



Effect of “Growol” on glucose levels and lipid profile of metabolic syndrome rats

Fransisca Shinta Maharini*, Maria Amrijati Lubijarsih, Bernadetta Eka Noviati, Ruth Surya Wahyu Setyaning

Department of Nutrition, Panti Rapih School of Health Science, Jalan. Tantular 401 Pringwulung, Condongcatur, Depok, Sleman, Yogyakarta 55584, Indonesia

*Correspondence: frshintamaharini@gmail.com

ABSTRAK

Latar Belakang: Growol merupakan makanan tradisional khas Kulon Progo, Yogyakarta yang terbuat dari singkong melalui proses fermentasi. Proses fermentasi ini melibatkan Bakteri Asam Laktat (BAL), khususnya *Lactobacillus casei* subsp. *rhamnosus* TGR2. Sifat sinbiotik pada growol menjadikannya pangan fungsional yang berpotensi memperbaiki profil lipid dan glukosa darah.

Tujuan: Penelitian ini bertujuan untuk mengetahui pengaruh pemberian growol terhadap kadar glukosa darah dan profil lipid pada hewan model sindrom metabolik.

Metode: Penelitian ini dilakukan di laboratorium pada 36 ekor tikus Wistar yang dibagi menjadi enam kelompok: dua kelompok kontrol dan empat kelompok perlakuan. Kelompok kontrol terdiri atas kontrol negatif yang diberi diet standar, dan kontrol positif yang diberi diet diet tinggi lemak dan fruktosa (DTLF). Kelompok perlakuan diberi DTLF selama 4 minggu, kemudian 4 minggu berikutnya diberikan intervensi pakan Growol dengan komposisi 25% (P1), 50% (P2), 75% (P3), dan 100% (P4).

Hasil: Hasil penelitian menunjukkan terdapat penurunan kadar kolesterol total, LDL, trigliserida, glukosa serta peningkatan HDL secara signifikan pada kelompok perlakuan yang diberi growol (P1-P4) ($p < 0,05$). Sedangkan kelompok kontrol negatif maupun positif mengalami peningkatan glukosa, kolesterol total, LDL, trigliserida, serta penurunan HDL secara signifikan ($p < 0,05$).

Kesimpulan: Diet berbasis growol secara signifikan memperbaiki profil lipid dan kadar glukosa pada model tikus sindrom metabolik. Temuan ini menyoroti potensi growol sebagai makanan fungsional dengan sifat sinbiotik untuk mengelola sindrom metabolik.

KATA KUNCI: growol; kadar glukosa; profil lipid; sindrom metabolik; tikus



ABSTRACT

Background: *Growol* is a traditional food from Kulon Progo, Yogyakarta, made from cassava through fermentation. This fermentation process utilizes Lactic Acid Bacteria (LAB), particularly *Lactobacillus casei* subsp. *rhamnosus* TGR 2. The synbiotic properties of "growol" make it a functional food with the potential to improve lipid profile and blood glucose levels.

Objectives: This study aims to assess the impact of "growol" administration on blood glucose levels and lipid profiles in an animal model of metabolic syndrome.

Methods: This study used an analytical observational method with a cross-sectional design. The sample of 150 adolescents aged 15-18 was selected using multistage random sampling. Adolescents' body fat was measured using Bio-Impedance Analysis (BIA) and their dietary intake was assessed using the Semi-Quantitative Questionnaire (SQ-FFQ). The research was conducted in Surakarta from May to June 2024.

Results: The findings indicated a significant decrease in total cholesterol, LDL, triglycerides, and glucose levels, along with an increase in HDL, in the treatment groups receiving growol (P1-P4) ($p < 0.05$). In contrast, the negative and positive control groups exhibited significant increases in glucose, total cholesterol, LDL, and triglycerides, along with a decrease in HDL ($p < 0.05$).

Conclusions: A growol-based diet significantly improves lipid profiles and glucose levels in a rat model of metabolic syndrome. These results highlight the potential of growol as a functional food with synbiotic properties that may aid in managing metabolic syndrome.

KEYWORD: *glucose levels; growol; lipid profile; metabolic syndrome; rats*

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INTRODUCTION

Growol, a traditional food from Kulon Progo, Yogyakarta, is made from cassava through a unique fermentation process involving Lactic Acid Bacteria (LAB), particularly *Lactobacillus casei* subsp. *rhamnosus* TGR 2. This LAB strain, known for its ability to survive in acidic digestive environments and resist bile salts, exhibits significant antimicrobial activity. Studies have reported high levels of LAB in growol, reaching 1.32×10^7 cfu/grams, highlighting its potential as a probiotic food source. Probiotics are live microorganisms that provide health benefits by colonizing the gut microbiota, particularly in the cecum, and must be present in sufficient amounts, typically between 10^6 and 10^8 cfu/grams (1).

In addition to probiotics, growol contains prebiotics – substrates enhancing the growth and resilience of probiotic bacteria and promoting gastrointestinal health. The fermentation of cassava into growol reduces sugar and sucrose levels while increasing dietary fiber, making it a valuable prebiotic source. Research into the prebiotic potential of Growol flour has shown that it can enhance the activity of probiotic bacteria

such as *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, although the effect is lower than inulin (2). Growol is a synbiotic food due to its probiotic and prebiotic content (3). Lactic acid bacteria serve as probiotic microorganisms in humans that can help lower total cholesterol, LDL, and triglyceride levels (29).

Metabolic syndrome encompasses a cluster of risk factors, including central obesity, dyslipidemia (elevated triglycerides and reduced High-Density Lipoprotein (HDL) cholesterol), hypertension, and high fasting blood glucose, which increase the risk of cardiovascular disease, stroke, and diabetes mellitus (4). These symptoms, linked to increased visceral fat, dyslipidemia, insulin resistance, and high blood pressure, can be managed non-pharmacologically through synbiotics, which combine probiotics and prebiotics (5). For instance, studies in hypercholesterolemic rats fed synbiotic products containing gembili flour demonstrated improved lipid profiles and reduced cholesterol levels (6). Similarly, consuming synbiotic yogurt with red bean prebiotics for 15 days lowered Low-Density

Lipoprotein (LDL) cholesterol in women with metabolic syndrome (7). Clinical trials also show that synbiotic supplementation can reduce fasting glucose, serum insulin, and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index while improving insulin sensitivity in women with gestational diabetes mellitus (8).

Investigated cookies made from modified growol flour enriched with glucomannan as a healthy snack for diabetes management (1). Adding glucomannan optimized dietary fiber, resistant starch content, and starch digestibility. The enhanced dietary fiber improved growol's prebiotic effects by serving as a carbohydrate source for lactic acid bacteria and reducing the glycemic index of the cookies. A low-glycemic-index diet is crucial for managing blood glucose levels in diabetes (30). Consistent with this, reported reduced fasting blood glucose and HOMA-IR levels and increased insulin in diabetic model rats after growol flour administration (10).

Low-glycemic-index diets also positively affect lipid profiles, reducing serum triglyceride by 15%–25% (11). Research (12) demonstrated growol flour's potential as a hypocholesterolemic agent across various age groups. While growol's effects on glycemic and lipid profiles are promising, studies on its impact on metabolic syndrome models remain limited. This study aims to evaluate growol's effects on blood glucose levels and lipid profiles in metabolic syndrome models.

MATERIALS AND METHODS

Design, location, and time

This study employed a true experimental design with a randomized controlled group pre-post design. The research was conducted between July and October 2024. The initial stabilization and adaptation phase lasted one week, during which all experimental animals were fed a standard diet. The intervention phase followed, lasting four weeks, during which metabolic syndrome was induced in the subjects. Subsequently, the subjects underwent dietary interventions based on the study groups for an additional four weeks. Glucose levels and lipid profiles were assessed before and after intervention. The study was conducted at the STIKes Panti Rapih Food Technology Laboratory,

the Laboratory of Experimental Animals at the Inter-University Food Center UGM Yogyakarta, and the Laboratory of Anatomic Pathology at FK-KMK UGM.

Sampling

The study sample consisted of 36 male Wistar rats weighing 180 and 200 grams. These rats were randomly allocated into six groups: a negative control group (K-) comprising normal rats fed a standard diet, a positive control group (K+) consisting of metabolic syndrome rats fed a standard diet, and four treatment groups (P1, P2, P3, and P4) in which metabolic syndrome rats were fed diets substituted with growol at 25%, 50%, 75%, and 100%, respectively. The standard diet was formulated based on the AIN-93 standard. Metabolic syndrome was induced using a high-fat and high-fructose (DTLF) diet. Rats were classified as having metabolic syndrome if their lipid profiles met specific criteria: total cholesterol levels above 100 mg/dL, triglycerides above 70.79 mg/dL, HDL cholesterol below 39.02 mg/dL, and LDL cholesterol above 27.34 mg/dL.

Data collection

Data were collected at two points: before and after the four-week intervention period. The collected data included glucose levels and lipid profiles, encompassing total cholesterol, triglycerides, HDL cholesterol, and LDL cholesterol. Measurements were taken to evaluate the impact of the dietary interventions on these parameters. Ethical approval for the study was obtained from the Panti Rapih Hospital Ethics Committee, Yogyakarta (No. 096/SKEPK-KKE/VII/2024), ensuring adherence to ethical standards in handling experimental animals.

In this study, the induction of a high-fat and high-fructose diet (DTLF) was carried out for four weeks. Each rat received a DTLF intake of 15 g/day. The high-fat diet combined with high fructose was intended to accelerate metabolic syndrome conditions, as the effects of a high-fructose diet cause insulin resistance, which can also increase lipogenesis in the liver, subsequently leading to an increase in triglyceride storage in adipose tissue. This condition accelerates the onset of metabolic syndrome in rats fed a high-fat and high-fructose diet.

The type of feed given to the treated rats was controlled with a high-fat and high-fructose diet (DTLF) to accelerate metabolic syndrome conditions for four weeks. This was then followed by an intervention with growol feed at varying proportions of 25%, 50%, 75%, and 100% for another four weeks. The control rats were given AIN-93 feed until the end of the study. Several factors influenced the condition of the test animals in this study, including the type of feed provided DTLF was used to accelerate metabolic syndrome, which could affect lipid and glucose levels. The amount of feed given could also contribute to increased lipid and glucose levels.

Data analysis

The collected data underwent statistical analysis to determine the effects of the interventions. Normality tests were conducted using the Shapiro-Wilk test. Paired T-tests were employed to analyze within-group differences before and after the intervention. Between-group differences were assessed using one-way ANOVA for normally distributed data, with statistical significance set at $p < 0.05$, followed by

post-hoc multiple comparisons. For data that did not meet normality assumptions, the Kruskal-Wallis test was applied. This multi-faceted approach ensured the robust and accurate interpretation of the experimental results.

RESULTS AND DISCUSSIONS

Characteristics of research subject

Healthy rats' fasting blood glucose levels range between 50-109 mg/dL (26). After being fed a high-fat and high-fructose diet (DTLF), the experimental rats developed metabolic syndrome characterized by significant deviations from these norms. The mean fasting glucose level increased to 267.24 ± 3.29 mg/dL, indicating hyperglycemia. Hypertriglyceridemia was evident with a mean triglyceride level of 138.64 ± 2.69 mg/dL (28). Total cholesterol levels rose to a mean of 204.15 ± 2.00 mg/dL, HDL cholesterol levels decreased to 30.96 ± 1.33 mg/dL, and LDL cholesterol levels increased to 78.94 ± 1.86 mg/dL. These findings confirm that the high-fat and high-fructose diet effectively induced metabolic syndrome in the rats, mirroring similar results in studies conducted by (13) (14).

Table 1. Pre and post-test total cholesterol levels

Group	Pre (mg/dL)	Post (mg/dL)	Δ Average (%)	<i>p</i>
K-	84.62 \pm 1.37 ^a	85.98 \pm 2.02 ^a	\uparrow 1.61	0.016 ^{**}
K+	207.33 \pm 3.27 ^{cd}	209.84 \pm 3.42 ^f	\uparrow 1.21	0.005 [*]
P1	202.81 \pm 3.51 ^b	132.6 \pm 1.59 ^e	\downarrow 34.62	<0.001 ^{***}
P2	206.84 \pm 3.7 ^{cd}	108.49 \pm 3.43 ^d	\downarrow 47.55	<0.001 ^{***}
P3	208.55 \pm 3.64 ^d	101.23 \pm .71 ^c	\downarrow 51.46	<0.001 ^{***}
P4	204.15 \pm 2.00 ^{bc}	95.08 \pm 1.83 ^b	\downarrow 53.43	<0.001 ^{***}
<i>p</i>	<0.001 ^{***}	<0.001 ^{***}		

The data is the average value \pm standard deviation (n=6).

\downarrow or \uparrow increase or decrease average value *pretest* and *posttest*

Significant value between group treatment by ANOVA test and next test with *post hoc* Tukey HSD (*Honest Significant Difference*) test (*p-value*)

Significant value before and after treatment to each group using *Paired t-test* (*p-value*)

*=0.005, **=0,01 ***=0.001;

a negative control group (K-) comprising normal rats fed a standard diet, a positive control group (K+) consisting of metabolic syndrome rats fed a standard diet, and four treatment groups (P1, P2, P3, and P4) in which metabolic syndrome rats were fed diets substituted with growol at 25%, 50%, 75%, and 100%, respectively

The treatment group received DTLF for 4 weeks, followed by 4 weeks of intervention with Growol feed containing 25% Growol (P1), 50% Growol (P2), 75% Growol (P3), and 100%

Growol (P4). Intervention using a Growol-based diet significantly affected the lipid profile and glucose levels of the treated rat. Total cholesterol levels significantly decreased

across all treatment groups (P1–P4), with the most pronounced reduction of 53.43% observed in the P4 group, which received a 100% growol substitution diet (**Table 1**). The hypocholesterolemic effect was attributed to the synbiotics in growol, which reduce intestinal cholesterol absorption by promoting the production of short-chain fatty acids (SCFAs). SCFAs inhibit the activity of HMG-CoA

reductase, a key enzyme involved in cholesterol synthesis (15). HDL cholesterol levels also increased significantly in the treatment groups, with the most notable improvement in the P4 group, showing a 143.75% rise (**Table 2**). This increase can be explained by the role of synbiotics in enhancing reverse cholesterol transport (RCT), facilitated by the upregulation of the ABCA1 and ABCG1 genes (16).

Table 2. HDL and LDL cholesterol levels pre and post-test

Group	Pre (mg/dL)	Post (mg/dL)	Δ Average (%)	p
HDL				
K-	82.21±1.33 ^c	81.04±1.76 ^f	↓ 1.42	0.007**
K+	30.49±1.05 ^{ab}	28.87±1.96 ^a	↓ 5.29	0.010**
P1	30.96±1.61 ^{ab}	58.24±1.92 ^b	↑ 88.12	<0.001***
P2	29.89±1.68 ^a	64.93±1.61 ^c	↑ 117.21	<0.001***
P3	32.15±2.36 ^b	68.03±1.39 ^d	↑ 111.62	<0.001***
P4	30.96±1.33 ^{ab}	75.46±1.39 ^e	↑ 143.75	<0.001***
p	<0.001***	<0.001***		
LDL				
K-	20.58±1.35 ^a	22.43±1.37 ^a	↑ 8.98	<0.001***
K+	77.5±1.56 ^b	78.8±1.43 ^f	↑ 1.68	<0.001***
P1	77.5±1.56 ^b	48.78±2.31 ^e	↓ 37.06	<0.001***
P2	77.74±2.02 ^b	37.99±1.59 ^d	↓ 51.13	<0.001***
P3	78.22±1.56 ^b	33.58±1.90 ^c	↓ 57.07	<0.001***
P4	78.94±1.86 ^b	29.78±1.38 ^b	↓ 62.28	<0.001***
p	<0.001***	<0.001***		

The data is the average value ± standard deviation (n=6).

↓ or ↑ increase or decrease average value *pretest* and *posttest*

Significant value between group treatment by ANOVA test and next test with *post hoc* Tukey HSD (*Honest Significant Difference*) test (*p-value*)

Significant value before and after treatment to each group using *Paired t-test* (*p-value*)

*=0.005, **=0,01 ***=0.001;

a negative control group (K-) comprising normal rats fed a standard diet, a positive control group (K+) consisting of metabolic syndrome rats fed a standard diet, and four treatment groups (P1, P2, P3, and P4) in which metabolic syndrome rats were fed diets substituted with growol at 25%, 50%, 75%, and 100%, respectively

Conversely, LDL cholesterol levels showed a significant decrease, with the P4 group exhibiting the greatest reduction of 62.28% (**Table 2**). This decrease is associated with the role of SCFAs in modulating transcription factors such as Sterol Regulatory Element-Binding Protein (SREBP). SREBP is a hepatic transcription factor that activates genes required for fatty acid synthesis and indirectly regulates various processes, including cholesterol biosynthesis, fatty acid uptake, and fatty acid biosynthesis (24). SREBP1 is one of the most important members of the

SREBP family and, when overexpressed, can increase fatty acid secretion and lead to hepatic steatosis. In this study, SREBP1 plays a role in regulating lipid metabolism by controlling lipogenesis. It is crucial in modulating the expression of lipogenic genes involved in fatty acid and cholesterol synthesis. Additionally, SREBP1 regulates the expression of genes encoding enzymes responsible for fatty acid (FA) and triglyceride (TG) synthesis (25).

The mechanism by which Growol lowers LDL cholesterol involves probiotic fermentation of

inulin to produce short-chain fatty acids (SCFAs), including propionic acid. Propionic acid lowers cholesterol synthesis in the liver by inhibiting the activity of HMG-CoA reductase. SCFAs, particularly acetate, propionate, and butyrate, are primarily produced through the anaerobic fermentation of gut microbes. They play a crucial role in regulating energy metabolism and supply while maintaining intestinal homeostasis. In lipid metabolism, SCFAs enhance fatty acid oxidation, inhibit fatty acid synthesis, increase heat production, and reduce fat storage. Furthermore, SCFAs contribute to glucose metabolism regulation, mainly by maintaining blood glucose stability. The secretion of insulin and glucagon is essential for regulating glucose metabolism (23).

The treatment also markedly impacted triglyceride levels, which decreased significantly in all treatment groups, with the P4 group demonstrating the most significant reduction of 39.4% (Table 3).

The reduction in triglyceride production leads to a decrease in LDL secretion. The decline in LDL synthesis and secretion in the liver impacts the reduction of serum LDL cholesterol levels, as LDL is converted into IDL (Intermediate Density Lipoprotein) after triglyceride breakdown. LDL can be directly absorbed by the liver through LDL receptors or converted into LDL. The consumption of growol, a probiotic-rich feed containing lactic acid bacteria, can help lower blood cholesterol levels.

Table 3. Pre and post-test triglyceride levels

Group	Pre (mg/dL)	Post (mg/dL)	Δ Average (%)	p
K-	73.36±2.10 ^a	74.43±2.02 ^a	↑ 1.46	0.001 ^{**}
K+	134.49±3.83 ^b	136.53±3.84 ^e	↑ 1.52	0.002 ^{**}
P1	135.76±2.75 ^{bcd}	104.11±2.12 ^d	↓ 23.31	<0.001 ^{***}
P2	135.07±3.08 ^{bc}	94.52±1.79 ^c	↓ 30.02	<0.001 ^{***}
P3	138.29±1.83 ^{cd}	93.04±2.19 ^c	↓ 32.72	<0.001 ^{***}
P4	138.64±2.69 ^d	84.02±2.32 ^b	↓ 39.40	<0.001 ^{***}
p	<0.001 ^{***}	<0.001 ^{***}		

The data is the average value ± standard deviation (n=6).

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Significant value between group treatment by ANOVA test and next test with *post hoc* Tukey HSD (*Honest Significant Difference*) test (*p-value*)

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Similarly, blood glucose levels decreased significantly in all groups, with the P4 group showing the most remarkable reduction of 68.74% (Table 4). The synbiotics in growol enhance SCFA production, which increases insulin sensitivity and reduces insulin resistance (18). SCFAs also activate the AMP-activated protein kinase (AMPK) pathway, reducing gluconeogenesis and stimulating the release of the GLP-1 hormone, which promotes insulin secretion and inhibits glucagon release (19).

Lactobacillus casei (the fermenting bacteria in Growol) plays a role in multiple mechanisms for lowering blood sugar levels. A study by (20)

showed improved glycemic control in patients with type 2 diabetes mellitus after supplementation with *Lactobacillus casei*, which involved the biomarkers Sirtuin 1 (SIRT1) and Fetuin-A. This supplementation increased SIRT1, which activates energy metabolism, while Fetuin-A is a protein expressed in adipocytes and hepatocytes that plays a role in insulin signaling.

In the study of giving yogurt to rabbits, it was found that fermented yogurt using lactic acid bacteria such as *Lactobacillus casei* had high antioxidant activity. *Lactobacillus casei* bacteria are also probiotic bacteria found in the human body that can reduce levels of total cholesterol

Table 4. Pre and post-test blood glucose levels

Group	Pre (mg/dL)	Post (mg/dL)	Δ Average (%)	p
K-	68.46±1.14	69.76±1.64	↑ 1.90	0.016**
K+	263.83±5.28	265.48±5.01	↑ 0.63	0.003*
P1	265.04±4.94	148.63±2.72	↓ 43.92	<0.001***
P2	265.17±4.2	99.87±1.40	↓ 62.34	<0.001***
P3	265.68±5.10	90.27±1.8	↓ 66.03	<0.001***
p	<0.001***	<0.001***		

The data is the average value ± standard deviation (n=6).

↓ or ↑ increase or decrease average value *pretest* and *posttest*

Significant value between group treatment by ANOVA test and next test with *post hoc* Tukey HSD (*Honest Significant Difference*) test (*p-value*)

Significant value before and after treatment to each group using *Paired t-test* (*p-value*)

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a negative control group (K-) comprising normal rats fed a standard diet, a positive control group (K+) consisting of metabolic syndrome rats fed a standard diet, and four treatment groups (P1, P2, P3, and P4) in which metabolic syndrome rats were fed diets substituted with growol at 25%, 50%, 75%, and 100%, respectively

LDL, and triglycerides (29). Lactic acid bacteria found in yogurt have the potential to reduce total cholesterol, LDL and triglyceride levels because they produce organic acids such as glucuronic acid, propionic acid, folic acid and lactic acid which can act as agents to lower total cholesterol, LDL, and triglycerides (29). The mechanisms underlying these beneficial effects can be attributed to the synbiotic properties of growol, which contains probiotics and prebiotics that work synergistically to improve lipid and glucose metabolism. The lactic acid bacteria in growol degrade cholesterol into coprostanol, which cannot be absorbed by the intestines and is excreted in feces. Additionally, the bile salt hydrolase (BSH) enzymes in probiotics facilitate bile salt deconjugation, reducing bile acid reabsorption and compelling the body to utilize cholesterol for bile synthesis, thereby lowering blood cholesterol levels (21). The production of SCFAs during the fermentation of prebiotics further enhances glucose and lipid metabolism by activating the AMPK pathway and increasing GLUT-4 expression, which improves insulin sensitivity and glucose uptake (22).

CONCLUSION AND RECOMMENDATION

In conclusion, growol-based diets significantly improved lipid profiles and glucose levels in syndrome metabolic rats, with the most pronounced effects observed in the P4 group, which received a 100% growol substitution diet. These findings highlight the potential of growol as

a functional food with synbiotic properties for managing metabolic syndrome.

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