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Exploring rice varietal effects on triglyceride/high density lipoprotein ratio in hyperlipidemia-induced wistar rats

Yusie Rosmalinda[1], Etika Ratna Noer[1,2], Mohammad Sulchan[1,2], Sya'bani Isnaen Khikmaturrohman[1], Martha Ardiaria[1], Firdaus Shadiqa Marsa[1], Via Anugrah Henny[1], Hartanti Sandi Wijayanti[1,3], Binar Panunggal[1,2,4*]

ABSTRACT

Background: Excessive intake of white rice has been associated with elevated triglyceride (TG) levels and decreased high density lipoprotein (HDL) cholesterol concentrations. Conversely, black and red rice varieties have demonstrated favorable impacts on lipid metabolism. However, despite these benefits, their palatability and texture are often met with aversion by consumers. Consequently, it is imperative to investigate rice blends that are both well-received by individuals and exhibit pronounced efficacy in reducing the TG/HDL ratio.
Objective: To investigate the differential effects on the TG/HDL ratio in rats administered different combinations of white, red, and black rice.
Methods: This research follows a true experimental design incorporating pre- and post-tests with a control group. Thirty rats were initially fed a hyperlipidemic diet for 14 days. Subsequently, the rats were subjected to interventions involving different rice combinations for a duration of 28 days. These combinations included K(+) (positive control), P1 (100% white rice), P2 (21 g red and 4 g white rice), P3 (16 g black and 4 g white rice), and P4 (8 g black, 8 g red, and 4 g white rice). Triglyceride and HDL levels were quantified using the GPO-PAP and CHOD-PAP methods, respectively. Statistical analysis was performed utilizing ANOVA tests followed by LSD post-hoc tests for comparison.
Results: Triglycerides decrease in P4 (-51.04±0.72) was the highest, followed by P3 (47.18±0.51), P2 (-34.60±1.07), P1 (-17.03±1, 06). Cholesterol HDL increase in P4 (49.36±1.68) was the highest, followed by P3 (42.71±0.41), P2 (38.09±0.76), P1 (30.64±0.90). The TG/HDL ratio in P1, P2, P3, and P4 were 0.21±0.092, -2.12±0.164, -2.45±0.123, -2.82±0.148, -3.18±0.371, respectively. The rice combination intervention had a significant decreased on the TG/HDL ratio (p=0.000).
Conclusion: The intervention involving rice combinations resulted in a greater reduction in the TG/HDL ratio compared to the consumption of white rice alone. Among the various combinations tested, the combination of white, red, and black rice demonstrated the most significant reduction in the triglyceride to HDL-cholesterol (TG/HDL) ratio.

Keywords *: black rice; HDL; red rice; triglyceride; white rice*

BACKGROUND

Metabolic syndrome represents a cluster of metabolic irregularities, including dyslipidemia, central obesity, hypertension, and glucose intolerance. Dyslipidemia within metabolic syndrome involves decreased HDL cholesterol levels and elevated blood triglyceride concentrations. In women, the syndrome manifests with abdominal obesity and reduced HDL levels, while men primarily exhibit hypertriglyceridemia.[1] The repercussions of low HDL cholesterol and high triglyceride levels pose a substantial risk for atherosclerosis and cardiovascular complications in type 2 diabetes mellitus.[2] Elevated triglycerides and cholesterol foster the generation of free radicals, which inflict damage upon endothelial cells. Endothelial cholesterol oxidation precipitates atherosclerosis, the thickening and stiffening of arterial walls due to lipid-laden lesions, thereby instigating coronary heart disease.[3]

Dyslipidemia is an abnormal status of lipid metabolism which is characterized by high concentrations of triglycerides, total cholesterol and LDL cholesterol (low density lipoprotein), as well as low concentrations of HDL cholesterol.[4] According RISKESDAS 2013 data shows that as many as 22.9% of the population aged ≥15 years has low HDL levels (≤40 mg/dl) and as many as 13% has high triglyceride levels (150-199 mg/dl).[5] The prevalence of low HDL cholesterol in the executive group of several companies in the community in Jakarta and surrounding areas aged 25-60 years is 91.4%, while the prevalence of hypertriglyceridemia in this

[1] Department of Nutrition Science, Faculty of Medicine, Universitas Diponegoro, Semarang, Central Java, Indonesia
[2] Center of Nutrition Research, Universitas Diponegoro, Semarang, Central Java, Indonesia
[3] Department of Epidemiology and Preventive Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan
[4] School of Nutrition and Health Sciences, Taipei Medical University, Taipei, Taiwan
[*]**Correspondence** : panunggalbinar@live.undip.ac.id

group is 31.5%.[6] The TG/HDL-C ratio is the result of a comparison between laboratory values for triglycerides and HDL cholesterol. The TG/HDL-C ratio is associated with an increased risk of cardiovascular disorders and metabolic syndrome even at low levels of LDL cholesterol and may also reflect low-density LDL particles that are more atherogenic.[7-8] Previous study found that a high risk of cardio metabolic factors is characterized by a TG/HDL ratio ≥ 3.5 in men and a TG/HDL ratio ≥ 2 in women.

One of the triggers for dyslipidemia is the wrong food consumption pattern, especially staple foods. Rice is a staple food for people in most countries in Asia, including Indonesia.[9] However, excessive consumption of white rice can contribute to the increasing of blood glucose because it has a relatively high glycemic index ranging from 64 to 93 depending on the variety and cooking method.[10] Apart from that, consumption of white rice is also associated with increased triglyceride levels and decreased HDL levels.[11] This effect is primarily due to its high glycemic index, low fiber content, and lack of essential nutrients found in the bran and germ.[12] White rice has a high glycemic index, leading to rapid spikes in blood glucose levels. This triggers an increase in insulin secretion, which can promote the synthesis of triglycerides in the liver and inhibit the breakdown of fats, thereby elevating TG levels and potentially lowering HDL cholesterol.[13] Dietary fiber plays a crucial role in lowering cholesterol by binding to bile acids and cholesterol in the digestive system, facilitating their excretion. Consuming drugs, such as statins, can also improve lipid metabolism, but the long-term use of drugs can cause side effects.[14] Therefore, it is needed to change diet into rich in fiber and antioxidants in order to reduce triglyceride levels and increase HDL cholesterol levels, such as brown rice and black rice.[15] These benefits are attributed to the presence of fiber, phytosterols, anthocyanins, and phenolic compounds in brown and black rice.[16]

Dyslipidemia, typified by elevated triglycerides, total cholesterol, and LDL cholesterol, coupled with diminished HDL cholesterol, underscores a significant metabolic disturbance.[4-7] Epidemiological data from RISKESDAS in 2013 underscores a substantial prevalence of low HDL levels and elevated triglyceride levels within the population.[8-10] Furthermore, within specific demographic subsets, such as executive cohorts, alarmingly high incidences of dyslipidemia are evident. The TG/HDL-C ratio, a pivotal metric, correlates with heightened cardiovascular risk and metabolic syndrome, even amidst normative LDL cholesterol levels.[11-14] Research underscores a threshold TG/HDL ratio indicative of heightened cardiometabolic risk.[14] Dietary habits, particularly the overconsumption of refined staples such as white rice, contribute to dyslipidemia onset. Overconsumption of refined staples like white rice promotes postprandial hyperglycemia and hyperinsulinemia, which stimulate hepatic lipogenesis and increase circulating triglycerides while lowering HDL cholesterol, thereby contributing to the onset of dyslipidemia. Alternately, integrating fiber and antioxidant-rich alternatives such as brown and black rice can mitigate triglyceride levels and enhance HDL cholesterol concentrations.[14-19] Previous studies validate the lipid-modifying effects of brown and black rice, attributing them to their fiber and anthocyanin content. However, widespread adoption of these alternatives is hindered by cost and palatability concerns, as white rice remains culturally entrenched.[20-23] Variations in rice composition, notably anthocyanin and fiber content, influence their metabolic impact. Soluble fiber aids in cholesterol excretion, while anthocyanin activates enzymes that curb fatty acid synthesis and enhance oxidation, ultimately bolstering HDL levels.[23-26] Nonetheless, reconciling the nutritional benefits of red and black rice with the widespread preference for white rice necessitates further exploration.[26-29] Experimental studies employing white rats as models offer insights into metabolic mechanisms and therapeutic interventions, leveraging their physiological parallels with humans to inform translational research endeavors.

MATERIALS AND METHODS

The research was conducted at the Center of Food and Nutrition Study Laboratory at Universitas Gadjah Mada University (UGM) in Yogyakarta. It involved 30 male Wistar rats aged 2 months with a body weight of approximately 200 grams. The rats underwent a 7-day adaptation period during which they were fed standard COMFEED AD II food and had access to distilled water ad libitum. Following the adaptation period, the rats were given hyperlipidic food consisting of a mixture of 2 ml pork oil, 1 ml duck egg yolk, and 1 ml fructose per 200 grams of rat body weight via gastric probe for 14 days to induce hyperlipidemia. The fructose intervention was included due to the study's focus on triglyceride and HDL cholesterol parameters.

Sample size determination was based on the WHO formula. Each group included 6 samples, including anticipation of drop out. The rats were then randomly divided into 5 groups and received different dietary treatments for 28 days. These treatments included 20 g white rice only (P1), a combination of 4 g white rice

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and 16 g red rice (P2), a combination of 4 g white rice and 16 g black rice (P3), a combination of 4 g white rice, 8 g red rice, and 8 g black rice (P4), and a control group (K(+)) fed standard feed. After the 28-day treatment period, the rats were fasted for 8-10 hours, and 2 ml of blood was collected via the retroorbital plexus on the 51st day to examine final triglyceride and HDL cholesterol levels..

The diet composition created in the study included 4 groups with a combination of rice and 1 positive control. The feed given to mice was 10% of the mice's body weight ad libitum. The experiment was carried out using Wistar rats with an average weight of ±200 gr, the feed given was 20 gr/day/rat. The rice dosage calculation is based on human fiber requirements which are then converted to the dosage for mice. Based on calculations, P1 was given 20 g of white rice, P2 consisted of a combination of 4 g of white rice and 21 g of red rice, P3 consisted of 4 g of white rice and 16 g of black rice, and P4 consisted of a combination of each red rice and black rice as much as 8 g, and white rice as much as 4 g. The positive control group was only given standard COMFEED AD II feed of 20 g/day/rat and was not given any intervention in the form of a combination of rice. COMFEED AD II standard food contains 51% carbohydrates, 15% crude protein, 12% crude fat, 6% crude fiber, 1.1% calcium and 0.9% phosphorus.[30]

The data collected for this study consists of primary data obtained from laboratory test results. Data collection occurred in two stages: at the beginning and the end of the intervention period. The collected data includes measurements of mouse body weight, triglyceride levels, and HDL cholesterol levels in the blood of experimental mice. Triglyceride levels were determined using the GPO-PAP method, while HDL cholesterol levels were determined using the CHOD-PAP method.[31]

The data that has been obtained is processed using the SPSS computer program. Data were tested for normality using the Shapiro-Wilk test. Analysis of differences in the effects of the five groups used the one-way ANOVA parametric statistical test and continued with the LSD post hoc test. Analysis of correlations used the Pearson test. This research has obtained ethical clearance approval from the Health Research Ethics Commission (KEPK) Faculty of Medicine, Diponegoro University/Dr. Kariadi Central General Hospital No. 88/EC/H/FK-UNDIP/VIII/2021.

RESULTS

The correlation between rat body weight and triglyceride and HDL cholesterol levels is a focal point of investigation. The analysis of body weight data is linked to the potential impact of rice combinations on body weight, which could subsequently affect triglyceride and HDL cholesterol levels. Specifically, there appears to be a positive correlation between rat body weight and triglyceride levels, while a negative correlation exists with HDL levels. This suggests that as rat body weight increases, triglyceride levels tend to rise while HDL levels decline.

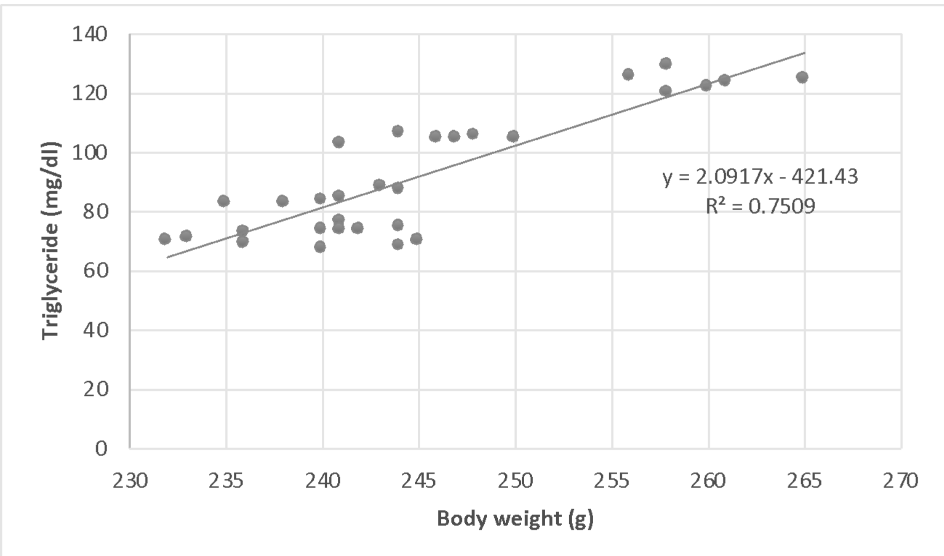

Figure 1. Relationship between Wistar Rat Body Weight and Triglyceride

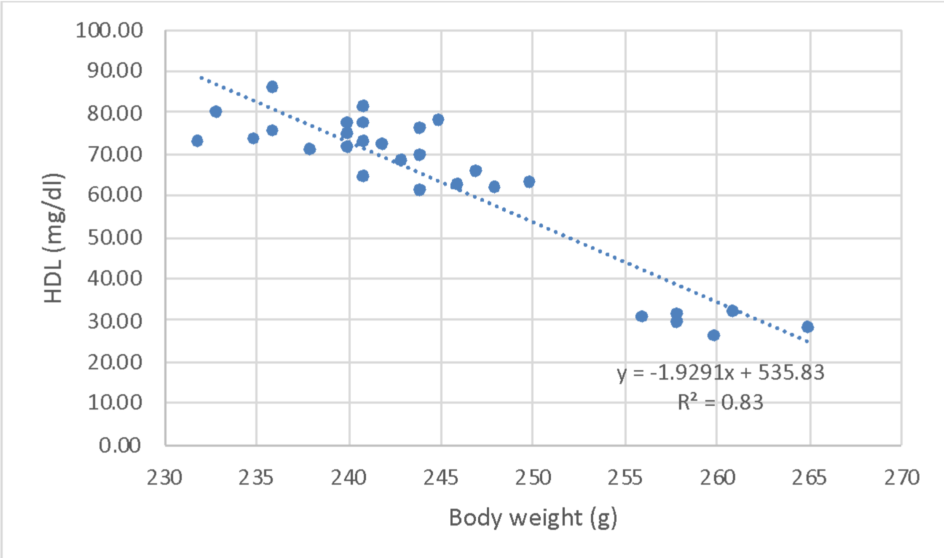

Figure 2. Relationship Between Wistar Rat Body Weight And HDL Levels

The K(+) group exhibits the highest mean body weight, accompanied by elevated triglyceride levels and diminished HDL levels. Conversely, the P3 group displays the lowest mean body weight, albeit not significantly different from P4. Despite comparable body weights between P3 and P4, notable disparities emerge in triglyceride and HDL levels. Specifically, triglyceride levels are lower in P4 compared to P3, while HDL levels are higher in P4. P4 demonstrates the lowest mean body weight alongside the lowest triglyceride levels and the highest HDL levels.

The Effect of Rice Combinations on Fiber Intake

According to the results of the ANOVA test, the rice combination treatment demonstrates a notable impact on feed consumption. The P2 group demonstrated the highest feed intake, with an average consumption of 23.18 grams. This consumption level differs significantly from that of the P3, P1, K(+), and P4 group, as indicated in Table 2. The P3 group produces the highest fiber intake, while the P1 and P4 groups have the same fiber intake and are lower than the P3 group. Group P2 has the lowest fiber intake. (Table. 2)

Table 2. Food and Fiber Intake

Groups	Food Intake (g)	Fiber Intake (g)
K(+)	18,13 ± 0,22[b]	-
P1	18,20 ± 0,23[b]	0,57
P2	23,18 ± 0,25[a]	0,53
P3	18,30 ± 0,17[b]	0,73
P4	18,12 ± 0,17[b]	0,57
p[1]	0,000	

p[1]: one way ANOVA Test; a,b,c,d) Different notations in the same column indicate significant differences in the LSD test.

The Effect of Various Rice Combinations on Reducing Triglyceride Levels

Normal rat triglyceride levels are in the range of 20 – 114 mg/dl. Before the intervention, all mice in the group experienced hypertriglyceridemia. After being given a combination of rice, triglyceride levels in groups P1, P2, P3 and P4 are in the normal category. Meanwhile, the K(+) group experiences an increase in triglyceride levels after being given standard feed. (Table. 3)

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Table 3. The Triglyceride Levels Before and After Rice Combination Intervention

Group	Before Intervention	After Intervention	Level Changes	p^2
K(+)	121,75±2,62	123,68±3,04[a]	1,93±0,98[a] (1,59)	0,005
P1	121,29±1,36	104,26±1,32[b]	-17,03±1,06[b] (14,04)	0,000
P2	119,06±1,70	84,46±2,31[c]	-34,60±1,07[c] (29,06)	0,000
P3	120,23±2,33	73,06±2,30[d]	-47,18±0,51[d] (39,24)	0,000
P4	120,47±1,52	69,42±2,21[e]	-51,04±0,72[e] (42,37)	0,000
p^1	0,188	0,000	0,000	

p^1: one way ANOVA test ; p^2: paired t test; a, b, c, d) different notations in the same column show significant differences in the LSD test.

The rice combination treatment shows a significant effect on triglyceride levels. All rice combination treatments, including P1, P2, P3, and P4, have triglyceride values that are significantly different and smaller than those in the K(+). It can be said that the rice combination treatment can reduce triglyceride levels. The highest triglyceride values are produced by the P1 group, followed by P2, P3, and P4. (Table. 3)

The reduction in triglyceride levels is also significantly influenced by the combination of rice. The greatest reduction in triglyceride levels is produced by the P4 group, followed by groups P3, P2, and P1. (Table. 3)

The Effect of Various Rice Combinations on Increasing HDL Cholesterol Levels

Normal HDL cholesterol levels in mice are ≥ 35 mg/dl. Before the rice combination intervention, the HDL levels of mice in all groups were in the low category, ranging from 29.9 – 32.65 mg/dl. After being given the intervention, mice in the rice combination had HDL levels in the normal category. In the K(+) control group, HDL levels decreased after being given standard feed. (Table. 4)

Table 4. The Average Changes in HDL Levels Before and After Rice Combination Intervention

Group	Before intervention	After Intervention	Level Changes	p^2
K(+)	30,24±2,22	29,26±2,28[a]	-0,98±0,59[a] (3,24)	0,010
P1	32,07±1,55	62,72±1,60[b]	30,64±0,90[b] (95,54)	0,000
P2	32,65±1,29	70,74±2,03[c]	38,09±0,76[c] (116,69)	0,000
P3	31,62±1,74	74,32±1,91[d]	42,71±0,41[d] (135,08)	0,000
P4	29,9±3,22	79,26±3,41[e]	49,36±1,68[e] (165,11)	0,000
p^1	0,145	0,000	0,000	

p^1: one way ANOVA test; p^2: paired t test; a, b, c, d) different notations in the same column indicate significant differences in LSD test

Based on the ANOVA test (Appendix 4), the initial condition of the mice before being given the rice combination did not show significantly different HDL levels. The combination of rice given shows a significant influence on HDL levels. All rice combination treatments in groups P1, P2, P3, and P4 have HDL levels that are significantly different and greater than those in the K(+) group. The rice combination treatment can increase HDL levels. Group P1 has the lowest HDL levels, followed by P2, P3, and P4. (Table. 4)

The increase in HDL levels is also significantly influenced by the combination of rice. The largest increase in HDL levels is produced by the P4 group, followed by groups P3, P2, and P1. (Table. 4)

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The Effect of Various Rice Combinations on the TG/HDL Ratio

The high risk of cardio metabolic disease is indicated by a TG/HDL ratio ≥ 3.5. The group before the intervention had a TG/HDL ratio in the high category, while after the intervention the group had a normal TG/HDL ratio except for the K(+) group where the ratio increased. (Table. 5)

Table 5. The Average of TG/HDL Ratio Before and After Rice Combination Intervention

Group	Before Intervention	After Intervention	Ratio Changes	p^2
K(+)	4,04 ± 0,32	4,25 ± 0,36[a]	0,21 ± 0,092[a]	0,000
P1	3,78 ± 0,20	1,66 ± 0,05[b]	-2,12 ± 0,164[b]	0,000
P2	3,65 ± 0,18	1,19 ± 0,06[c]	-2,45 ± 0,123[c]	0,000
P3	3,81 ± 0,15	0,98 ± 0,02[d]	-2,82 ± 0,148[d]	0,000
P4	4,05 ± 0,40	0,87 ± 0,03[d]	-3,18 ± 0,371[e]	0,000
p^1	0,068	0,000	0,000	

p^1: one way ANOVA test; p^2: paired t test; a, b, c, d) different notations in the same column show significant differences in the LSD test.

Based on the ANOVA test (Table 5), the TG/HDL ratio before the rice combination intervention did not show a significant effect. After being given the intervention, the rice combination has a significant effect on the TG/HDL ratio. The K(+) control group has a higher TG/HDL ratio and is significantly different from the P1, P2, P3, and P4 groups. Group P4 is not significantly different from group P3. The lowest TG/HDL ratio is produced by the P4 group, followed by the P2, P1, and K(+) control groups. (Table. 5)

DISCUSSION

The Effect of Various Rice Combinations on Wistar Rat Body Weight

Wistar rats in the K(+) group has the highest average body weight (Table 3). The high body weight in this group is caused by mice that have previously been given a high fat and high fructose diet, only given standard food and not given the rice combination treatment. In this standard food, nutritional composition has been considered that suits the needs of mice both in terms of nutrients and quantity to support optimal mouse growth.[32] P3 and P4 have the lowest average body weight and are not significantly different. The low average body weight of mice in the P3 and P4 groups could be due to the high dietary fiber content in black rice, which ranges from 4.5 to 6 grams per 100 grams and makes the mice feel fuller.[33]

The Relationship among Wistar Rat Body Weight and Triglyceride and HDL Cholesterol Levels

Based on the regression correlation analysis test, body weight is positively correlated with triglyceride levels and negatively correlated with HDL cholesterol. In obesity, there is an increase in free fatty acids which will be stored in subcutaneous adipose tissue, but when this tissue reaches its capacity, it will cause fat accumulation in ectopic tissue and visceral adipose tissue. Excessive fat accumulation in ectopic tissue will trigger insulin resistance.[34] Insulin resistance will trigger the increase of lipolysis, which will cause an increase in free fatty acids. The increase in free fatty acids will be used as a material to form triglycerides in the liver and produce VLDL rich in triglycerides so that triglyceride levels in the blood will increase.[35] This is supported by a case-control study in Vietnam which shows that the obese group has triglyceride levels 1.4x higher than the group with normal weight. Apart from that, the results of this study also show that an increase in abdominal fat occurring along with an increase in triglyceride levels.[36]

Hypertriglyceridemia is associated with low HDL levels and the increase of HDL catabolism. In conditions of hypertriglyceridemia, CETP will mediate the transfer of triglycerides from triglyceride-rich VLDL to HDL particles and produce triglyceride-rich HDL. The lipase enzyme in the liver will hydrolyze triglyceride-rich HDL and be cleaned by the liver so that HDL levels in the body decrease.[37] A cross-sectional study in India aimed at explaining the relationship between obesity and decreased HDL shows that someone who is centrally obese had HDL levels 4x lower than the non-obese group.[38]

Effect of Rice Combinations on Wistar Rat Food and Fiber Consumption

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Based on rat food consumption, group P2 has the highest feed consumption, followed by P3, P1, and P4. The high food consumption in group P2 occurred due to low fiber intake in the combination of white and red rice. The P4 group has the lowest food consumption and is not significantly different from the P3 group. The low food consumption in the P3 group is due to the high fiber consumed, namely 0.73 grams so that the mice felt full and had lower the appetite.[39]

The Effect of Various Rice Combinations on Reducing Triglycerides

The triglyceride content shows some differences between before and after treatment in both the K(+) control and the rice combination treatment. In the K(+) group there is an increase in triglyceride levels after being given standard food, while in the P1, P2, P3 and P4 groups there is a decrease in triglyceride levels after being given the rice combination intervention (Table 1). The increase in triglycerides in the K(+) group is caused by the rats standard diet containing 51% carbohydrates and yellow corn which is the main carbohydrate source. Yellow corn has a moderate glycemic index value so it can increase fasting sugar levels in mice.[30] There is a relationship between fasting blood sugar levels and triglyceride levels, that is, if fasting sugar levels increase, triglyceride levels will also increase.[40] Apart from that, this group is only given standard food and is not given intervention in the form of a rice combination.

The triglyceride content in the rice combination treatment shows significant differences in all treatments, both in the combination of white rice only, white rice and red rice, white rice and black rice, white rice, red rice and black rice with K(+) control and between the rice combination treatments. P4 has the smallest triglyceride levels, followed by groups P3, P2, and P1. The decrease in triglycerides after the rice combination intervention may be due to the influence of active compounds contained in rice in the form of dietary fiber and anthocyanin's.[20,21,41,42] Based on the analysis test for dietary fiber content carried out in this research, white rice has a higher dietary fiber content than red rice, but lower than black rice. The dietary fiber content of white rice, red rice and black rice respectively is 3.105%, 2.143% and 4.213% (Appendix 2). Group P1 which is given white rice only is able to reduce triglyceride levels, but the reduction is lower than groups P2, P3 and P4. This may be due to the dietary fiber content in the form of resistant starch in white rice.[43] Resistant starch is part of the starch that is not digested by amylase in the small intestine, but is fermented by the microbiota in the large intestine and produces short chain fatty acids or SCFA (short chain fatty acids) consisting of acetate, propionate and butyrate.[44] Short chain fatty acids produced from fermentation of resistant starch in the large intestine are able to inhibit the expression of ACC-1 in the liver which plays a role in fatty acid synthesis so that triglyceride levels in the liver and plasma can decrease.[45]

Resistant starch has the potential to reduce triglyceride levels. This is in accordance with the research by Zhou, et al (2015) which states that consuming resistant starch for 4 weeks is able to reduce triglyceride levels significantly by 65.4%.[46] Previous study show that black potato flour rich in type three resistant starchs given for 28 days to Wistar rats is also able to reduce triglyceride levels significantly by 32.90%.[47]

The decrease in triglycerides in red rice and black rice may also be due to the anthocyanin content. Red rice contains proanthocyanidin of 72.7 µg/g, while black rice contains cyanidin-3-glucoside of 72.7 µg/g.[17] The research results of Wei, et al (2011) shows that cyanidin-3-O glucoside supplementation for 12 weeks was able to reduce triglyceride levels by 41.7%. In blood vessels and skeletal muscles, cyanidin-3-O glucoside is able to increase the activity of the LPL (lipoprotein lipase) enzyme which will hydrolyze free triglycerides resulting in a decrease in plasma triglycerides. Meanwhile, LPL activity in adipose tissue can hydrolyze triglycerides in capillary blood vessels and produce free fatty acids. Free fatty acids ia transported and stored in the form of triglycerides in adipose tissue. Therefore, cyanidin-3-O glucoside plays a role in inhibiting LPL activity in adipose tissue so that the accumulation of triglycerides in adipose tissue can be reduced. This causes the mobilization of triglycerides to tissues that have a high level of oxidation, such as skeletal muscle.[26] Proanthocyanidins is able to reduce triglyceride levels by reducing triglyceride secretion, increasing fatty acid oxidation, and increasing plasma triglyceride catabolism in the liver. Proanthocyanidins can inhibit SREBP-1c in the liver so that fatty acid synthesis is inhibited and increase the expression of CPT-1 (carnitine palmitoyltransferase-1) which controls the oxidation of free fatty acids resulting in a decrease in the secretion of VLDL-rich triglycerides. Proanthocyanidins can also increase the expression of the Apo-A5 gene which activates the hydrolysis of triglyceride-rich VLDL by liver lipase.[48] The research by Quesada, et al (2009) regarding Proanthocyanidins extract in grapes against dyslipidemia explained that 25 mg/kg BW of Proanthocyanidins extract given for 10 days is able to reduce triglyceride levels by 40% in Wistar rats after being given high-fat food.[49]

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The dietary fiber content in white rice has the potential to reduce triglyceride levels. Even though white rice has higher dietary fiber than red rice, the bioactive compound content of white rice is lower than red rice and black rice so the decrease is the smallest when compared with P2, P3, and P4. Consuming 91.00% of white rice alone in P1 can reduce triglyceride levels by 14.04%. Meanwhile, even though P2 (combination of white rice and red rice) has the highest food consumption at 92.72% and has the lowest fiber intake, it could reduce triglycerides by 29.06%. This may be because red rice has a high Proanthocyanidins content. In the P3 group (combination of white rice and black rice) with food consumption of 91.50%, triglycerides can be reduced by 39.24%. Groups P3 and P4 (a combination of white rice, red rice and black rice) has almost the same food consumption, but group P3 has a higher fiber intake than group P4. Even though the P4 group had lower fiber intake than P3, the reduction in triglycerides is higher than P3, namely 42.37%. This could be because the combination of Proanthocyanidins content in red rice and cyanidin-3-O glucoside in black rice has a complementary effect so that it has a strong influence on triglyceride levels.[50] This research is in line with research by Khattab HA, et al (2013) which proves that the combination intervention of ginger and cur cumin is more effective in reducing triglycerides and increasing HDL than ginger or cur cumin intervention separately in mice with diabetes.[51]

The Effect of Various Rice Combinations on Increasing HDL Levels

HDL levels before being given the rice combination food show values that are not significantly different in the control groups K(+), P1, P2, P3, and P4. In the K(+) group, HDL levels decrease after being given standard food, while HDL levels in the rice group increase after being given the rice combination intervention. Group P4 is the group with the highest HDL levels, followed by groups P3, P2, and P1. The largest to smallest increase in HDL levels result from groups P4, P3, P2, and P1 (Table 2). The P4 group, which is a combination of white rice, red rice and black rice, contains bioactive compounds and dietary fiber which have the potential to increase HDL levels.

The main bioactive compound that plays a role in red rice is Proanthocyanidins, while black rice contains the compound cyanidin-3-O glucoside. Procyanidin and cyanidin-3-O glucoside can increase HDL and ApoA-1 levels through a CETP inhibition mechanism.[25,52] Previous study show that supplementation with oligomeric proanthocyanidins of 150 mg/day for 8 weeks can significantly increase HDL levels in COPD (chronic obstructive pulmonary disease) patients.[53]

Dietary fiber can also influence HDL cholesterol metabolism. Short chain fatty acids produced from fermentation of dietary fiber play a role in the production of Apo A-1 (the main protein in HDL) which can increase HDL function so that HDL can play a protective effect against cardiovascular disease.[54] Previous study show that black potato flour which is rich in type 3 resistant starch can increase HDL by 100% and black potato crackers can significantly increase HDL levels by 156%.[47] In a randomized controlled clinical study, inulin supplementation of 10 g/day for 8 weeks is able to significantly increase HDL levels by 19.90%.[55] In addition, dietary fiber intake given 30 g/day can increase HDL levels by 10.1% in factory workers in China in a cross-sectional design study.[56]

The increase in HDL levels in groups P1, P2, P3, and P4 can be due to the presence of dietary fiber and anthocyanin's. In group P1, consumption of 91.00% white rice alone could increase HDL by 95.54%, possibly due to the resistant starch content and adequate fiber intake, namely 0.57 gr. In group P2 (combination of white rice and red rice) there was an increase in HDL of 116.69% even with high feed consumption (92.72%) and low fiber intake of 0.53 gr. This increase can occur due to the high Proanthocyanidins content in red rice. Group P3 (combination of white rice, red rice and black rice) with food consumption of 91.50% and high fiber intake of 0.73 grams can increase HDL levels by 135.08%. The P4 group had the largest increase in HDL levels, namely 165% with feed consumption of 90.60% and fiber intake of 0.57 grams which is sufficient for needs. Even though group P4 has lower fiber intake than P3, the increase in HDL in group P4 is higher. These results indicate that increasing HDL is not only influenced by fiber intake, but can also be influenced by anthocyanin content. In the P4 group, the combination of red rice and black rice contains Proanthocyanidins and cyanidin-3-O glucoside which provide a complementary effect on increasing HDL.[50]

The Effect of Various Rice Combinations on the TG/HDL Ratio

The TG/HDL ratio is a prediction factor for metabolic syndrome and insulin resistance.[8] A person with insulin resistance has a high TG/HDL ratio. High triglyceride levels and low HDL cholesterol can occur due to the insulin resistance. Insulin resistance can lead to increase synthesis and secretion of triglyceride-rich VLDL by the liver. Insulin resistance can also cause an increase in CETP activity resulting in an exchange

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between triglycerides in VLDL and cholesterol esters in HDL. This results in triglyceride-rich HDL being catabolized by liver lipase resulting in a decrease in HDL cholesterol levels. Insulin resistance also reduces lipoprotein lipase activity so that triglyceride-rich VLDL does not undergo lipolysis and triglyceride levels in the blood will increase.[57] After being given the rice combination intervention, the P4 group has the lowest TG/HDL ratio among the other groups. This may be caused by low body weight and food consumption, while fiber intake is sufficient, as well as the presence of a combination of Proanthocyanidins and cyanidin-3-O glycoside in the P4 group. Low body weight can reduce the TG/HDL ratio due to low free fatty acid production and lipoprotein synthesis resulting in low triglyceride levels and high HDL levels.[58] Low food consumption can be caused by adequate fiber intake so that mice feel full more quickly.[39] Dietary fiber can produce short chain fatty acids which can inhibit the ACC-1 enzyme in the liver so that fatty acid synthesis is hampered and triglycerides decrease.[45] Short chain fatty acids are able to increase the production of Apo-A1 so that HDL cholesterol production increases.[54] In the P4 group there is also a combination of anthocyanin compounds in red rice and black rice which has a complementary effect on reducing triglyceride levels and increasing HDL levels.[59] Anthocyanin's are able to reduce triglyceride levels by activating AMPK in hepatocytes and inhibiting ACC-1 and ACC-2 activity which can increase fatty acid oxidation and decrease fatty acid synthesis resulting in a decrease in triglyceride levels.[23] Procyanidin and cyanidin-3-O glucoside in red rice and black rice can increase HDL levels through inhibiting CETP in the liver.[25,52]

CONCLUSIONS

In this study, the P3 group, which received a combination of white and black rice, demonstrated the highest fiber intake among all groups. Conversely, both the P1 and P4 groups showed identical fiber intake levels, which were lower compared to the P3 group. Notably, the P2 group had the lowest recorded fiber intake among all groups. These findings are summarized in Table 2, providing a clearer understanding of the variations in fiber intake across the different groups.

Based on the results, the P4 produced the best outcomes, showing the greatest reduction in triglyceride levels and the most significant increase in HDL levels. A daily fiber intake starting from 0.57 grams was shown to reduce triglyceride levels and increase HDL cholesterol levels.

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