-0.749*	-0.445
TF	-0.768*	-0.844*	0.449

\*Significantly correlated at  $p < 0.05$

Consumption of analogue rice without the addition of rice bran (F1) also revealed a relatively high TDF of rat plasma although it was lower than that of F2, F3 and F4. The significant decrease of TC, LDL-C, AI and TF in AR1 group compared with C+ group was associated with the role dietary fiber. The dietary fiber derived from mixed materials in the chow (cellulose, cassava flour, sago flour and coconut dregs flour).

Rice bran from Campoireng (black rice) contained the highest  $\gamma$ -oryzanol, while rice bran from Cere rice was the lowest (Table 3), which corresponded to the  $\gamma$ -oryzanol content present in analogue rice (Table 4). The  $\gamma$ -oryzanol significantly related to the decrease of TC, LDL-C, TG, AI and TF and the increase of HDL-C (Table 8). This result confirms the previous research which showed a significant role of  $\gamma$ -oryzanol to hypocholesterolemic effect.<sup>18,46</sup> The possible mechanism of cholesterol-lowering effect related to the role of  $\gamma$ -oryzanol to interfere with cholesterol absorption as well as increase excretion of cholesterol and its metabolites in feces. The digestive enzymes may metabolize  $\gamma$ -oryzanol in the digestive tract, which breaks it into free ferulic and sterols. The free ferulic is absorbed and acts as an antioxidant in the plasma along with the action of free sterols that prevents the absorption of cholesterol in the digestive tract.<sup>18</sup>

This study suggests the significant role of dietary fiber (especially SDF) and  $\gamma$ -oryzanol present in

rice bran to decrease the blood cholesterol level of hypercholesterolemic rats. The CHD risk of the rats is also lowered as indicated by the significant decrease of AI. Consumption of analogue rice added with rice bran from white rice (Ciherang) had the highest hypocholesterolemic effect followed by that of black rice (Campoireng) and red rice (Cere). The rice analog intake containing 15% coconut dregs flour also revealed a hypocholesterolemic effect owing to the role of dietary fiber.

### Conclusion

Intake of rice cassava-based analogue rice with the addition of 15% coconut dregs flour (F1), 5% coconut dregs plus 10% rice bran from white rice (F2), red rice (F3) or black rice (F4) exhibited significant hypocholesterolemic effect on experimental rats as characterized by the decrease of TC, LDL-C, AI and liver fat as well as increase of HDL-C. Rats at AR2 group experienced the highest hypocholesterolemia followed by AR4, ARF1 and AR3. The hypocholesterolemic effect is associated with the significant contribution of SDF and/or  $\gamma$ -oryzanol in rice bran. This study suggests that analogue rice added with rice bran and/or coconut dregs flour is a potential functional diet that is beneficial to lower the CHD risk.

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### Conflict of Interest

The authors have no conflict of interest.

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