

Original Research Article

Effects of Betamelor (Black Rice, Red Beans and Moringa Leaves) Consumption on Hypercholesterolemic Rats

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Abstract: Human health risk increases with higher exposure to free radicals. An imbalance between the number of free radicals and antioxidants in the body results in oxidative stress. Oxidative stress triggers a variety of diseases, including cardiovascular disease. Betamelor contains anthocyanins, flavonoids and isoflavones, it is expected to reduce oxidative stress. The purpose of this study was to analyze the potential of betamelor (black rice, red beans, and moringa leaves) on cholesterol levels in *rattus norvegicus* rats induced by a high-fat diet. This is an experimental study with pre and post control group design. A total of 30 female of *rattus norvegicus* strains aged 5-6 months with a weight of 150-200 g were induced orally using a high-fat diet about two ccs/day. Experimental animals were divided into five groups, namely standard feed (K0), standard feed with 2cc/day of simvastatin (K1), 20% betamelor-based feeding (K2), 50% betamelor-based feeding (K3), and 80% betamelor-based feeding (K4). Betamelor was intervened about 5% of bodyweight for 14 days. Blood sampling and analysis were carried out twice, at week 0 (before the intervention) and week 2 (after the intervention). Blood was drawn directly from the eye vein (ocular artery) \pm 2 ml using microhaematocrit capillary tubes after anesthetized with 10 mg/kg body weight of ketamine. Cholesterol data were analyzed by Analysis of Variance (ANOVA) at a 95% confidence level. If the results of the analysis show a significant effect on the response variable, then a Tukey test will be conducted. Data processing and analysis were performed using Microsoft Excel and SPSS for Windows version 22.0. **Result:** There were significant differences ($p < 0.05$) in the K2, K3, and K4 groups, with total blood cholesterol levels of 10.1 mg/dl, 18.0 mg/dl, and 22.02 mg/dl, respectively, compared to the control group (K0). There were significant differences found in the K2 and K3 groups compared to the positive control group (K1). Comparison among 80% betamelor group, 50% betamelor group, and 20% betamelor group showed a significant difference ($p > 0.05$). Betamelor (black rice, red beans, and Moringa leaves) seem to possess a potential effect on the condition of hypercholesterolemia. **Conclusion:** There was a significant effect on the intervention of a mixture of flour of black rice, red beans, and Moringa leaves on blood cholesterol levels in hypercholesterolemia rats.

Keyword: Black rice, red beans, moringa leaves, total cholesterol.

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INTRODUCTION

The prevalence of non-communicable diseases in Indonesia has increased compared to 2013. These diseases including cancer (1.4% to 1.8%), stroke (7% to 10.9%), chronic kidney disease (2% to 3.8%), diabetes mellitus (6.9% to 8.5%), and hypertension (25.8% to 34.1%) (Ministry of Health of the Republic of Indonesia, 2018). Non-communicable diseases such as hypertension, atherosclerosis, cardiovascular disease, metabolic syndrome, obesity, hypercholesterolemia, and diabetes mellitus have been linked to increased levels of cholesterol in the body (Medhat, 2017).

Health risks from non-communicable diseases can be minimized by adopting a healthy lifestyle, such as eating foods rich in bioactive compounds. Bioactive compounds in food can act in a variety of biological activities, for example, as antioxidants in the body. The role of food is now not only as of the fulfillment of nutritional needs and giving the feeling of satiety but also expected to be useful for health (functional food).

Three types of food that familiar to Indonesian people are black rice, red beans, and Moringa leaves. There are several antioxidants in black rice, red beans and moringa leaves that improving metabolic disorders

and overcoming intracellular inflammation and reducing the occurrence of oxidative stress.

Goufo and Trindade (2013) stated that rice contains antioxidants, namely phenolic acids, flavonoids, tocopherols, tocotrienols, anthocyanins, proanthocyanidins, γ -oryzanol, and phytic acids. Hosoda *et al.* (2018) reported that black rice and brown rice contain anthocyanin and proanthocyanidin, which are potentially used as sources of antioxidants. Bioactive compounds in pigmented rice can reduce cardiovascular disease (Walter and Marchesan, 2011), whereas red beans contain dietary fiber. Regular consumption of dietary fiber may contribute to lowering the risk of several diseases such as heart disease, stroke, and obesity, also helps in decreasing blood cholesterol levels (Anderson *et al.* 2009). Moringa contains antioxidants such as alkaloids, saponins, phytosterols, tannins, phenolics, flavonoids. Besides, Moringa leaves also contain vitamin C, about 120 mg/100 g of its leaves (Tejas *et al.*, 2012). The high vitamin E content in Moringa leaves can prevent lipid peroxidation so as to prevent the onset of oxidative stress disorders (Astuti, 2016).

This study aims to determine the feeding effect of a mixture of black rice, red beans, and Moringa leaves on rat blood hypercholesterolemia. Specifically, this study aims to 1) determine the differences in body weight (BW), 2) determine the average consumption of rat feed during the intervention period; 3) analyze the effect of treatment on reducing feed consumption; 4) investigate the impact of intervention on rat blood cholesterol levels.

METHODS

The design used in this experimental study was a random blok design (RBD) with a pre-post control group. This research was conducted in several laboratories, from March-May 2020. The production of flour or powder of black rice, red beans, and Moringa leaves was carried out in the Food Processing Laboratory of Health Polytechnic of the Health Ministry's - Kupang, the making of feed was carried out at the Animal Feed Industry Laboratory, Agricultural Polytechnic - Kupang. Maintenance and blood collection of the experimental animal were executed at the Biosciences Laboratory of the University of Nusa Cendana - Kupang. Blood analysis conducted at the Health Laboratory of the East Nusa Tenggara Province, while proximate composition analyzed at the Saraswanti Indo Genetech Laboratory in Bogor, West Java.

The animals used were *rattus norvegicus* female rats met the inclusion criteria, such as normal and healthy, aged 5-6 months, the weight of 150-200 g, open eyes, reddish-white skin, agile, and have soft, clean, dense, smooth, not fall, and shiny-haired. The exclusion criteria were that the rat died during the

intervention, behavior changes (did not want to eat, drink and limp), weight loss of > 5%. Equipment used for rat care consists of a cage filled with husks equipped with food containers and drinking bottles, iron cage enclosures, digital scales for weighing rats and leftovers, and a set of cage cleaning tools. This study had received ethical approval from the Animal Ethics Commission, Faculty of Veterinary Medicine, University of Nusa Cendana No.168 / UN15.8.1 / PP / 2020.

The first stage of the research was producing feed formulations with black rice, red beans, and Moringa leaves. The composition of ingredients based on diet composition in people with high cholesterol (Almatsier, 2010).

Adaptation and hypercholesterolemia phase. During the adaptation period, intended to ensure the experimental animals are in a healthy condition before the intervention. This phase was carried out for ten days in individual cages and given standard feed and ad libitum drinking water. Giving high-fat feed about two ccs/200g BW/day through sonde for four weeks to create hypercholesterolemia states. Bravo 512 standard feed content 12% water, 19.5-21.5% crude protein, min 5% crude fat, max 5% crude fiber, max 7% ash, 0.9 - 1.1% calcium, 0.6 - 0.9% phosphorus, and 3125kcal/Kg energy.

Intervention phase. The intervention or treatment period was carried out for two weeks and given a standard feed (K0), standard feed and 2cc/day of simvastatin (K1), 20% betamelor-based feed (K2), 50% betamelor-based feed (K3), and 80% betamelor-based feed (K4). The data of feed intake and residual were weighed and recorded every day, and weighing is done once a week.

Blood sampling and collecting. Blood sampling and analysis were performed twice, at week 0 (before the intervention) and week 2 (after the intervention). Rats fasted for 10 - 12 hours and two ccs of blood drawn through the eye veins (ocular arteries) using microhematocrit capillary tubes after anesthetized with 10 mg/kg of ketamine.

Data processing and analyzing. Analysis of Variance (ANOVA) analyzed blood cholesterol data at a 95% confidence level. If the results show a significant effect on the response variable, then Tukey's Mutual Range Test conducted. Data processing and analysis were performed using Microsoft Excel and SPSS for Windows version 22.0.

RESULT AND DISCUSSION

Feed Intake

Feed intake of rats during the experiment was calculated daily from the period of acclimatization to the intervention phase and obtained average value, as

shown in Table 2. It can be seen that at the beginning of the experiment or the adaptation and a provision of a high-fat diet or Hypercholesterolemia intake on all groups were not significantly different. On day 52 or 2 weeks of the intervention period, the consumption of the standard feed group (K0) and 20% betamelor group

(K2) was significantly higher than the 50% betamelor group (K3), 80% betamelor (K4) and positive control group (K1). The consumption of the intervened group seemed to increase during the intervention but not equal to the beginning of the adaptation (Table 1).

Table-1: Mean feed intake in grams of Betamelor and standard feed

Phase		Mean ± SD (g)					P
		Standar feed (K0)	Feed of Medicine (K1)	Betamelor 20% (K2)	Betamelor 50% (K3)	Betamelor 80% (K4)	
Adaptation phase	Acclimatitition (Day 10 th)	12,53±0,70	12,97±0,71	12,59±0,96	12,66±0,80	12,87±0,55	0,936
High fat diet	Day 24 th	11,57±0,82	11,64±0,82	11,77±0,62	11,57±0,87	11,71±0,66	0,079 ¹
	Day 38 th	11,71±0,44 ^a	11,94±0,53 ^b	11,69±0,84 ^b	11,62±0,81 ^c	11,68±0,88 ^c	
Intervention phase	Day 52 th	10,89±1,41 ^c	8,12±1,12 ^a	10,65±1,25 ^c	9,27±1,36 ^b	8,98±1,46 ^b	0,039 ¹
P1 Hiperkolesterolemik		0,362 ²	0,023 ²	0,095 ²	0,019 ²	0,032 ²	
P2 Adaptation		0,000 ²					

¹) One way Anova test, ²) Paired t-test, ^{a,b,c,d}) Mean scores with the same superscripts are statistically different at p < 0.05

Body Weight

The weighing was done weekly to determine the amount of standard feed, high-fat feed (weeks 1, 2, 3, and 4) and betamelor-feed (weeks 5 to 8). The effect of feeding treatment on the body weight of rats can be seen in Table 2. On the day 17th, and 45th, or one week after blood sampling, there was a decrease in body

weight in all diet groups. It was probably caused by the influence of blood drawn through the eye veins (ocular arteries), which results in pain and decreased appetite that can be seen from the feed intake reduction (Table 1) in all diet groups. In the following days, the slow improvement of body weight was in line with the increase in feed intake.

Table-2: Body weight of rats fed a high-fat diet and treated with betamelor for 10 weeks

Group	Acclimatization mean ± SD (g)	HFD ³ mean ± SD (g)	Intervention of Betamelor mean ± SD (g)	P acclimatization	P HFD ¹ - Intervention
K0	190,82±12,40	209,22±16,71	217,03±17,17	0,000 ²	0,000 ²
K1	192,43±9,34	209,77±15,36	207,67±13,78		
K2	189,91±23,87	207,78±16,07	217,37±13,15		
K3	190,38±22,03	209,50±13,36	214,01±11,78		
K4	192,27±14,84	210,88±17,41	210,76±18,50		
P	0,309 ¹	0,681 ¹	0,002 ¹		

¹) One way Anova test, ²) Paired t-test ³) HFD : High Fat Diet

The average Blood Cholesterols before and after Intervention

The mean of rat blood cholesterol level at the end of the adaptation stage and after giving a high-fat diet for each group can be seen in Table 3. The elevation in blood cholesterol occurred in all groups

significantly after a given high-fat diet with a significant value (p> 0.05) based on Anova one way test analysis. The betamelor treatment group tended to experience a decrease but the large decrease was below K1.

Table-3: Concentration (mg/dL) of cholesterol that were administered graded doses of high fat diet.

Group	Acclimatization mean ± SD (mg/dl)	HFD ³ mean ± SD (mg/dl)	P ²	• Increase (mg/dl) (%)	P ¹
K0	60,75 ± 6,62	135,23 ± 6,59	0,000	74,48 ± 0,93 (122,6%)	0,000
K1	66,33 ± 4,55	133,33 ± 5,05		67,00 ± 1,38 (101,0%)	
K2	61,83 ± 11,67	131,52 ± 5,55		69,69 ± 4,92 (112,7%)	
K3	64,40 ± 4,68	135,96 ± 4,33		71,56 ± 0,82 (111,1%)	
K4	68,50 ± 11,33	136,97 ± 3,80		68,47 ± 5,56 (99,95%)	
P	0,835	0,198			

¹) One way Anova test, ²) Paired t-test ³) HFD : High Fat Diet

There were differences in rat blood cholesterol levels among groups after the administration of betamelor based on the Kruskal Wallis test. The K1 treatment group experienced the most substantial decrease in blood cholesterol levels, about -27.10 ± 0.82 (-20.32%) mg/dl (Table 4). In this study, the intake of the experimental animal during the adaptation in all groups did not have a significant difference with an average consumption of ± 12 g/day. The changes in intake occurred when hypercholesterolemia diets or

high-fat diets with a dose of two ccs/day, feed intake reduced to an average of ± 11 g/day.

Alteration in feed intake also occurred at the time of intervention by betamelor 80%, 50%, 20%, and simvastatin treatment; feed intake decline to $\pm 9-10$ g/day. It caused by nutrients contained in the betamelor and side effects of simvastatin. The decrease in feed intake was significantly different in the simvastatin treatment and betamelor intervention groups with a p-value < 0.05 , but the decline in feed intake was not significantly different in the control group.

Tabel-4: Concentration (mg/dL) of fasting blood cholesterol that were administered graded doses of betamelor

Group	HFD ³ mean \pm SD (mg/dl)	Betamelor mean \pm SD (mg/dl)	P ²	i. Increase (mg/dl) (%)	P ¹
K0	135,23 \pm 6,59	140,24 \pm 5,13	0,043	5,01 \pm 1,91 (3,70%)	0,000
K1	133,33 \pm 5,05	106,23 \pm 5,42	0,000	-27,1 \pm 0,82 (-20,32%)	
K2	131,52 \pm 5,55	121,42 \pm 5,97	0,000	-10,1 \pm 0,74 (-7,67%)	
K3	135,96 \pm 4,33	117,96 \pm 4,01	0,000	-18,0 \pm 1,02 (-13,23%)	
K4	136,97 \pm 3,80	114,95 \pm 4,09	0,000	-22,02 \pm 1,13 (16,07%)	
P	0,198	0,000			

¹) Kruskal Wallis test, ²) Paired t-test ³) HFD : High Fat Diet

There were also significant differences in feed intake between treatment groups at the time of the intervention, while in acclimatization and conditioning to hypercholesterolemia, there were no significant differences, with p values > 0.05 . It is due to the administration of betamelor in the treatment group contains a high fiber composition, so that the stomach capacity of the rat would full more quickly compared to the standard feed group. The reduction of feed intake in the treatment group may also due to bioactive compounds found in red beans, such as isoflavones. Isoflavones in the body can increase the levels of cholecystokinin, a digestive hormone (Zhang, et al., 2009). Genistein and daidzein compounds are also found in red beans that known able to reduce cholesterol levels, appetite, and fat deposits in the body (Ulbricht and Seamon, 2010). Decreased appetite may affect body weight.

The rat's weight in this study was changed in all groups, following a significant change in treatment. Changes in treatment from the adaptation phase were followed by a hypercholesterolemia conditioning phase and the intervention phase resulted in body weight changes, but there were no significant differences between groups during the acclimatization and high-fat diets. Weight gain may occur due to the consumption of standard feed and high-fat diets before the intervention.

In the intervention time, there were differences in treatment that caused a significant effect on the amount of energy intake triggered the excess consumption which would be stored as fat and increase body weight of experimental animals. The body weight

of the control group (K0) seemed improved; the simvastatin treatment group (K1) and 80% betamelor-based feed group (K4) experienced weight loss. Similarly, the intervention betamelor group gained weight but not as much as in the control group (K0). Bodyweight changes in this intervention group are possible because of feed developed from Moringa leaves and red beans. Moringa leaves contain active compounds, i.e., chlorogenic acid. The presence of chlorogenic acid active compounds found in Moringa can increase the body's metabolism and increase the oxidation of fatty acids (Cho, 2010), cause no significant weight gain occurs.

The average total cholesterol level in rats before the intervention was not significantly different between treatment groups (p < 0.05). This means that the initial conditions are not a confounding factor in the intervention process (Table 4). ANOVA test results showed that the treatment groups had a significant effect on cholesterol levels after the intervention (p < 0.05). In K4, intervened with the highest composition of black rice, red beans, and Moringa leaves experiences the cholesterol reduction effect was 16.07%. The decrease in cholesterol may cause by the active substances present in each ingredient, such as chlorogenic acid, ferulic acid, caffeic acid, polyphenols, anthocyanins, flavonoids, and vitamin C.

The chlorogenic acid content in moringa leaves, also ferulic acid and caffeic acid proven efficacious as an anti-inflammatory and antioxidant. Chlorogenic acid may prevent the absorption of cholesterol and inhibit the release of glucose into the

bloodstream after meals (Ong, 2013). In addition to chlorogenic acid, polyphenol content in black rice and red beans also has the potential to reduce the accumulation of visceral fat. Pinontoan (2015) reports that the administration of black rice extract can reduce cholesterol levels in Wistar rats that are treated by the prodyslipidemia diet. The anthocyanin in black rice has also influenced the reduction in cholesterol levels (Wang *et al.*, (2007).

The reduction of cholesterol levels of female *rattus norvegicus* rats in this study is possible because the cholesterol reduction can be through the binding of free radicals mechanism by antioxidants. This is due to the presence of flavonoids and vitamin C, which increase LCAT activity. LCAT described as an enzyme that can convert free cholesterol into more hydrophobic cholesterol esters (Aprilia, 2010). This is in accordance with the finding of Winarti (2010); the action mechanism of flavonoids is to reduce free radicals in the body. Chemically, the antioxidant is an electron-donate compound to those oxidants, so the activity of these oxidant compounds can be inhibited. Moringa leaves, red beans, and black rice contain flavonoids and vitamin C, which are useful for increasing LCAT activity.

Another mechanism caused by oxidative stress in the body, which is reduced by antioxidant activity, can reduce MDA levels (Bais 2014 (Sikder). Reactive oxygen species (ROS) are very reactive in damaging biological molecules, including DNA, proteins, carbohydrates, and lipids. Thus, causing an increase in oxidative stress leads to Non-Alcoholic Fatty Liver Disease (NAFLD) (Ling *et al.*, 2002). The anthocyanin content in black rice contains the main bioactive phytochemicals, including cyanidin-3- glucoside, peonidin-3-glucoside, cyanidin-3, 5-diglucoside, cyanidin-3-routineoside, and protocatechuic acid (Choi *et al.*, 2012) (Hu *et al.*, 2003; Yoshimura *et al.*, 2012). These compounds showed a high activity in reducing stress oxidative by lipid peroxidation inhibition due to hyperlipidemic conditions, thereby reducing ROS, decreasing synthesis, and regulation of Inducible Nitric Oxide Synthase and Cyclooxygenase-2. This is supported by Anthony *et al.* (2012) and Carroll's (1991) that legume peptides are suggested for its anti-hyperlipidemic effect and a decrease in blood cholesterol concentrations. Further research is necessary to test the complete phytochemical contents of betamelor and to analyze its effect on other anti-inflammatory and antioxidant parameters.

CONCLUSION

In conclusion, the betamelor made from black rice, red beans and moringa leaves flour added to three formulas dough with bravo II. The administration of the selected betamelor formula to reduce cholesterol significantly, in the experimental rats. Thus, betamelor may be considered a local-based potential alternative

food that is rich in nutrients, especially antioxidant, to help reduce blood cholesterol in human subjects in the future.

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