Effect of Black Glutinous Rice Fermented Beverage on Short-Chain Fatty Acid Levels in Metabolic Syndrome Rats

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ABSTRACT

Metabolic Syndrome (MetS) represents a combination of metabolic factors that can elevate the likelihood of developing type 2 diabetes mellitus (T2DM) and coronary heart disease (CHD). The high prevalence of MetS results in a significant socio-economic burden. However, current management approaches have limitations, prompting the exploration of functional foods and nutraceuticals as promising alternatives. The primary objective of this study is to investigate the impact of Black Glutinous Rice Fermented Beverage (BGRFB) on Short Chain Fatty Acids (SCFA) concentration in a MetS rat model. Rats were induced with a high-fat diet (HFD) and streptozotocin (STZ)-nicotinamide (NA) to induce MetS conditions. BGRFB was administered as a treatment to the MetS rat group. The results showed that the Negative Control (NC) group exhibited a significant decrease in SCFA levels compared to the normal group (N). However, administration of BGRFB to the T2 group resulted in a significant increase in SCFA levels. The SCFA levels in the T2 group were higher but not significantly different from the Positive Control (PC) group treated with metformin. Increasing SCFA production could be an effective strategy in addressing Mets. This study demonstrates the potential of BGRFB as a therapy to enhance SCFA production and improve MetS. The outcomes of this investigation are anticipated to provide insights into novel dietary approaches for MetS management

Keywords: Black Glutinous Rice Fermented Beverage; Coronary Heart Disease; Metabolic Syndrome; Short Chain Fatty Acids; Type 2 Diabetes

INTRODUCTION

MetS represents a combination of metabolic factors that can elevate the likelihood of developing type 2 diabetes mellitus (T2DM) and coronary heart disease (CHD). These factors encompass abdominal obesity, high blood sugar levels, elevated lipid levels, and high blood pressure (Wang et al., 2020). The global occurrence of MetS is approximated to be between 20% and 25% of the population, with comparable proportions observed in the Asia-Pacific area (International Diabetes Federation, 2006; Ranasinghe et al., 2017). In Indonesia, the prevalence of MetS is also high, with rates of 28% in males and 46% in females (Sigit et al., 2020). Considering the widespread occurrence of MetS, it is crucial to implement effective actions in managing and preventing MetS to reduce the socio-economic burden and improve quality of life.

The management of MetS involves a combination of pharmacological interventions and lifestyle modifications. However, the long-term use of multiple medications can lead to non-compliance and potential side effects (Rochlani et al., 2017). As an alternative, the development of medicinal plants has shown potential in modulating gut microbiota and managing MetS, to reduce the use of medications and minimize side effects (Nyakudya et al., 2020). One food known to have the potential to improve MetS is fermented black glutinous rice, and a study by Fauziyah (2020) demonstrated that individuals consuming more than 11.5 grams of fermented black glutinous rice per day had a protective effect against the risk of MetS (Fauziyah & Putri, 2020).

Furthermore, the fermentation of fermented black glutinous rice also produces another product called BGRFB. The International Scientific Association for Probiotics and Prebiotics (ISAPP) states that some types of fermented foods contain probiotics, which can be beneficial for health (Marco et al., 2021). In addition, the deep purple color of BGRFB indicates a high concentration of anthocyanins and antioxidant capacity (Rodríguez et al., 2013). Both

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anthocyanins and antioxidants have been proven to have numerous health benefits, including in metabolic diseases (Ayivi et al., 2020; Xu et al., 2021).

SCFA is produced in the colon through the bacterial fermentation of complex carbohydrates. These compounds play a vital role in linking nutrients, the gut microbiota, and diverse physiological and pathological processes within the body (Alexander et al., 2019). Imbalances in gut microbiota and disrupted SCFA production can contribute to health problems, including MetS (Festi et al., 2014). Therefore, the influence of BGFRB beverage on SCFA levels in a rat model of MetS needs to be investigated to understand its potential benefits and the underlying mechanisms.

The utilization of BGRFB is currently limited and further research is needed to optimize its use, especially in the management of MetS. Therefore, we are interested in studying the effect of BGRFB administration on SCFA levels in *the Sprague Dawley* (SD) rat model of MetS. This study aims to explore the potential of BGRFB in the management of metabolic syndrome and provide a basis for the development of functional food products based on BGRFB that can help control SCFA levels in the prevention and treatment of MetS. Thus, this research is expected to make a significant contribution to the development of new interventions for the management of MetS.

MATERIALS AND METHODS
Research design
The research took place at the Experimental Animal Laboratory of the Food and Nutrition Postgraduate Research Center, Universitas Gadjah Mada (UGM), employing a randomized post-test-only control group experimental design. A total of 25 SD rats were selected using a simple random sampling method and allocated into 5 groups. Ethical clearance for this study was granted by the Ethics Committee of the Faculty of Medicine, Sebelas Maret University, under reference number 43/UN27.06.11/KEP/EC/2023.

Sample selection
SD rats were chosen as the sample based on predetermined inclusion and exclusion criteria. For inclusion, the criteria consisted of rats that were 8-9 weeks old, weighed between 150-200 grams, were of male gender, displayed normal behavior, and showed no signs of abnormalities. On the other hand, rats were excluded if they exhibited diarrhea or refused to eat. Randomization was performed using a completely randomized design, where each rat had an equal chance of being assigned to the treatment or control group.

Interventions
The rats were given different treatments for 28 days. The treatment group consisted of rats with MetS who received BGFRB at a dose of 4.5 ml/kg BW (T1) and 9 ml/kg BW (T2) using a gastric tube. The control group consisted of normal rats without any treatment (N), rats with MetS without any treatment (NC), and rats with MetS receiving metformin at a dose of 45 mg/kg BW (PC). The process of inducing MetS in the rats involved the administration of a high-fat diet (HFD) and subsequent injection of streptozotocin (STZ) and nicotinamide (NA).

Preparation of BGFRB
BGRFB was made from steamed black glutinous rice, fermented with yeast, and squeezed to extract the fermented water. This water was stored as BGRFB for use during the intervention.

Creation of the MetS Rat Model
The MetS rat model was induced by providing an HFD for 2 weeks, followed by the administration of STZ at a dose of 45 mg/kg BW and NA at a dose of 110 mg/kg BW. The HFD composition consisted of Partially Autolyzed Yeast-supplemented (PAR-S) comfeed, wheat flour, cholesterol, cholic acid, and lard, which were provided ad libitum for 2 weeks. The criteria for MetS were determined based on the assessment of the rat's fasting blood glucose (FBG) > 119 mg/dl, High-Density Lipoprotein (HDL) < 40 mg/dl, and triglyceride (TG) >125 mg/dl (Ghezzi et al., 2012). FBG, HDL, and TG levels were measured by enzymatic methods.

Analysis of SCFA Levels
SCFA in the rat's cecum was analyzed in the Food Technology and Agricultural Product Laboratory, Faculty of Agricultural Technology, UGM, using the SHIMADZU GC-2010 PLUS instrument. SCFA samples were injected into the instrument using the appropriate injection technique, followed by the identification and quantification of SCFA by comparing the chromatogram peaks of the samples with the corresponding internal SCFA standards.

Data Analysis
The statistical analysis was performed using SPSS 25 software. The normal distribution of the data was assessed using the Shapiro-Wilk test. The equality of variances across groups was

assessed using Levene's test. One-way analysis of variance (ANOVA) was conducted to compare the group's means. To determine significant differences between pairs of groups, post hoc Tukey's test was applied.

RESULTS

The mean results of MetS components in rats after being fed HFD and STZ-NA induction can be seen in Table I. The measured components include FBG, HDL, and TG levels. The N group showed significantly lower FBG and TG levels and significantly higher HDL levels compared to the NC, PC, T1, and T2 groups. These results indicate that the administration of HFD and STZ-NA successfully induced MetS conditions in rats, characterized by increased FBG and TG levels and decreased HDL levels.

The occurrence of hyperglycemia decreased HDL levels, and elevated TG levels, as indicated by National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III), have been identified as MetS criteria since they fulfill 3 out of 5 MetS criteria (National Cholesterol Education Program, 2001). Statistical analysis showed significant differences in all of these components ($p < 0.001$), indicating that the administration of HFD and STZ-NA has a significant impact on the occurrence of MetS in rats. These findings are consistent with a study conducted by Sudargo (2021), where the administration of HFD for 2 weeks along with STZ-NA doses (STZ 45mg/KgBB and NA 110 mg/KgBB) significantly increased FBG levels and affected lipid profiles (Sudargo et al., 2021). Another study by Niture (2014), involving HFD administration for 4 weeks and STZ injection (35mg/KgBB), also showed increased GDP and TG levels and decreased HDL levels (Niture et al., 2014). HFD administration is associated with increased fat mass, mesenteric adipocyte size, and GDP levels (Marques et al., 2016). On the other hand, STZ induction plays a role in causing massive damage to pancreatic β cells, while NA is given before STZ administration to partially protect pancreatic β cells, resulting in milder damage (Eleazu et al., 2013; Fukaya et al., 2013).

In Table II, the effect of BGRFB administration on SCFA levels is described. The measured SCFAs include acetate, propionate, and butyrate. A study conducted by Marques (2016) revealed that HFD administration significantly reduced the abundance of *Firmicutes*, *Bacteroidetes*, *Lactobacillus*, and *Prevotella* (Marques et al., 2016). These findings indicate the occurrence of gut microbiota dysbiosis resulting from HFD consumption. The presence of gut microbiota species is linked to the production of SCFAs in the host's body (Alexander et al., 2019). Our research results are in line with these findings. In the N group, SCFA levels remained relatively stable as the rats did not experience MetS. However, in the group of rats with MetS (NC), we found a significant decrease in SCFA production compared to the N group. This indicates that MetS in rats is associated with reduced SCFA production.

Other studies have also shown that decreased plasma SCFA levels and gut microbiota dysbiosis occur in male Tsumura Suzuki obese diabetes (TSOD) rats that spontaneously develop MetS (Nishitsuji et al., 2017). This indicates consistency with our research findings. Studies in humans have also shown that obese women have lower levels of acetate, propionate, and butyrate, as well as a significantly higher risk of MetS compared to the normal weight group (Teixeira et al., 2013). These findings indicate that decreased SCFA production and changes in gut microbiota composition may contribute to the development of MetS.

Meanwhile, the PC and T2 groups showed a significant increase in SCFA production compared to the NC group. Post hoc Tukey HSD test results showed significant differences in SCFA production between the PC and T2 treatment groups and the NC group ($p < 0.001$), indicating that BGRFB administration in the PC and T2 treatment groups has a significant impact on increasing SCFA levels in rats with MetS. However, in the T1 group, there was no significant increase in SCFA production compared to the NC group.

Metformin has been identified as a promising therapeutic option for addressing MetS (Ladeiras-Lopes et al., 2015). The findings of this study align with research conducted by Mueller (2021) in humans, which demonstrated that metformin administration for 6 and 12 months at doses of 500mg-2000mg can improve gut microbiota composition and increase SCFA production (Mueller et al., 2021). Additionally, research by Ermolenko (2022) showed that metformin administration in a rat model of MetS can lead to weight loss, decreased total cholesterol and blood glucose levels, along with improvements in gut microbiota composition (Ermolenko et al., 2022). The mechanism of action of metformin involves reducing glucose production by the liver, increasing fat oxidation, and effects on the gastrointestinal tract, including the suppression of oxidative phosphorylation and alterations in endocrine cells and gut microbiota (Szymczak-Pajor et al., 2022).

The BGFRB dosage refers to the study conducted by Fauziyah (2020), which found that the administration of black glutinous rice tapai

Table I. Components of MetS in rats after HFD and STZ-NA induction

Groups	Components of MetS (mg/dl)		
	FBG	HDL	TG
N	69.09 ±1.77[bcde]	80.28 ±1.24	78.22 ±2.09[bcde]
NC	270.66 ±2.18	25.84 ±1.79[a]	141.25 ±3.60
PC	269.71 ±2.38	25.03 ±0.52[a]	141.61 ±3.64
T1	269.76 ±4.11	25.84 ±1.43[a]	144.04 ±1.58
T2	267.40 ±2.17	24.46 ±1.84[a]	143.31 ±3.44
p	<0.001	<0.001	<0.001

N=Normal, NC=Negative Control, PC=Positive Control, T1= Treatment 1, T2= Treatment 2; Values in the same column with a superscript were significantly ($p < 0.05$) lower than the N group; Values in the same column with b superscript were significantly ($p < 0.05$) lower than NC group; Values in the same column with c superscript were significantly ($p < 0.05$) lower than the PC group; Values in the same column with d superscript were significantly ($p < 0.05$) lower than the T1 group; Values in the same column with e superscript were significantly ($p < 0.05$) lower than the T2 group.

Table II. Effect of BGRFB administration on SCFA levels

Groups	SCFA (mmol/L)		
	Acetate	Propionate	Butyrate
N	52.39 ±16.42[bcde]	24.58 ±7.26[bcd]	15.47 ±3.43[bcde]
NC	12.96 ±3.27	6.13 ±1.61	3.56 ±0.78
PC	24.14 ±2.90[b]	12.20 ±1.88[b]	6.23 ±0.82[b]
T1	16.18 ±2.68	7.35 ±1.00	4.50 ±1.01
T2	32.19 ±15.79[bd]	15.40 ±7.46[bd]	8.81 ±3.52[bd]
p	<0.001	<0.001	<0.001

N=Normal, NC=Negative Control, PC=Positive Control, T1= Treatment 1, T2= Treatment 2; Values in the same column with a superscript were significantly ($p < 0.05$) higher than the N group; Values in the same column with b superscript were significantly ($p < 0.05$) higher than NC group; Values in the same column with c superscript were significantly ($p < 0.05$) higher than the PC group; Values in the same column with d superscript were significantly ($p < 0.05$) higher than the T1 group; Values in the same column with e superscript were significantly ($p < 0.05$) higher than the T2 group.

extracts at a dosage of 200g/day can decrease LDL levels in adult age groups (Fauziyah *et al*., 2020). This dosage was then calculated based on the value of BGFRB consumption generated in the process of making black glutinous rice tapai, which amounts to 50%. Subsequently, the dose of 100 ml for a 70 kg human was converted into a dose for a 200g body weight rat, resulting in a dose of 1.8 ml/200gBW or 9 ml/kgBW. In this study, we compared the effects of BGFRB at half the dose, which is 4.5 ml/kgBW (T1), and the full dose, which is 9 ml/kgBW (T2). Our research results showed a significant increase in SCFA production in the T2 group, while the T1 group showed a non-significant increase in SCFA production.

DISCUSSION

Several previous studies have shown that certain foods can increase SCFA production, such as kombucha, which is a fermented product of LAB, and anthocyanin extract from black rice (H. Wang *et al*., 2020; Xu *et al*., 2022). It is also known that

there is an interaction between LAB and anthocyanin that mutually affects each other. LAB influences anthocyanin metabolism, while anthocyanin affects LAB activity (Tian et al., 2019).

Previous literature has documented that the administration of LAB with different *Lactobacillus* strains significantly improves lipid profiles, and gut microbiota composition, and reduces pathogenic bacteria (Ai et al., 2021; Linninge et al., 2019). These findings are also supported by another study conducted by Yang (2021), which demonstrated that the administration of two different *Lactobacillus* strains can significantly stimulate gut microbiota composition and SCFA production (Yang et al., 2021).

Gut microbiota can enhance the bioavailability of anthocyanins through their role in modifying polyphenols, including anthocyanins (Cardona et al., 2013). The majority of anthocyanins are not absorbed in the small intestine but reach the large intestine intact, where gut microbiota metabolize them (Fernandes et al.,

2014). Certain types of gut microbiota, such as *Bifidobacteria spp*, *Lactobacillus spp*, and various other LABs, possess enzymes involved in the breakdown of anthocyanin structures (Mattioli et al., 2020; Verediano et al., 2021). When anthocyanins reach the large intestine, bacteria containing β-D-glucosidase and α-L-rhamnosidase enzymes remove the sugar from the compound, resulting in aglycone forms that are more easily absorbed by the body (Gui et al., 2022). These aglycone anthocyanins are then converted back into phenolic compounds by gut microbiota. This process increases the availability of anthocyanins to the body and facilitates their absorption (Mattioli et al., 2020). Additionally, gut microbiota plays a role in the heterocyclic cycle (in ring C) to phloroglucinol derivatives (in ring A) and benzoic acid (in ring B), further facilitating their absorption (Fernandes et al., 2015).

Meanwhile, anthocyanins are known to exhibit prebiotic activity by modulating the growth of gut microbiota and improving the gut environment by inhibiting pathogenic bacteria (M. Wang et al., 2022). For example, in vitro research conducted by Zhu (2018) revealed that black rice extract rich in anthocyanins showed potential as a prebiotic by increasing the levels of *Bifidobacteria* and *Lactobacillus* (Zhu et al., 2018). Both types of bacteria play a crucial role in SCFA production and possess β-glucosidase enzymes involved in anthocyanin metabolism (Verediano et al., 2021). Furthermore, meta-analyses provide strong evidence that anthocyanin supplementation enhances gut health biomarkers in rats by increasing the presence of *Firmicutes* and *Bacteroides*, and promoting SCFA production while reducing gut dysbiosis associated with obesity (Kapoor et al., 2023).

Our study showed that the administration of the full dosage of BGFRB (T2) had a more significant effect on increasing SCFA levels compared to the half dosage (T1). There were no significant differences between the T1 group and the NC and PC groups. However, the results indicated that the mean SCFA levels in the T1 group were higher than in the NC group but lower than in the PC group. This suggests that T1 administration increased SCFA production compared to the untreated group (NC), but it was still lower than the group receiving metformin (PC). In the T2 group, there were no significant differences compared to the PC group. However, the results showed that the mean SCFA levels in the T2 group were higher, and even the propionate levels showed no significant difference compared to the N group. This indicates that administering the full dose of BGFRB in the T2 group can increase SCFA production to a level close to the normal group.

SCFAs are known to play a role in adipose tissue metabolism, such as adipogenesis, lipolysis, and inflammation (May & Den Hartigh, 2021). In vitro studies have shown that acetate in SCFAs has an antilipolytic effect by reducing basal glycerol release in hMADS (human Multipotent Adipose tissue-derived Stem cells) adipocytes (Jocken et al., 2018). Human studies have also indicated that circulating SCFAs are associated with GLP-1 concentration, whole-body lipolysis, and peripheral insulin sensitivity (Müller et al., 2019). These findings support the idea that increasing SCFA production can be an effective strategy in addressing MetS. However, further research is needed to understand the mechanisms of interaction between SCFA and components of MetS, as well as the potential for their use in the treatment of MetS in humans.

CONCLUSION

The administration of BGFRB can increase SCFA production. The T2 group, which received the full dosage, showed the highest increase compared to other treatment groups. The half dosage of BGFRB (T1) did not significantly increase SCFA production. This indicates that BGFRB has the potential as a therapy to enhance SCFA production and address MetS, Providing a foundation for future studies in the domain of functional food and the interactions between gut microbiota.

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