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Lipid Lowering Effect of Brown Pigmented Rice (*Oryza Sativa L*) in Hyperlipidemic Sprague Dawley Rats

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***Abstract* –The study investigated the hypolipidemic effect on diet induced hyperlipidemic Sprague Dawley rats of three brown pigmented rice (BPR) varieties namely, Inpari 24, Mawar and Segreng from Indonesia. Study phases included: 1) chemical analysis of the proximate composition, total phytosterol, amylose and dietary fiber contents of BPR, estimated glycemic index (EGI), and the fecal fat content; 2) intervention phase 1 where effective dose of BPR was determined; and 3) intervention phase 2 where the *in vivo* evaluation of the effective dose was established. Segreng variety exhibiting relatively high phyrosterol, dietary fiber and amylose contents and moderate glycemic index (GI) was the variety used. Highest lipid lowering effect was observed in rats fed with of 75% cooked BPR in the effective dose determination. The 75% cooked BPR exhibited significant reduction of 53.48% in total cholesterol (TC), 53% in LDL-cholesterol (LDL-c), 37.4% in triglycerides and more than 131.96% increase in HDL-cholesterol (HDL-c) in the in vivo evaluation of the effective dose. The observed mean TC, LDL-c, triglycerides and HDL-c in hyperlipidemic rats fed with 75% BPR were comparable with those of the hyperlipidemic-statin-treated rats at 20 mg/Kg of body weight. Significant positive association for TC, LDL-c and triglyceride levels with increased calorie and fat intakes were also observed. Therefore, the study concludes that consumption of cooked BPR can lower lipid levels in diet-induced hyperlipidemic Sprague Dawley rats. The lipid lowering effect could possibly be due to the phytosterol, amylose and dietary fiber contents present in brown pigmented rice. Further study utilizing humans as subjects is recommended.**

***Keywords* – Brown Pigmented Rice, Hypolipidemic Effect, Phytosterol, Segreng Variety.**

I. Introduction

The prevalence of coronary heart disease (CHD) is increasing along with the rapid advancement where people's dietary and lifestyle pattern are changing. According to the latest World Health Organization (WHO) data published in May 2014, deaths due to coronary heart disease in Indonesia reached 138,380 or 9.89% of total deaths, ranking 97th in the world. Bittner (2011) states that dyslipidaemia is an important risk factor in the occurrence of atherosclerosis which is closely associated with cardiovascular diseases such as CHD.

Hyperlipidemia is a condition characterized by elevated levels of lipids that includes increasing one or more of normal levels of cholesterol, cholesteryl esters, phospholipids or triglycerides. Based on Household Health Survey in 2004, the prevalence of hyperlipidemia in Indonesia at the age of 25 to 34 years increased to 9.3% while those aged 55-64 years approximately increased to 15.5% (DOH 2004). With this growing concern, the consumption of natural foods like whole grains or brown rice is being promoted for the prevention and management of heart diseases since they have no side effects and are relatively cheaper.

In terms of nutrient profile, researches have shown that the bran in brown rice is superior over white rice for its crude ash, protein, dietary fiber, B-vitamins, vitamin E, minerals, polyphenols and fatty acid content (Dinesh Babu et al. 2009, Juliano 2007). The major components of brown rice which are believed to exhibit cholesterol and triglyceride lowering effects are the fiber, phytosterols and vitamin E contents.

Researchers believed that insoluble fiber in brown rice not only speeds intestinal transit time but also lowers triglycerides. Soluble dietary fiber was also seen for its potential to decrease the levels of total cholesterol and LDL cholesterol in the serum (Glore et al. 1994). Investigators believed that the viscosity and the ion-change capacity of the dietary fibers are important in lipid absorption (Guillon and Champ 2000). Furthermore, the absorption of bile salts by dietary fibers and the products of bacterial fermentation of dietary fibers play key roles in the changes in lipid and cholesterol levels (Thebaudin et al. 1997).

Dehulled or brown pigmented rice (BPR) is seen as a potential solution, it is thought to contain dietary fiber and phytosterols that can lower blood cholesterol. The Food and Drug Administration (FDA) and the Indonesian Food and Drug Administration have approved the claims of phytosterol health in reducing the risk of heart disease with a minimum of 1.3 g/day ester of fitosterol intake in two serving as part of a low-saturated and low-cholesterol diet (FDA 2010, BPOM 2011). Increasing body of evidence indicates that brown rice constituents such as sterols (e.g. β-sitosterol, stigmasterol and γ-oryzanol), resistant starch (e.g. amylose) and dietary fibers (e.g. β-glucan, pectin and gum) have anti-hyperlipidemic and anti-hyperglycemic effects.

The purpose of this study was to evaluate the effect of diet with BPR on the lipid profile of Sprague Dawley rats induced with hyperlipidemic conditions. Efforts are currently being undertaken to address the acceptability and promote awareness on consumption of brown rice and one way to convince them is to provide evidence on the potential health claims and benefits through this study.

II. Materials and Methods

Three pigmented rice varieties, namely, Inpari 24, Mawar, and Segreng, were procured from Yogyakarta Assessment Institute for Agriculture Technology, Indonesia. The rice grains were cleaned then dehulled in the Food and Nutrition Gadjah Mada University. The dehulled BPR was packed in moisture proof containers and stored in a clean, cool and dry place for later use. The rice grains were analyzed for proximate composition, carbohydrate profile, phytosterol and dietary fiber contents using the standard procedures of Association of Official Analytical Chemists - AOAC (1995) while glycemic index was determined in vivo experiment in human with the approval of the Committee of Ethical Clearance for Pre-Clinical Research of the Integrated Laboratory of Research and Testing, Gadjah Mada University, Yogyakarta (Ref. 00119/04/LPPT/11/2017.

Preparation of Animals

Sprague Dawley rats were used since many genes of mice are relatively similar to humans. The ability of breeding the mice is very high, relatively suitable for use in a mass experiment; the physique of small rodents is easily maintained and drugs used in the body can be quickly manifested. The use of female rats was avoided because of hormonal (estrogen/progesterone) fluctuation during menstruation cycle which might influence the results of the experiment. Clearance from the ethical committee of Food and Nutrition Study Center, Universitas Gadjah Mada Yogyakarta was obtained prior to the start of the study.

Acclimatization

Eight healthy male Sprague Dawley rats, 6-8 weeks old, weighing between 150-250g were acclimatized for a week (7 days) for adaptation in captivity and feed before the experiment, then placed in a plastic enclosure with a lid made of wire. Feeds and drinks were given ad libitum. Environmental enclosures were maintained with relative humidity of 60-70% at temperature of about 25^{o}C and with alternating exposures to light and dark conditions at 12 hours interval. Each group of 2 mice was placed in separate cages and maintained so as not to interact with each other.

Preliminary Observation

Usual food intake was observed using the same rats in acclimatization. Sprague Dawley rats were grouped into four and assigned a specified test food; the first group was fed with cooked pigmented rice; the second group with cooked white rice; the third group was fed with high fat (HF) rodent feed; and the fourth group was given a feed of 50% BPR and 50% HF. The feeding was done for 7 days. Blood lipid levels were measured before and after the intake of the test foods. All animals were killed at the end of the experiment.

Induction of Hyperlipidemia

A total of 24 Sprague Dawley rats were induced with hyperlipidemia. A high fat (HF) rodent feed from the Food and Nutrition Laboratory in Gadjah Mada University in Indonesia as hyperlipidemic inducer were given ad libitum. Baseline lipid profile of the rats, including TC, triglyceride, HDL and LDL was collected before the induction process. The rodent feed was administered for 30 days and the blood lipid level was again measured to determine the effect of the feed.

Determination of Effective Dose

The diet-induced hyperlipidemic Sprague Dawley rats were grouped into four, with 6 rats per group. One group was given pure HF rodent feed (hyperlipidemic control, HC). The three other groups were given various mixtures of cooked BPR and HF rodent feed as follows: Hyperlipidemic Dose 1 (HD1) group was given 25% cooked pigmented rice and 75% HF rodent feed; Hyperlipidemic Dose 2 (HD2) with 50% cooked pigmented rice and 50% HF rodent feed; and Hyperlipidemic Dose 3 (HD3) with 75% cooked pigmented rice and 25% HF rodent feed. Blood lipid level was measured at baseline and every week thereafter for one month. Daily changes in feed intake and physical change were observed. Weight was also monitored every week.

Determination of the Lipid Lowering Effect of the Effective Dose

Induction of Hyperlipidemia

For this phase, another set of 24 Sprague Dawley rats were induced with hyperlipidemia implementing the same method in effective dose determination. Blood lipid level was collected before and after the induction to establish baseline levels and determine the effect of the feed.

A total of 30 Sprague Dawley rats, 6 non-hyperlipidemic and 24 feed-induced hyperlipidemic rats were used. Five (5) groups were created with 6 rats per group which included four control groups and one treatment group. The four control groups were composed of non-hyperlipidemic rats which received commercial feed only (NHC), hyperlipidemic rats (negative control) fed with HF rodent feed only (HC), hyperlipidemic rats (negative control) fed with 50% HF rodent feed and 50% cooked pigmented rice (HPR), and hyperlipidemic rats (positive control) were given HF rodent feed plus 20 mg/kg BW of simvastatin drug (HST). The treatment group was the hyperlipidemic rats that were given the 75% cooked pigmented rice and HF rodent feed from the effective dose experiment (HCBPR). The control groups were intended for comparison of lipid lowering effects and total fat present in the feces.

Statistical Analysis

Data were analyzed using Statistical Package for Social Science (SPSS) version 22.0 and Microsoft Office Excel software. Analysis of Variance (ANOVA) was used to compare groups and T-statistics was used to determine the level of significance. The probability value of <0.05 was considered as significant result. Bivariate correlation analysis was also done using SPSS.

III. Results

Chemical Composition of Brown Pigmented Rice

Segreng variety showed high amounts of phytosterol and dietary fiber at 2.50 and 5.59%, respectively, moderate amylose content of 17.22% and also moderate glycaemic index value of 55 (Table 1). Thus, it was selected as the test food for the intervention phase of the study.

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Table 1. Glycemic index, phytosterol, dietary fiber, amylose and amylopectin contents of brown pigmented rice.

Variety	Glycemic Index (Gi)*	Phytosterol (%)**	Dietary Fiber (%)	Amylose (%)
Mawar	59.79	2.39^{b}	4.78a	19.65^{a}
Inpari 24	59.95	2.49^{a}	4.37a	15.06^{b}
Segreng	55.00	2.50^{c}	5.59b	17.22^{c}

* moderate (55-70) classification of GI value
** only β-sitosterol and stigmasterol

Acclimatization

The mean food intake regardless of groupings was 12.52 g/day. Generally, the rats experienced weight gain between 9.5 to 10.5g and all were healthy. This indicated that the room temperature and humidity conditions during acclimatization provided proper growth and development of the rats. No significant difference was observed in the lipid profile of all groups, due to standard rodent feed and drink. Regardless of grouping, the mean TC observed in the rats was 79.37 mg/dL, triglycerides of 69.71 mg/dL,

Table 2. Lipid profile (mg/dL) and feed intake (g) of the rats during acclimatization

Treatment Groups	Total Cholesterol (Mg/Dl)	Triglycerides (Mg/Dl)	Hdl-C(Mg/Dl)	Ldl-C(Mg/Dl)	Feed Intake (G)
Group 1	78.47	68.47	65.75	26.60	12.28
Group 2	79.79	70.54	65.75	25.60	13.25
Group 3	80.14	71.37	62.99	26.60	12.25
Group 4	79.09	68.46	65.75	26.60	12.31
Average	79.37	69.71	65.06	26.35	12.52

Preliminary Observation

Feed Intake

Feed intake was highest in rats fed with high fat (HF) rodent feed and cooked white rice at 12.36g/day, followed by rats fed with cooked brown rice. Feed intake was observed to be lowest in rats fed with 50% BPR and 50% HF rodent fed at only 12.07 g/day. The latter observation could have been caused by the early satiety (fullness) experienced by the rats since brown rice contains dietary fiber and rodent feed contains fat that is high enough compared to white rice (Table 3).

Table 3. Mean feed intake (g) and body weight gain (g) during the observation.

Treatment	Feed Intake (G)	Weight Gain (G)
Cooked brown pigmented rice	12 .14^{a}	7.0^{a}
High fat (HF) rodent feed	12. 36^{b}	10.5^{b}
Cooked white rice	12.36^{b}	8.5^{c}
50% cooked brown pigmented rice and 50% high fat rodent feed	12. 07^{c}	7.0^{a}

Means having the same superscript(s) within same roware not significantly different at α=0.05using t-test.

Weight Gain

Body weight gain was within the range of 7.0-10.5 g/week. The highest weight gain of 10.5 g/week was observed in rats fed with HF rodent feed and the lowest at 7.0 g/week in those fed with pure BPR as well as those fed with 50% BPR and 50% HF feed. Results of statistical analysis revealed the body weight gain in rats fed with pure BPR was not significantly different with rats fed with 50% feed cooked BPR and 50% HF rodent feed but both differed significantly in those fed with either pure rice or high fat rodent feed. These imply the potential weight reducing effect of BPR over white (well milled/polished) rice (Table 3).

Lipid Profile

The highest TC, triglyceride and LDL-c levels were observed in rats fed with HF rodent feed at 159.39 mg/dL, 150.20 mg/dL and 68.53mg/dL while the lowest levels were observed in rats fed with cooked BPR at 83.14 mg/dL, 69.48 mg/dL and 29.60 mg/dL, respectively. On the other hand, the highest HDL-c was found in rats fed with cooked BPR at 64.18 mg/dL and the lowest in those fed with HF rodent feed at 25.17 mg/dL. Results were found to be significantly different from each other indicating the potential lipid lowering effect of the consumption of pigmented rice (Table 4).

Table 4. Mean* level of lipid profile in rat blood during the observation period.

Treatment Groups	Total Cholesterol (Mg/Dl)	Triglyceride (Mg/Dl)	Hdl (Mg/Dl)	Ldl (Mg/Dl)
Cooked brown pigmented rice (HBR)	83.14^{a}	69.48^{a}	64.18^{a}	29.60^{a}
High fat(HF)rodent feed	159.39^{b}	150.20^{b}	25.18^{b}	68.53^{b}
Cooked white rice	129.89^{c}	131.73^{c}	32.62^{c}	58.85^{c}
50% cooked pigmented rice, 50% high fat rodent feed	106.51^{d}	106.83^{d}	62.06^{d}	35.02^{d}

- Means having the same superscript within the column are not significantly different at α=0.05 using t-test.

Effective Dose of Brown Pigmented Rice in Hyperlipidemic Rats

In this phase, three doses of cooked BPR rice were used, namely, Dose 1 (HD1): 25% HF rodent feed and 75% BPR, Dose 2 (HD2): 50% HF rodent feed and 50% BPR and Dose 3 (HD3): 75% HF rodent feed and 25% BPR. No significant difference in the mean food intake was found among and between the different rice doses and the control.

The caloric intake of HD3 rats (159 kcal) was significantly higher compared with the other groups and the lowest caloric intake was observed in the control (71 kcal). No significant difference was observed in the protein intakes between groups. HD3 treated group recorded the highest mean total carbohydrate intake and was significantly higher compared with the other groups. It must be noted that with increasing amounts of BPR in the rodent feed resulted in an increasing amounts of carbohydrate and energy intakes while decreasing the fat and protein intakes.

Total Cholesterol

Different doses of PBR on lipid profile, levels of TC, LDL-c, HDL-c, and triglycerides were measured weekly for one month. Baseline (Week 0) mean levels of total cholesterol were high in four rat groups exhibiting hyperlipidemia in the early phase of the study. HD3 rats had the largest reduction since the beginning of the intervention phase, this decrease was statistically significant against the control and HD1and HD2 (Figure 1a). The percentage change in total cholesterol weekly was computed cumulatively from the baseline Week 0.

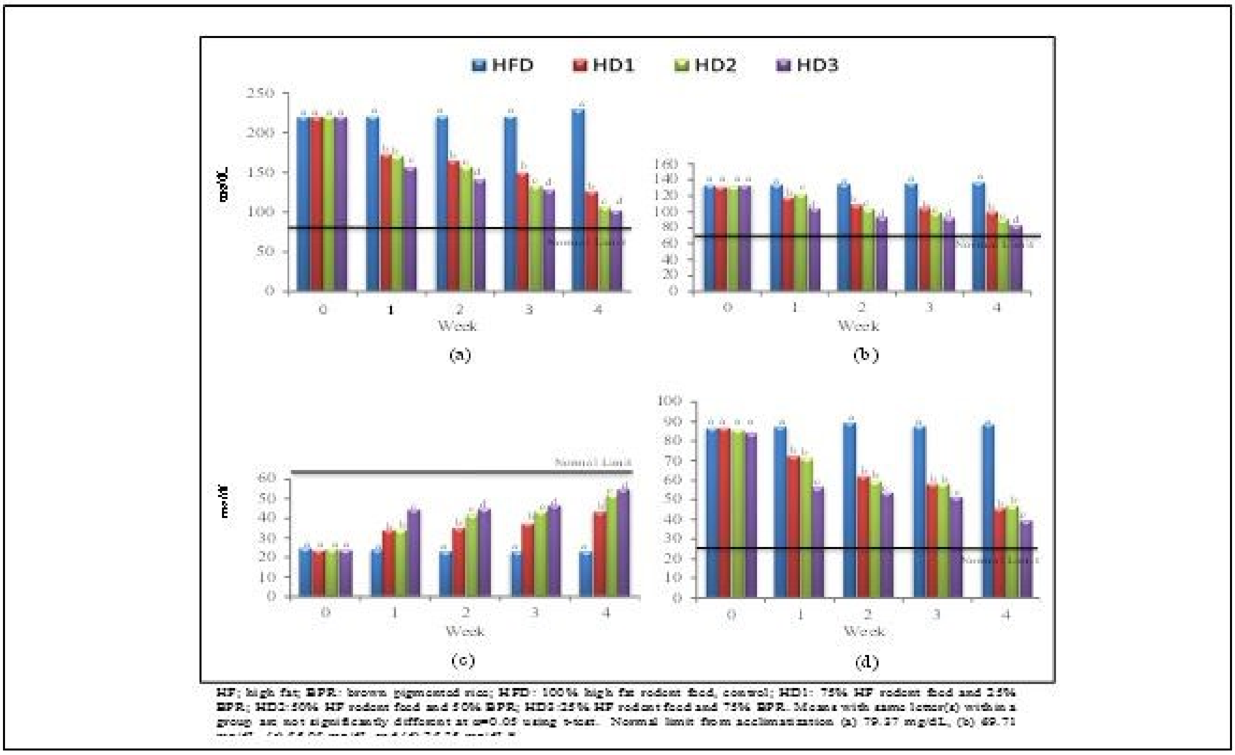

Fig. 1. Weekly mean (a) total cholesterol, (b) triglyceride, (c) high density lipoprotein and (d) low density lipoprotein level of feed-induced hyperlipidemic Sprague Dawley rats in effective dose determination

Decreased level of total cholesterol was observed in HD1, HD2, and HD3 rats (Table 5). All rats given rice doses significantly decreased in TC level from baseline until week 4, and reduction was highest in HD3 rats. Figure 1a further reveals that TC levels were above the normal limit of 79.37 mg/dL which was observed in the acclimatization, and only the total cholesterol level of HD3 rats was observed nearing the said normal limit.

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Table 5. Weekly mean percent change in total cholesterol, triglyceride, HDL-c and LDL-c level from the baseline measurement in feed-induced hyperlipidemic Sprague Dawley rats for effective dose determination.

Treatment Group	Mean Percent Change + Sd			
	Total Cholesterol			
	Week 1	Week 2	Week 3	Week 4
HFD	-0.27(±0.25)^{a}	-0.^{57}(±0.57)^{a}	-0.28(±0.28)^{a}	-0.30(±0.44)^{a}
HD1	4.89(±1.54)^{b}	24.86(±2.32)^{a}	8.98(± 0.60)^{b}	21.0(±2.01)^{a}
HD2	7.91(± 2.50)^{b}	28.74(±1.51)^{c}	15.23(±1.11)^{d}	22.6(±1.27)^{a}
HD3	9.80(±3.27)^{b}	35.74(±2.27)^{c}	19.27(± 1.65)^{b}	28.75(±0.75)^{a}
	Triglycerides			
HFD	-0.47(±0.40)^{a}	-0.52(±0.61)^{a}	0.77(±0.37)^{a}	-0.94±0.56^{a}
HD1	9.92(±1.98)^{a}	15.46(±4.15)^{b}	4.29(±0.60)^{c}	8(± 5.28)^{d}
HD2	9.08(±2.99)^{a}	7.97(± 2.72)^{a}	2.95(±1.89)^{c}	4.75(±3.31)^{d}
HD3	21.29(± 2.66)^{a}	10.58(±1.96)^{b}	0.88(±1.13)^{c}	10.14(±3.56)^{b}
	High density lipoprotein-cholesterol (HDL-c)			
HFD	3.34(±6.55)^{a}	4.51(±2.41)^{a}	0.02(±1.37)^{b}	0.18(±3.07)^{b}
HD1	-44.86(±13.16)^{a}	-2.93(±2.32)^{b}	-6.49(±5.15)^{c}	-16.36(± 2.91)^{d}
HD2	-41.75(±14.73)^{a}	-23.13(± 10.48)^{b}	-3.87(± 2.94)^{c}	-19.76(±2.81)^{d}
HD3	-67.88^{a}(±32.42)a	-2.30^{b}(±11.01)b	-3.97^{c}(±1.84)c	-18.20^{d}(±7.70)d
	Low density lipoprotein-cholesterol (LDL-c)			
HFD	-0.85(±0.82)^{a}	-2.38(±1.56)^{b}	1.90(±0.96)^{c}	-1.04(±1.41)^{d}
HD1	16.55(±2.86)^{a}	13.94(±3.10)^{a}	6.51(±2.95)^{c}	21.37(±1.27)^{d}
HD2	13.58(±3.93)^{a}	1.70(±4.17)^{b}	18.85(± 3.63)^{c}	-16.64(±1.21)^{d}
HD3	4.84(±6.04)^{a}	8.13(±6.46)^{b}	8.13(±7.95)^{b}	19.77(±2.73)^{c}

HF; high fat; BPR: brown pigmented rice; HFD: 100% high fat rodent feed, control; HD1: 75% HF rodent feed and 25% BPR; HD2: 50% HF rodent feed and 50% BPR; HD3: 25% HF rodent feed and 75% BPR. Values with positive (+) sign mean a reduction. Means within the row followed by the same letter(s) are not significantly different at α = 0.05 using t-test.

Triglycerides

High triglyceride levels were observed at the beginning of the intervention period (Figure 1b). The triglyceride level did not decrease in the control (HFD) rats during the entire duration of the intervention period. However, significant decline was observed for the HD1, HD2, HD3 rats, from week 1 to 4, the highest of which was observed in the HD3 rats. Figure 1b further shows that HD3 rats exhibited triglyceride level that was nearest to the normal TG limit (69.71 mg/dL) observed during the acclimatization period. Moreover, consistent increase in the triglyceride level was observed in the control group until week 4 (Table 5).

HDL-c

Mean levels of HDL-c for the control and treatment groups did not differ significantly at baseline measurements (Figure 1c) but was observed to increase at the end of the intervention period except in the HFD rats (control). In HD3 rats, the increase in HDL-c was significantly higher than in HD1, HD2 and the HFD rats. Mean HDL-c levels in the control group decreased while it increased in the treated rats (Table 5). HD2 rats had the highest increase (19.76%), which is more than in HD3 rats (18.20%) and HD1 rats (16.36%). Figure 1c also shows that HD3 almost reached the normal blood HDL-c level limit at 65.06 mg/dL from the acclimatization experiment.

LDL-c

High levels of LDL-c were observed for all groups before the start of the intervention period (Figure 1d). Mean LDL-c did not differ significantly between HD1 and HD2 rats but differed with HD3 rats. LDL-c of the treated rats was significantly different from the control.HD3 rats had a consistent reduction trend throughout the intervention period, with the reduction of almost 19.77% while in HFD rats the level increased to 1.04 at week 4 (Table 5).

In-vivo lipid lowering effect of effective dose of brown pigmented rice

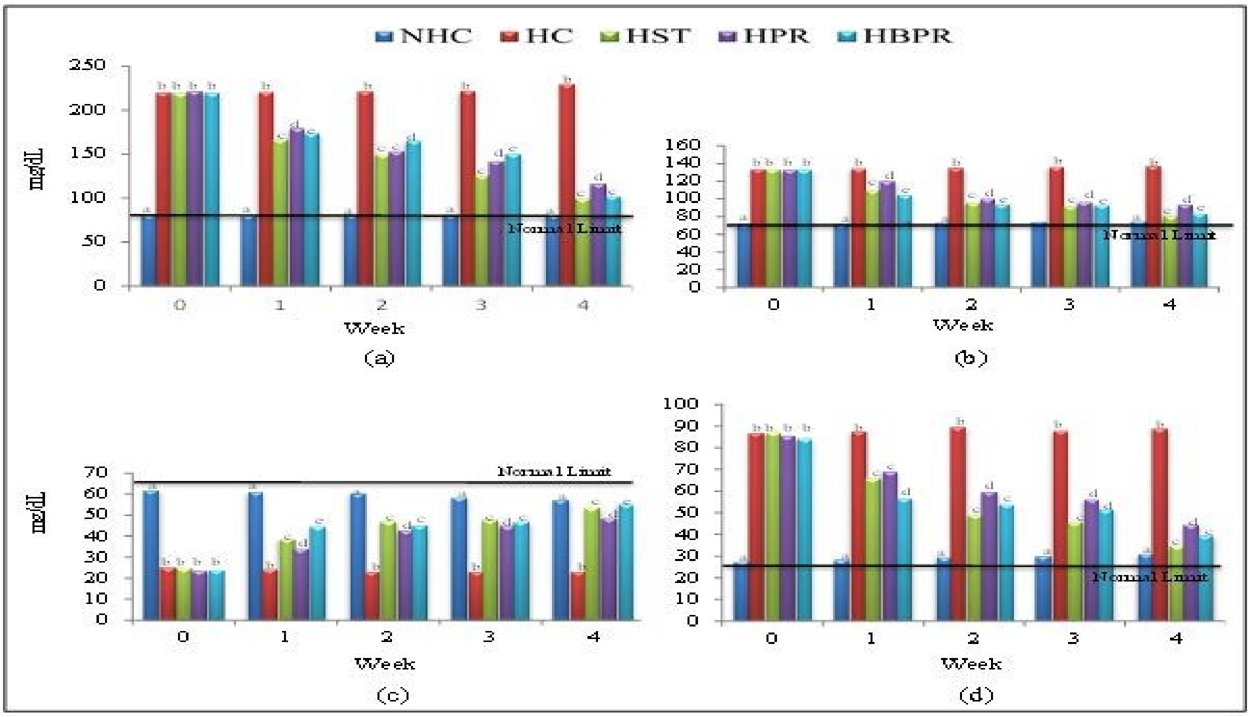
Mean Feed Intake

The non-hyperlipidemic control (NHC) group received commercial feed of 12.56 g/day, while the hyperlipidemic control (HC) group was given 12.64 g/day of HF rodent feed and the hyperlipidemic Simvastatin treated (HST) was given 12.42 g/day of HF rodent feed plus simvastatin. Meanwhile, the hyperlipidemic brown pigmented rice group (HBPR) received 12.61 g/day of 75% BPR and 25% HF rodent feed and hyperglycemic pigmented rice group (HPR) at 12.18 g/day of 50% well milled PR and 50% HF rodent feed. The calorie content of the food given to respective groups differed significantly, with the highest in HC group and lowest in the NHC group.

Total Cholesterol

The mean TC of the NHC rats was maintained during the entire intervention period. Meanwhile at baseline (Week 0), the average levels of TC in the HST, HPR, and HBPR significantly differed with the NHC group. Figure 2a presents that none of the level of cholesterol in treated rats i.e., HST, BPR and HBPR met the cholesterol level in NHC rats. Lowest reduction in cholesterol level was observed in HST and HBPR rats however, it was not significantly different. Both observed TC levels were either nearest to NHC rats at 80.59 mg/dL or the normal limit of 79 mg/dL in the acclimatization period.

Continuous decrease was observed for HST and HBPR group with close mean percent (%) change of 21 and 20 respectively at week 4, found to be significantly lower than the HC group (Table 6). No reduction in mean TC levels was observed for HC group; in fact it even increased at the end of the intervention period. This is expected because this group was only given 100% HF feed. While the NHC group



HF-high fat; BPR-brown pigmented rice; NH: non-hyperlipidemic, control fed with commercial rodent feed; HC: hyperlipidemic control fed with HF rodent feed; HST: hyperglycemic simvastatin treated; HPR: 50% well milled pigmented rice and 50% HF rodent feed; HBPR: 75% brown pigmented rice and 25% HF rodent feed. Means within a group with the same letter(s) are not significantly different at α=0.05 sing t-test. Normal limit from acclimatization (a) 79.37 mg/dL, (b) 69.71 mg/dL, (c) 65.06 mg/dL and (d) 26.35 mg/dL*

Fig. 2. Weekly mean (a) total cholesterol, (b) triglyceride, (c) high density lipoprotein and (d) low density lipoprotein level of non-hyperlipidemic control and feed-induced hyperlipidemic Sprague Dawley rats for *in vivo* evaluation of the effective dosemaintained low TC levels from the beginning to the end of the intervention period since the group was not given a HF rodent feed.

Table 6. Weekly mean percent change in the lipid profile and fat content of feces of non-hyperlipidemic and feed-induced hyperlipidemic Sprague Dawley rats for *in vivo* lipid lowering effect of the effective dose

Treatment group	MEAN PERCENT CHANGE + SD				
	Total Cholesterol				
	Week 1	**Week 2**	**Week 3**	**Week 4**	**% Red.**
NHC	0.68(± 1.07)[a]	-0.99(± 1.14)[a]	-0.29(± 0.37)[b]	-0.62(± 0.78)[b]	-0.31
HC	-0.30(± 0.44)[a]	-0.27(± 0.25)[a]	-0.28(± 0.28)[a]	-0.354(± 0.33)[a]	-4.41
HST	24.13(±1.81)[a]	10.09(± 2.35)[b]	15.97(±0.84)[c]	21.08(±1.08)[d]	54.78
HPR	19.02(±1.81)[a]	14.61(±2.20)[b]	7.20(±3.62)[a]	18.24(±1)[c]	47.57
HBPR	88.60(± 1.24)[a]	9.80(±3.27)[a]	9.27(± 1.65)[b]	20.34(±0.86)[c]	53.48
	Triglycerides				
NHC	0.15(± 0.79)[a]	-1.68(± .69)[b]	-1.60(± 1.29)[b]	-0.77(± 0.62) [c]	-3.94
HC	-0.74(± .0.40)[a]	-0.52(±0.61)[a]	-0.77(± 0.37)[a]	-0.94(± 0.56) [a]	-2.75
HST	17.69(±2.21)[a]	-0.52(±0.61)[a]	12.93(± 3.37)[a]	3.87(± 0.87) [a]	39.36
HPR	9.44(±6.71)[a]	16.28(±6.98)[a]	3.80(±1.18)[b]	3.18(± 1.21)[c]	29.75
BPR	20.36(± 2.09)[a]	10.58(±1.96)[b]	0.88(± 1.13)[b]	10.14(±3.56)[c]	37.40
	High density lipoprotein-cholesterol (HDL-c)				
NHC	1.30(± 1.28)[a]	1.03(± 0.51)[a]	3.26(± 1.29)[a]	2.01(± 2.06)[a]	7.45
HC	3.34(±6.35)[a]	4.51(±2.41)[a]	0.02(±1.37)[a]	0.18(±3.07)[a]	7.85
HST	-56.44(± 8.09)[a]	-21.69(±6.10)[a]	-1.79(± 1.95)[b]	-12.39(±6.34)[b]	-116.75
HPR	-45.42(±14.94)[a]	-26.62(±15.50)[b]	-4.74(±2.99)[c]	-8.87(±2.99)[d]	-106.66
HBPR	-44.86(±13.16)[a]	-2.92(±2.32)[b]	-6.49 (±5.15)[a]	-16.36 (± 2.90)[c]	-131.96
	Low density lipoprotein-cholesterol (LDL-c)				
NHC	-3.22 (±1.76)[a]	-4.21(±5.42)[a]	-1.50(±3.27)[a]	-3.19(± 1.61) [a]	-12.53
HC	-0.85 (± 0.82)[a]	-2.38(± 5.42)[b]	-1.90(± 0.96)[b]	-1.04 (± 1.41)[b]	-2.39
HST	24.42(± 3.02)[a]	25.66(±3.93)[b]	-6.77(±3.55)[b]	29.79(± 0.96)[c]	60.11
HPR	19.27(±3.37)[a]	13.17(±9.39)[b]	5.86(±3.29)[c]	20.91 (±1.88)[a]	48.05
HBPR	20.46(±6.10)[a]	9·35(±3.10)[a]	6.51(±2.95)[a]	35.29 (± 5.58)b	53.00

	Fat content (g/100g)		
	First 10 Days	Middle 10 Days	Last 10 Days
NHC	1.98 (±0.99)[a]	1.08 (± 0.63)[a]	1.96 (± 0.98)[a]
HC	2.16(±0. 71)[a]	3.19 (± 1.05)[b]	2.09 (± 1.06)[c]
HST	2.20 (±1.00)[a]	3.39 (± 1.54)[b]	2.56 (± 1.58)[c]
HPR	2.25 (± 1.00)[a]	4.05 (±1.61)[b]	2.72 (± 1.32)[a]
HBPR	2.91(±1.16)[a]	4.83 (± 1.92)[b]	3.37 (± 1.92)[b]

HF-high fat; BPR-brown pigmented rice; NH: non-hyperlipidemic, control fed with commercial rodent feed; HC: hyperlipidemic control fed with HF rodent feed; HST: hyperglycemic simvastatin treated; HPR: 50% well milled pigmented rice and 50% HF rodent feed; HBPR: 75% brown pigmented rice and 25% HF rodent feed. Values with positive (+) sign mean a reduction. Means within same row with the same letter(s) are not significantly different at α=0.05 sing t-test.

Triglycerides Level

High triglyceride levels for the HC, HST, HPR and HBPR groups were observed at baseline. The mean triglyceride level of the HC group was significantly higher than in any of the other groups. It must be noted that the triglyceride levels of the simvastatin and BPR treated groups continued to decrease significantly from the baseline. Further, at week 4 the triglyceride levels of HST and HBPR rats did not differ significantly, implying the potential affectivity of BPR in decreasing the levels of triglyceride in the blood but still unable to meet the normal limit of 69 mg/dL observed in the acclimatization period (Figure 2b). Mean triglyceride level of HC group increased continuously with 0.94% recorded at the end of the intervention period (Table 2). HST group showed about 4% reduction at the end of the intervention period, while HBPR rats exhibited about 10% reduction.

HDL-C – Cholesterol Level

There was no significant difference in the levels of HDL-c of rats in the HC, HST, HPR and HBPR groups at baseline (Figure 2c). However, the HDL-c of these groups was significantly lower than the NHC group. The continued cumulative percent change increase in the HDL-c levels of HBPR and Simvastatin treated groups may be due to the phytosterol present in the brown pigmented rice (Table 2) and statin in Simvastatin. Mean HDL-c values obtained at the start were more than doubled by the end of the intervention period. The HDL-c level in HBPR rats was not significantly different from that of the statin treated group implying that BPR even at 75% substitution for white rice in the feed can enhance the protective effect of HDL-c against dyslipidemia. Figure 2c further shows that HDL-c levels in HST and HBPR rats were near the normal limit (65 mg/dL) in the acclimatization experiment.

LDL-c

The average level of LDL-c in the NHC group was significantly lower than the other groups during the entire intervention period (Figure 2d). High values were obtained for the HC, HST, HPR and HBPR groups at the start of the intervention period but are not significantly different from each other. In the HC group, LDL-c remained high while simvastatin and BPR treated groups decreased. It can be observed that HBPR ranked next to simvastatin treated group in lowering LDL-C levels in the blood of the rats, although not significantly different, this indicates the potential of BPR in lowering LDL-c in the blood. Both exhibited LDL-c level proximate to the NHC rats and normal limit of 26.35 mg/dL in the acclimatization period. The percent change in average weekly LDL-c levels based on initial measurements, reduction was observed only for groups given statin and pigmented rice (Table 6).

Weight Assessment

No significant difference in body weight was observed in the four treatment groups at the start of intervention period. During weeks 2 to 4, HBPR (253g) group achieved the highest mean in weight, followed by the HC (251g), HPR (250g) and HST (245g) groups. Non-hyperlipidemic rats (NHC) retained its relatively low body weight (189g). HST rats had lower body weight than the other groups. This group was given statin drugs by gavage method and required more handling, which put much stress on them and affected their weight gain.

Fat Content

Analysis showed that HBPR rats had the highest fat content in the feces at each collection period, ranging from about 3.37 g/100 g (Table 6), followed by HST group at 2.56 g/100 g. Manure from the HC rats approximately contained 2.09 g/100 g fat. The group with the lowest fat content was in the NHC rats, with only about 1-2 g/100g. This is expected, because the NHC group was not given a HF rodent feed. The phytosterols and dietary fiber in cooked brown pigmented rice consumed by HBPR rats may have helped in the excretion of lipids.

IV. Discussion

Total cholesterol and triglyceride levels increased with increased high fat feed intake. The abnormally-elevated levels of total cholesterol and triglycerides characterized hyperlipidemia. The bivariate analysis undertaken in this study ascertained the positive relationship between calorie with the levels of TC, triglycerides and LDL-c, and a positive relationship between fat intake with TC and triglycerides.

Results of the analysis showed that calorie intake was positively correlated significantly with TC (total cholesterol), triglycerides and LDL levels (Table 7) while fat was also positively correlated significantly with TC, and triglycerides. This indicates that fat intake is a strong predictor of TC and triglycerides while calorie was a strong predictor of TC, triglycerides and LDL-c levels. As fat intake increases (particularly saturated and Trans fats) the TC level also increases. The negative relationship observed between fat intake and HDL-c level implies that high fat intake lowers HDL-c level in the body.

Table 7. Bivariate correlation of intake with lipid levels.

COMPOSITION	TOTAL CHOLESTEROL	TRIGLYCERIDES	HDL-c	LDL-c
	Correlation Coefficient			
Intake				
Calorie	0.81**	0.72*	0.69	0.69**
Carbohydrate	-0.29	-0.21	-0.22	0.02
Protein	-0.19	0.12	-0.44	-0.21
Fat	0.82**	0.74**	0.22*	-0.46

*Correlation is significant at p-value < 0.05; ** Correlation is significant at p-value < 0.01

In the study, the positive correlations between fat intake and lipid levels were observed in the HC and NHC groups. The HC group, as the negative control, was given 100 % high fat (HF) feed and had a mean fat intake of 12.66 g/day, the highest fat intake among the four study groups and maintained lower HDL-c and high cholesterol and triglycerides levels above the desirable amount all throughout the intervention period (Figures 1 and 2). The positive association between dietary fat intake and cholesterol and triglyceride levels observed in this study was in agreement with the results obtained by Ueshima et al. (2000).

Although the HST and HBPR groups were likewise given high fat (HF) rodent feed, and both had mean fat intakes not significantly different with that of the HC group, these two groups were given interventions of statin drug for HST and cooked brown pigmented rice containing phytosterols for HBPR that could presumably account for the lowering of cholesterol and triglyceride levels.

The intervention drug given to HST group was Simvastatin, a synthetic drug considered very potent among other statin drugs (Lopez 2003). It was given to the HST group at a dosage of 20 mg/kg body weight from 5 to 6 in the afternoon. The statin was given at such time so as to account for the greater intake of rats at night time. In addition, since cholesterol synthesis appears to occur mostly at night (Mietinnen 1982), the statin drug was given at night to maximize its effect.

The mechanism of action of statin drugs is attributed to the inhibition of HMG- CoA reductase, the first committed enzyme in cholesterol synthesis. Statins block the pathway for synthesizing cholesterol in the liver. Aside from competing with the normal substrate in the enzymes active site, they alter the conformation of the enzyme when they bind to its active site. This prevents HMG-CoA reductase from attaining a functional structure. The change in conformation at the active site makes these drugs very effective and specific (Stancu and Sima 2001).

In this study, it was observed that the HST group given the statin drug had 54.78% reduction in total cholesterol, 60.11% in LDL-c level and 39.36% reduction in triglycerides at the end of the intervention period (Table 8). The observed decrease in

TC was proportional to the value obtained by other researchers (Kleemannetal.2003). However, Vargas (2014) reported 25 % reduction in total cholesterol when rats were fed with 75 % brown rice which was lower that what was obtained in the study.

Table 8. Summary of results for *in vivo* evaluation of lipid-lowering effect of cooked brown pigmented rice in non-hyperlipidemic and hyperlipidemic Sprague Dawley rats.

Parameter	Study Group							
	NHC		HC		HST		HBPR	
Intake (mean)*								
Food (g)	12.40^{a}		12.66^{a}		12.42^{a}		12.50^{a}	
Energy (kcal)	47.31^{a}		77.23^{b}		69.18^{c}		37.08^{d}	
Carbohydrates (g)	6.09^{a}		3.67^{b}		3.60^{b}		5.09^{c}	
Proteins (g)	5.06^{a}		2.28^{b}		2.24^{b}		0.61^{c}	
Fats (g)	0.29^{a}		5.69^{b}		5.59^{b}		1.52^{c}	
Lipid Levels, after 4 weeks (mg/LDL-c)								
TC	80.59^{a}	-0.31*	222.76^{b}	-4.41*	151.97^{c}	54.78*	162.15^{d}	53.48*
LDL-c	29.31^{a}	-12.53*	88.10^{b}	-2.39*	56.45^{c}	60.11*	57.16^{a}	53.00*
Triglycerides	72.99^{a}	-3.94*	135.18^{b}	-2.75*	102.14^{c}	39.36*	101.31^{c}	37.40*
HDL-cholesterol	59.73^{a}	7.45*	23.65^{b}	7.85*	42.29^{c}	-116.75*	43.11^{a}	-131.96*
Weight(mean,g)**	188.60^{a}		251.37^{b}		245.00^{c}		252.77^{d}	

Fecal Fat Content (mean, g/100g)				
First 10 days	0.96[a]	1.23[b]	1.13[c]	1.42[d]
Mid 10 days	1.00[a]	1.28[b]	1.17[c]	1.78[c]
Last 10 days	0.90[a]	1.32[b]	1.23[c]	1.84[d]

NHC - non- hyperlipidemic control group; HC - hyperlipidemic control group; HST – hyperlipidemic statin-treated group; HBPR – hyperlipidemic brown rice-fed group; *Food intake composed of 9.38 g cooked brown rice and 3.13 g high fat feed; **Weight at the end of intervention period. Means in the same row followed by the same letter(s) are not significantly different at α = 0.05; *Values following the mean in lipid level is the % reduction, negative (-) sign means anincrease.

The HBPR group fed with 75% BPR and 25% rodent HF rodent feed recorded a decrease in TC 53.48%, 53% in LDL-c and a 37.40% reduction in triglyceride (Table 8). This reduction is caused by brown pigmented rice consumed by this group that contains phytosterols that are believed to have a cholesterol-lowering effect (Lichtenstein et al 1994; Ausman et al. 2003; Vargas, 2014). The amount of phytosterol in HBPR was45 mg with % bioactive adequacy of about 647 but with only about 13% maximum effective dose had contributed to its relatively low reduction in TC, triglycerides and LDL-c levels but not enough to reach the normal limit values. Vargas (2014) showed that phytosterol in brown rice can be bioactive in humans even at a dose of 41 mg phytosterol per day produces maximum effectiveness to lower lipids. In this study, the phytosterol intake of the HBPR group was 23.45 mg with cooked brown pigmented rice as the main source. The phytosterol intake of the HBPR group met only half of the standard requirement for bioactive dose in rats of about 48 mg. In the study only about 13.36 % of the required maximum effective dose was met by the HBPR group (Table 9). Although the amount of phytosterol consumed is small, the study clearly shows that it can decrease significantly the levels of TC, triglycerides and LDL-c in the HBPR rats.

Table 9. Phytosterol standard requirement, phytosterol content of intake, and percent adequacy of phytosterol intake in feed-induced hyperlipidemic Sprague Dawley rats for effective dose determination

Treatment Group	Phytosterol Standard Requirement		Mean Intake of Bpr (G)	Phytosterol Contentof Intake (Mg)	% Adequacy Of Phytosterol	
	Bioactive Dose (Mg)	Maximum Effective Dose (Mg)			Bioactive Dose	Maximum Effective Dose
HFD	3.66[a]	48.89[a]	-	-	-	-
HD1	3.62[a]	48.36[a]	3.11[a]	7.78[a]	214.92[a]	13.36[a]
HD2	3.63[a]	48.49[a]	6.13[b]	15.33[b]	421.89[b]	13.36[a]
HD3	3.62[a]	48.36[a]	9.38[c]	23.45[c]	647.79[c]	13.36[a]

HFD: high fat rodent feed; BPR: brown pigmented rice; HD1: 25 % high fat (HF) rodent feed and 75 % brown BPR; 50 % HF rodent feed and 50 % BPR; HD3: 75 % HF rodent feed and 25 % BPR. Means having the same superscript within a column are not significantly different at α=0.05 using t-test.

The lowering of blood cholesterol has been accounted to the phytosterols' effect on cholesterol absorption. The mechanisms of action include the ability of phytosterols to displace cholesterol within intestinal micelles decrease binding of cholesterol to the brush border membranes, decrease intracellular esterification of cholesterol, and decrease incorporation of cholesterol into chylomicrons, thus resulting in less cholesterol absorption and its increased excretion in the feces (Ikuo et al. 1988, Lichtenstein et al. 1994, Ostlund 2002, Brown 2011).

High fat excretion was observed in the feces of HBPR rats (Table 4). Among the four groups, only the HBPR group was given cooked brown pigmented rice, and results of fecal analysis showed that this group had the highest fat content in the feces. The high fat content can presumably be the effect of the phytosterols present in brown pigmented rice, since these inhibit the absorption of cholesterols in the intestinal lumen and increase its excretion in the feces. Several studies such that of Racette et al. (2009), in their study on the effects of phytosterols on cholesterol metabolism, observed that phytosterol intakes significantly increased total fecal cholesterol excretion.

The results of this study to humans, the amount of cooked brown rice of the Segreng brown pigmented rice variety that should be consumed by humans in order to observe the same effects on lipid levels is 229.81 g/day (Appendix 5). This amount, when translated into house hold cup at 195 g/cup is equivalent to approximately 1 ¼ cups of cooked brown rice. This amount can be considered manageable for daily consumption, especially among adults whom recommendations to modify feed to improve blood lipid concentrations are most commonly made.

V. Conclusion

Results revealed that increasing the amounts of brown pigmented rice in the feed of the rats resulted in the lowering of the lipid levels of TC, triglycerides and LDL-c but increasing the amount of HDL-c. Specifically, the study showed the following: (a) Segreng exhibited relatively high amounts of phytosterol and dietary fiber at 2.50 and 5.59%; (b) inclusion of 75% cooked BPR in the feed of the rats for 4 weeks resulted in the reduction of TC level by 53.48%, triglyceride level by 37.40% and LDL-c level by 53% and an increase in HDL- c level by 131.96%; however, the amounts were insufficient to bring the lipid levels of the rats to normal values; (c) mean TC, LDL-c, triglycerides and HDL-c in hyperlipidemic rats fed with 75% BPR rats were comparable with those of the hyperlipidemic-statin-treated rats at 20 mg/Kg of body weight; (d) higher amount of excreted fat was found in rats fed with 75% BPR and (e) bivariate correlation analysis indicated higher significant positive correlation of fat between TC, triglyceride and LDL-c than carbohydrate and protein. In addition, the amount of cooked Segreng brown pigmented rice variety that should be consumed by humans in order to observe the same effects on lipid levels is 229.81g/day or approximately 1¼ cups. This amount is considered manageable for daily consumption, especially among adults whom recommendations to modify feed to improve blood lipid concentrations are commonly made. This study may also be replicated using other grains like corn, adlai, sorghum and root crops such as arrowroot, cassava, sweet potato and banana or any indigenous grain or root crop flours. A further study on human subjects is also recommended.

References

[1] [AACC] American Association of Cereal Chemists. 2000. Approved Methods of the AACC. 10th ed. American Association of Cereal Chemists. St. Paul, MN.

[2] [AOAC] Association of Official Analytical Chemist. 1990. Official Methods of Analysis.17th ed. AOAC International. Arlington, VA.

[3] [AOAC] Association of Official Analytical Chemist. 1995. Official Methods of Analysis of the Association of Analytical Chemists, Washington D.C.

[4] BALKAYA M, VOYVODA H, UNSAL C., CELER H. 2001. Some hematological and biochemical characteristics of male and female Sprague Dawley rats. J. Faculty

[5] BITTNER V.2005. Perspectives on Dyslipidemia and Coronary Heart Disease in Women. Journal of American College of Cardiology 46(9):1628-35.

[6] [BPOM RI] Indonesian Food and Drug Supervisory Agency. 2011. Regulation of Head of BPOM RI concerning monitoring of claims in label and advertisement of foodstuff processed number HK.03.1.23.11.11.09909 year 2001 appendix IV. Jakarta.

[7] BROWN AW, HANG J, DUSSAULT PH, CARR TP. 2011. Phytosterol ester constituents affect micellar cholesterol solubility in model bile. Lipids 45(9):855-862.

[8] DINESH BABU P, SUBHASREE RS, BHAKYARAJ R, VIDHYALAKSHMI R. 2009. Brown rice: Beyond the color reviving a lost health food- A review: American-Eurasian Journal of Agronomy, 2009: 2 (2): 67-72.

[9] [DOH] Department of Health. 2004. Household Health Survey. Retrieved from http://www.depkes.go.id.

[10] [FDA] Food and drugs administration. 2010. Food labeling; Health claim; Phytosterols and risk of coronary heart disease; Proposed rule. FDA, US.

[11] FERNANDEZ ML, WEBB D. 2012. The LDL to HDL Cholesterol Ratio as a Valuable Tool to Evaluate Coronary Feart Disease Risk. Journal of American College of Nutrition 27(1): 1-5.

[12] GIACCO R, PARILLO M, RIVELLESE AA, LASORELLA G, GIACCO A, D'EPISCOPO L, RICCARDI G. 2000. Long-term dietary treatment with increased amounts of fiber-rich low–glycemic index natural foods improves blood glucose control and reduces the number of hypoglycemic events in type 1 diabetic patients. Diabetes care, 23(10), 1461-1466.

[13] HALLIKAINEN M. 2001. Role of plant stanol ester and sterol ester enriched margarines in the treatment of hypercholesterolemia. Kuopio University Publication D. Medical Science. 90p.

[14] HARNAFI H, AZIZ M, AMRANI S. 2009. Sweet Basil (Ocimum basilicum L.) improves lipid metabolism in hypercholesterolemic rats. E- SPEN European e-J. Clinical Nutr. Metabolism 4:e181-e186.

[15] IKOU L, TANAKA K, SUGANO M, VAHOUNY GV, GALLO LL. 1988. Inhibition of cholesterol absorption in rats by plant sterols. J Lipid Res 29:1573-1582.

[16] LICHTENSTEIN AH, AUSMAN AM, CARRASCO W, GUALTIERI LJ, JENNER JL, ORDOVAS JM, NICOLOSI RJ, GOLDBIN BR & SCHAEFFER EJ. 1994. Rice bran oil consumption and plasma lipid levels in moderately hypercholesterolemic humans. J Atheroscler Thromb 14(4): 549-556.

[17] LOPEZ LM. 2005. Rosuvastatin: a high-potency HMG-CoA reductase inhibitor. J Am Pharm Assoc 45(4):503-13.

[18] MOMUAT LI. 2001. Palm oil accelerates the regression of the atheroscler aortic osis in severe hypercholesterolemia rabbits [MS Thesis]. Bogor: Graduate Program, IPB Bogor.

[19] OSTLUND RE. 2002. Phytosterols in human nutrition. Ann Rev Nutr 22:533–549.

[20] RACETTE SB, LIN X, LEFEVRE M, SPEARIE CA, MOST MM, MA L. 2009. Dose effects of dietary phytosterols on cholesterol metabolism: a controlled feeding study. Am J Clin Nutr 91: 32-38.

[21] RAMACHANDRAN HD, NARASIMHAMURTHY K, RAINA PL. 2003. Modulation of cholesterol induced hypercholestero--lemia through dietary factors in Indian desert gerbils (Meriones hurricinae). Nutr. Res 23: 245-256.

[22] REAGAN-SHAW S, NIHAL M, AHMAD N. 2007. Dose translation from animal to human studies revisited. FASEB J 22(3):659-661.

[23] SIALVERA TE, POUNIS GD, KOUTELIDAKIS AE, RICHTER DJ, YFANTI G, KAPSOKEFALOU M, ET AL. 2012. Phytosterol supplementation decreases plasma small and dense LDL levels metabolic syndrome patients on a westernized type diet. Nutrition, metabolism and cardiovascular diseases 22:843-848.

[24] SOUPAS L, HUIKKO L, LAMPI AM & PIIRONEN V. 2005. Esterification effects phytosterol oxidation. Eur J Lipid Sci Technol 107, 107-118.

[25] WILDMAN R, MEDEIROS D. 2000. Advanced Human Nutrition. CRC Press.

[26] WU TM, FU J, YANG Y, ZHANG L, HAN J. 2009. The effects of phytosterol/stanol on blood lipid profile: A systematic review with meta-analysis. Asia Pac J. clin. Nutr. 18(2), 179-186.

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