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Original Research Article

Effects of Tempeh Fermentation with *Rhizopus oligosporus* and *Lactobacillus rhamnosus* GG on Body Weight, Lee Index, hs-CRP, and Lipid Profile of Obese Rats

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Abstract

Background: Tempeh is a fermented soybean containing isoflavones that shows good benefits against obesity. Co-fermentation of tempeh using *Lactobacillus rhamnosus* GG could increase the bioavailability of isoflavones.

Objective: This study aimed to determine the effect of co-fermented tempeh using *Lactobacillus rhamnosus* GG (tLGG) on body weight (B.W.), Lee Index, high sensitivity C Reactive Protein (hs-CRP), and lipid profile of obese rats.

Methods: A total of 30 Male Sprague Dawley obese rats were divided into five groups and were given different diets for four weeks: the high-fat high sucrose (HFHS) diet (negative control group), HFHS diet + orlistat (positive control group), HFHS diet + standard tempeh fermented with *Rhizopus oligosporus* (tS group), HFHS diet + 60 mg/kg B.W./day tLGG (low tLGG group), and HFHS diet + 120 mg/kg B.W./day tLGG (high tLGG group). Body weight and Lee Index were measured before and after the intervention; meanwhile, hs-CRP, total cholesterol (TC), triglyceride (TG), HDL, and LDL were measured after the interventions. In addition, the total flavonoid and genistein of tempeh were also analyzed.

Results: Compared to the negative control, all diet interventions on obese rats prevented further increase in Lee index level and were able to deplete it significantly. Importantly tLGG group demonstrated a better Lee index decrease compare to tS group; yet, there was no significant weight gain differences. Additionally, the tLGG group also exhibited lower hs-CRP levels, TG, TC, and LDL and higher HDL ($p < 0.001$). Finally, we confirmed the total flavonoid and genistein levels were higher in co-fermented tempeh than in standard one.

Conclusion: co-fermented tempeh tLGG showed a beneficial effect on obese rats by preventing weight gain, Lee index reduction, lipid profile improvement and systemic inflammation due to its high flavonoid and genistein content

Keywords: Tempeh; Lactic acid bacteria; High fat high sucrose diet; Obesity

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INTRODUCTION

The prevalence of obesity has increased more than four times since 1975 along with increasing high-fat high-sucrose diet consumption.¹ Obesity is defined as excessive fat accumulation in the body (Body Mass Index, BMI ≥ 30), which can increase the risk of metabolic syndrome; among which the most common are dyslipidemia, cardiovascular disease, and insulin resistance.²⁻³ Excessive fat accumulation in the body

generate a high level of free fatty acid; thus, obese people have high triglyceride level. It is also associated with chronic inflammation of white adipose tissue. Adipocytes secrete pro-inflammatory cytokines and induce the production of C-reactive protein.

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Together, these processes could lead to impaired immune systems.³ Several drugs for obesity have been developed. One of the most commonly used is orlistat, yet the existing drugs are costly and have side effects such as vitamin deficiency and gastrointestinal and kidney disorders.⁴ Improving an active lifestyle and a healthy diet can be an alternative strategy to overcome obesity.

Food containing bioactive compounds that are beneficial for obesity has been studied extensively. Tempeh is a traditional food made from the fermentation of soybean and *Rhizopus spp.* Soybean contains bioactive substances, such as peptides, phytosterols, soy saponin, and isoflavones.⁵ Isoflavones are the most abundant substances contained in soybean. Isoflavones, such as genistein and daidzein, could reduce lipid accumulation in the early stages of adipogenesis. They act upon the attenuation of the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and increase brown adipose activity through the induction of uncoupling protein 1 (UCP-1); thus it could inhibit body weight gains in obesity.⁶ Isoflavones could improve the lipid profile and inflammatory status in obesity through several mechanisms. They can decrease the expression of stearoyl coenzyme A desaturase 1 (SCD1), peroxisome proliferator-activated receptors (PPAR) α and γ protein, adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK), tumor necrosis factor (TNF)- α , interleukin (IL)-1, and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) in white adipose tissue and liver.⁶ Apart from their benefits, intestinal epithelium hardly absorbs Isoflavones. Yet, several studies have demonstrated that fermentation can increase the bioavailability of soy isoflavones.⁶

Recently, lactic acid bacteria (LAB) have been used to optimize soy isoflavones' bioavailability while inhibiting pathogens and mycotoxins.⁷ *Lactobacillus rhamnosus* G.G. shows the highest β -glucosidase activity compared to other *Lactobacillus* bacteria, which could increase the hydrolysis of isoflavone glucosides into more optimal aglycones.⁸ Several studies have shown the benefits of *Lactobacillus rhamnosus* G.G. against obesity. *L. rhamnosus* demonstrated the ability to suppress several lipogenic and pro-inflammatory genes, normalizing intestinal microbiota dysbiosis, and improving leptin response, triglyceride, and cholesterol levels in blood.⁹⁻¹¹ Research using *Lactobacillus rhamnosus* G.G. in whole soybean fermentation showed that it could grow stably up to 10 hrs of fermentation at 37°C. This bacterium continued to show its metabolic activity even during storage.¹² These bacteria are relatively stable and can survive up to a temperature of 54°C.¹³ Importantly, *Lactobacillus rhamnosus* G.G., in live or dead state, still shows the same benefits in reducing pro-inflammatory mediators and increasing anti-inflammatory mediators.¹⁴

Tempeh is a fermented soybean product using *Rhizopus oligosporus* as a starter that is widely consumed daily in Asia, especially in Indonesia. This fungus can grow fast at 30-42°C so tempeh can be made at room temperature.¹⁵ Tempeh is rich in nutrients, can decrease phytic acid (nutrient absorption inhibitor), metabolizing 5-hydroxy isoflavones (genistein, biochanin A) and 5-deoxyisoflavones (daidzein,

formononetin), and producing α -galactosidase.¹⁵ Despite the improved nutritional value of co-fermented tempeh, the effect of co-fermentation of tempeh using *Rhizopus oligosporus* and *Lactobacillus rhamnosus* G.G. on obese rats has not been demonstrated yet. Therefore, this study aimed to analyze the effect of co-fermentation tempeh using *Lactobacillus rhamnosus* G.G. on diet-induced obesity rats.

MATERIALS AND METHODS

Research design

The research design was a true experiment with a randomized pre-test post-test for body weight, body weight gains, and Lee Index data, while post-test-only control group design was used for lipid profile and systemic inflammation measurements. Sample preparation was conducted in the Universitas Diponegoro Integrated Laboratory. Experimental animal treatment and biomarker testing were conducted in the Experimental Animal Laboratory at Gadjah Mada University. Tests for total flavonoid and genistein were conducted at Satyawacana Christian University.

Sample preparation

Lactobacillus rhamnosus were obtained from the Center for Food and Nutrition Studies, Gadjah Mada University. The soybeans used were local organic yellow soybeans from Sleman, Yogyakarta, obtained through the Lingkar Organik. *Rhizopus oligosporus* used were Ragi Raprima. The procedure for making probiotic fermented tempeh was based on Nout and Kiers.¹⁵ Soybeans were washed and soaked for 90 min, and the outer membranes were removed. The beans were then cooked in water at 100°C for 30 min. The hot water was then removed and cooled at room temperature to 25°C. Afterward, 10⁵ CFU/g of *L. rhamnosus* G.G. and 10⁴ CFU/g of *R. oligosporus* were inoculated at 30°C and mixed with beans homogeneously into polythene bags, followed by incubation for 48 hrs at 25°C. Determination of total bacterial levels of *L. rhamnosus* G.G. based on research by Petrušáková and Valík that showed 10⁵ CFU/g *L. rhamnosus* G.G. could grow well on soybeans.¹² Standard tempeh preparation is the same as the probiotic fermented tempeh preparation procedure without adding *Lactobacillus rhamnosus* G.G. The tempeh samples obtained were then cut into 0.5 cm and freeze-dried for 48 hrs. The samples were ground with a grinder and stored until reuse at -20°C.

Animal and diets

Male Sprague Dawley (n=36, 200 to 215-gram, age eight weeks) was obtained from the Experimental Animal Laboratory at Gadjah Mada University in a healthy condition. The selection of male rats was based on the sensitivity of the rats to a high-fat high-sucrose diet and metabolic syndrome and to eliminate the variation of metabolic outcomes. Rats were housed in an individual cage with a temperature of 25±2°C, a humidity of 55-60%, light/dark cycle of 12 hrs. All groups were adapted for one week and given a standard diet of 10 gr/day with free access to water. After acclimatization, rats were divided into six groups. One group was designated as normal control and was fed the standard diet. The rest of the groups were orally administered high fat and high

sucrose diet (HFHS diet) for two weeks to induce obesity. The composition of the HFHS diet was based on AIN-93M formulation with some modifications to provide a high-fat and high-sucrose diet (Table 1). A total of 30 g/day HFHS diet was administered orally to rats at three meal time points. After obesity was confirmed (Lee Index > 300), rats were divided into five groups and fed different diets for four weeks. The negative control: HFHS diet; Positive control: HFHS + 120 mg/kg B.W./day of Orlistat; tS group: HFHS + 60 mg/kg B.W./day standard tempeh; low dose tLGG: HFHS + 60 mg/kg B.W./day tLGG; high dose tLGG: HFHS + 120 mg/kg B.W./day tLGG. M Body weight was measured once every week using a digital analytical scale. Body length and lee index were measured before and after intervention (Figure 1). The body length of the rats was measured from nose to anus or vice versa using a standard measuring tape with an accuracy of 0.01 cm. Lee index was obtained from the following calculation by Lee formulation¹⁶:

$$\text{Lee index} = \frac{\sqrt[3]{\text{body weight in gram}}}{\text{body length in cm}} \times 1000.$$

Lee index > 300 indicates that the rats are obese.¹⁶ Before weighing and measuring the body length of the rats, the rats were anesthetized using ketamine 75–100 mg/kg + xylazine 5–10 mg/kg. On the last day of the experiment, the blood was collected through the rat's orbital eye and centrifuged at 3500 rpm for five min to obtain blood plasma. The blood was then stored at -20°C until used for further analysis.

Inflammatory marker

Hs-CRP was analyzed using the ELISA method (Enzyme-linked immunosorbent assay). ELISA kits were obtained from Elabscience Biotechnology Inc., and the assay was performed according to the protocols provided by the commercial kits.

Table 1. Experimental diet composition (g/kg)

Ingredient	AIN 93M (standard diet)	HFHS
Cornstarch	465.692	365.692
Casein	200	200
L-Cystine	2	2
Sucrose	100	300
Cellulose (CMC)	50	50
Soybean oil	50	50
Cholin Chloride	2	2
Tert-Butylhydroquinone (TBHQ)	0,014	0,056
Lard	100	200
Mineral mix AIN-93-MX	35	35
Vitamin mix AIN-93-VX	10	10

HFHS (High-fat High-Sucrose); AIN 93M (American Institute of Nutrition 93 Maintenance)

Lipid profile measurements

Total cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were analyzed using CHOD/PAP calorimetry method (Cholesterol Oxidase Phenol Amino Phenazone). Triglycerides were analyzed using GPO-PAP (Glycerol-3-Phosphatase Oxidase-Phenol Amino Phenazone). Kits for determining lipid profile were obtained from Diagnostic System GmbH & Co Kit or DiaSys of 500 nm. The assay was performed according to the protocols provided, and absorbance was measured by spectrophotometer (Merck-Microlab) at a wavelength of 500 nm.

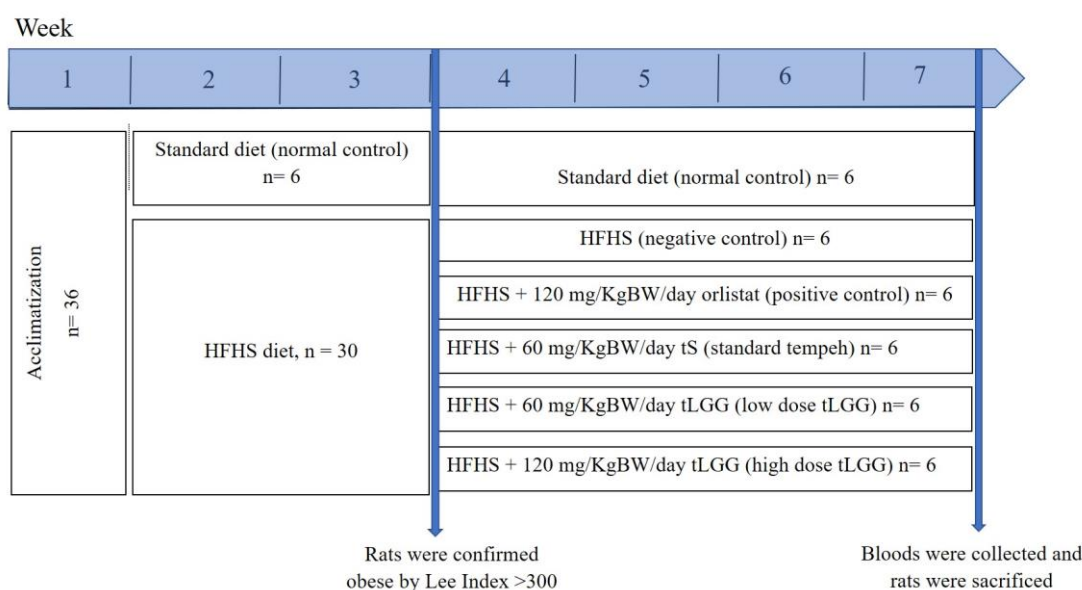


Figure 1. Schematic of the experimental timeline. HFHS (High fat high sucrose) diet, tS (standard tempeh), tLGG (co-fermented tempeh with *L. rhamnosus* GG).

Tempeh extraction

One gram of tempeh was mixed with 25 mL of 60% ethanol v/v in a glass container. Extraction was carried out using an ultrasonicator at 40°C for 15 min. The extract solution was filtered, and the residue was re-extracted with 25 mL 60% ethanol v/v. These stages were repeated three times. The obtained filtrate was combined into a 100 mL volumetric flask, and 60% v/v ethanol solvent was added until it met the measuring line, and 10 mg/mL tempeh extract was obtained.

standard was obtained from HWI pharma service GmbH Germany. One mL of tempeh extract was pipetted, added to four mL of distilled water, and homogenized with a vortex mixer. Afterward, 0.3 mL NaNO₃ of 0.5%, 0.3 mL of 10% AlCl₃, and 2 ml of 1 M NaOH were added consecutively and mixed for another five min. Distilled water was added to the mixture so that the end volume was 10 mL and left at room temperature for 15 min before measurement. The absorbance was determined using the UV-Vis spectrophotometry method at a maximum wavelength of 510 nm (the result of running the quercetin standard). Conversion of absorbance values into total

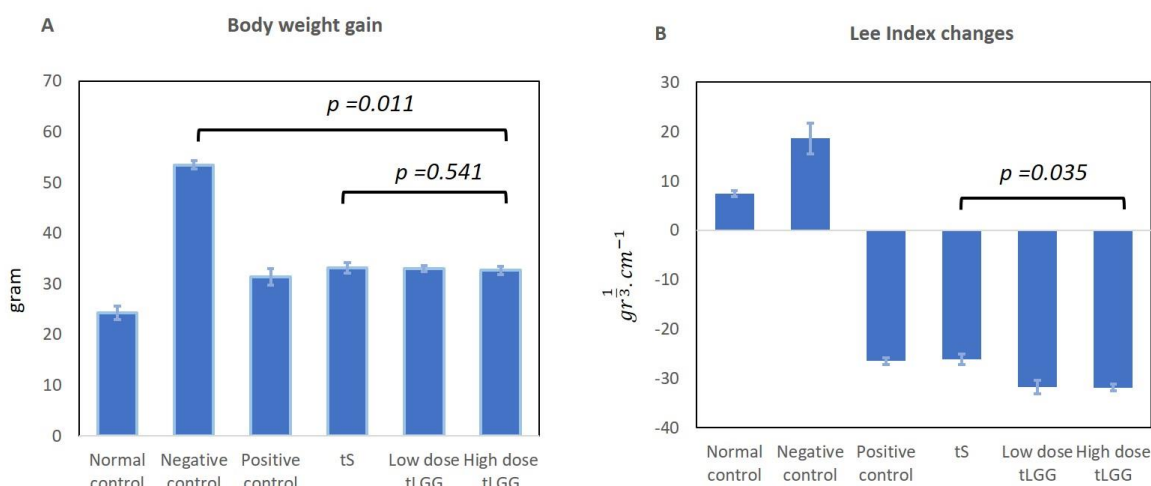


Figure 2. Effect of different diet interventions on body weight gain and Lee index changes in obese rat. Values were presented as mean ± standard deviation (SD). Normal control (standard diet); Negative control (HFHS diet only); Positive control (HFHS diet+120 mg/kg BW/day orlistat); tS (HFHS diet + 60 mg/kg BW/day standard tempeh); Low dose tLGG (HFHS diet +60 mg/kg BW/day probiotic fermented tempeh); High dose tLGG (HFHS+120 mg/kg BW/day probiotic fermented tempeh).

Table 2. Changes in weight and Lee index of obese rats in different groups which were fed with various diets for four weeks.

Group	Initial BW (gram)	Week 1 (gram)	Week 2 (gram)	Week 3 (gram)	Week 4 / Final BW(gram)	Initial Lee index	Lee index at week 4
Normal control	226,00±3,16 ^a	232,00±3,58 ^a	238,50±3,83 ^a	244,17±3,49 ^a	250,33±3,44 ^a	281,73±2,70 ^a	289,20±2,79 ^a
Negative control	239,17±2,48 ^b	251,67±2,58 ^b	264,83±2,93 ^b	279,50±1,87 ^b	292,67±2,16 ^b	328,75±4,98 ^{bd}	347,38±2,22 ^b
Positive control	238,83±4,54 ^b	247,67±4,59 ^b	254,83±4,07 ^c	262,17±4,62 ^c	270,17±5,12 ^{ce}	332,99±5,00 ^{bc}	306,51±4,49 ^c
tS	244,00±3,16 ^b	253,33±3,08 ^b	260,67±2,74 ^{bc}	269,17±3,76 ^d	277,17±3,71 ^d	334,07±3,48 ^b	307,945±2,60 ^c
Low dose tLGG	244,67±3,56 ^b	253,83±4,26 ^b	261,67±3,50 ^{bd}	269,67±3,88 ^d	277,67±3,56 ^d	334,87±5,06 ^b	303,14±3,76 ^c
High dose tLGG	241,17±2,79 ^b	250,50±3,27 ^b	257,50±2,74 ^{cd}	264,83±3,19 ^{cd}	273,83±3,19 ^{de}	327,70±2,39 ^{acd}	295,89±1,81 ^d
	<0,001*	<0,001*	<0,001*	<0,001*	<0,001*	<0,001**	<0,001*

*One-way ANOVA ** Kruskal wallis. All parametric data was measured using one-way ANOVA test and continued using post hoc Bonferroni test for homogen data or Games Howell test for non homogen data. Kruskal wallis was used for non-parametric data and continued using post hoc Dunn test. Means with different superscripts (a,b,c,d) are significantly different at p-value <0.05 between groups while those with similar letters are non-significant. Normal control (standard diet); Negative control (HFHS); Positive control (HFHS+120 mg/kg BW/day orlistat); tS (HFHS + 60 mg/kg BW/day standard tempeh); Low dose tLGG (HFHS+60 mg/kg BW/day probiotic fermented tempeh); High dose tLGG (HFHS+120 mg/kg BW/day probiotic fermented tempeh).

Total flavonoids assay

Total flavonoid content was determined using a PG T60 spectrophotometer. Standard reagents and markers were obtained from E-Merck Germany. The quercetin

flavonoid concentrations based on the quercetin calibration curve, i.e., $y = 0.00060x + 0.00070$. Calibration curves were made based on a series of quercetin concentrations of 20, 40, 60, 80, and 100 µg/ml.

Total genistein assay

Total genistein was measured using isocratic elution reversed-phase HPLC (High-Performance Liquid Chromatography) method with HPLC instrument Knauer, GMBH Germany. All standard reagents and markers were obtained from E-Merck, Germany. Ten mg/mL of tempeh extract was filtered using a 0.45 µm Whatman micro membrane filter prior to HPLC analysis. Twenty µL of the tempeh filtrate was injected using a microinjector and separated in the column based on the difference in polarity at a temperature of 30°C. Components were detected at a wavelength of 254 nm and displayed as peaks that appeared based on the retention time. The column phase (stationary) was Eurosphere C-18 (250 × 4.6 mm, 5µm) GJ 95. The mobile phase used 0.1% v/v acetic acid: methanol in a ratio of 52:48 v/v, and the pH was adjusted to pH 3.

Statistical analysis

SPSS 27 were used for statistical analysis. All data are presented as means ± S.D. (Standard Deviation). Data were analyzed using repeated measures ANOVA for body weight variables. Weight changes were analyzed using a t-test. Other variables used one-way ANOVA. Next, Post Hoc Bonferroni and Games Howell were used to compare variables. Post Hoc Bonferroni was used for homogeneous data; otherwise, Games Howell was applied. Means with different superscripts (a,b,c,d) significantly differ at *p*-value <0.05.

Ethical consideration

This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Diponegoro, Semarang, Indonesia No.125/EC/H/FK-UNDIP/XI/2022.

RESULTS

Body Weight and Lee Index

In this study, the obese rats (Lee index >300) were intervened with different diets for four weeks. Table 2

shows the body weight of all groups during the diet intervention. By the end of the intervention, obese rats receiving no diet intervention shows the highest body weight among other groups. On the other hand, rats receiving co-fermented tempeh (tLGG group) showed lower body weight gain against negative control (*p*=0.011). Furthermore, the weight gain in the high-dose tLGG group was comparable with the positive control group (*p*> 0.001); yet no significant difference was observed between tLGG against the tS group (*p*=0.054) (Figure 2A). Importantly, the effect of diet intervention using co-fermented tempeh was demonstrated in Lee index reduction. Figure 2B showed that intervention of high dose tLGG significantly reduced the lee index compare to intervention with the standard tempeh (tS group) (*p*=0.035).

Inflammatory marker

After four weeks of intervention, rats administered tLGG showed lower hs-CRP compared to the negative control, positive control, and tS group (*p*< 0.001) (Table 3). Rats in the negative control group demonstrated the highest level of hs-CRP, while rats in the normal control group showed the lowest level of hs-CRP among all groups (*p*<0,001) (Table 3). hs-CRP levels of rats in the positive control group, standard tempeh (tS), or tLGG tended to be lower than those of the negative control group (*p*<0,001) (Table 3). Rats administered a high-dose probiotic fermented tempeh (tLGG) group had the lowest Lee Index at week four among all the HFHS groups (*p* < 0,001) (Table 3).

Lipid profile

Rats administered tLGG for four weeks showed lower triglyceride, total cholesterol, and LDL levels but a higher HDL level compared to the negative control, positive control, and tS group (*p*> 0,001) (Table 3). The lipid profile of rats administered orlistat group (positive control), standard tempeh (tS), or probiotic fermented tempeh (tLGG) tended to be better than those of the

Table 3. Serum biochemical parameters in rats fed a high-fat diet for 4 weeks and administered standard tempeh and different dose of *Lactobacillus rhamnosus* co-fermented tempeh orally during the last 4 weeks.

Group	hsCRP (pg/ml)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
Normal control	2,99±0,125 ^a	66,93±2,94 ^a	86,89±3,14 ^a	27,24±1,69 ^a	83,61±2,59 ^a
Negative control	17,45±0,84 ^b	129,12±7,05 ^b	196,81±3,28 ^b	87,93±1,34 ^b	21,00±2,02 ^b
Positive control	4,24±0,42 ^c	77,87±3,03 ^c	104,66±3,88 ^{cd}	36,44±2,92 ^c	69,24±2,32 ^c
tS	7,78±0,44 ^d	98,42±3,77 ^d	118,75±3,14 ^e	51,73±5,47 ^d	63,55±2,59 ^d
Low dose tLGG	5,07±0,24 ^e	81,29±4,89 ^c	100,49±3,56 ^{cd}	37,75±4,34 ^c	66,94±1,76 ^{cd}
High dose tLGG	3,98±0,31 ^c	74,57±2,38 ^{ac}	95,22±2,01 ^d	30,59±2,16 ^{ac}	74,79±2,12 ^e
	<0,001*	<0,001*	<0,001*	<0,001*	<0,001*

* One Way ANOVA test and continued using post hoc Bonferroni test for homogen data or Games Howell test for non homogen data.

Means with different superscripts (a,b,c,d) are significantly different at *p*-value <0.05 between groups while those with similar letters are non-significant. Normal control (standard diet); Negative control (HFHS); Positive control (HFHS+120 mg/kg BW/day orlistat); tS (HFHS + 60 mg/kg BW/day standard tempeh); Low dose tLGG (HFHS+60 mg/kg BW/day probiotic fermented tempeh); High dose tLGG (HFHS+120 mg/kg BW/day probiotic fermented tempeh).

negative control group ($p < 0,001$) (Table 3). Rats administered a high-dose probiotic fermented tempeh (tLGG) group showed the best improvement in all lipid profile parameters among all the HFHS groups ($p < 0,001$) (Table 3).

Table 4. Total Isoflavones and Genistein in standard and *Lactobacillus rhamnosus* co-fermented tempeh

Group	Total flavonoid	Total genistein
	%w/w	%w/w
tS	0,095	2,63
tLGG	0,137	3,88

tS: standard tempeh; tLGG: co-fermented tempeh with *L. rhamnosus* GG: w/w: weight/weight

Total flavonoid and genistein

The results of the analysis showed that probiotic fermented tempeh (tLGG) had higher levels of total flavonoid (0.137% w/w) and genistein (3.88% w/w) than standard tempeh (tS) (Table 4).

DISCUSSION

Obesity is characterized by disorder of the lipid profile due to fat accumulation, an increase in inflammatory status, higher body weight, and body mass index (BMI) ≥ 30 . In rats, the Lee index was used instead of BMI. Lee Index >300 shows that rats are obese. The goal of obesity therapy is to lose weight and improve lipid profile and inflammatory status, including high sensitivity C Reactive Protein as a sensitive inflammatory marker for obesity. The anti-obesity effect of soy isoflavones has been widely documented; hence soy isoflavones have low bioavailability.⁵⁻⁶ The bioavailability of soy isoflavones can be increased through the fermentation process. Through fermentation, isoflavone glycosides are hydrolyzed into aglycones, which more easily absorbed in the intestine. This study demonstrated for the first time that during four weeks of treatment, probiotics co-fermented Tempeh with *Lactobacillus rhamnosus* G.G. could improve the obesity parameters (body weight, Lee index, hs-CRP, and lipid profile) of HFHS-induced obesity rats. Probiotic fermented tempeh also increased total flavonoid and genistein compared to standard tempeh.

In this study, obesity is induced by the HFHS diet for two weeks. During this period, rats showed body weight gain and obesity was confirmed by Lee Index >300 . Increased body weight is related to increased fat accumulation in adipocytes due to chronic HFHS administration. Chronic administration of HFHS increases fat storage in adipocytes, leading to adipocyte proliferation and enlargement.¹⁷ In addition, HFHS diet increases insulin secretion, suppresses lipolysis, induces free fatty acids release from adipocytes, and directs fat to be stored.¹⁸ Administration of high dose tLGG resulted in better Lee Index reduction in rats compared to negative control and tS group. Improvement in Lee Index depletion of tLGG group most probably due to bioavailability increase of isoflavone in co-fermented tempeh (tLGG). Increasing the bioavailability of isoflavones enhances the benefits of isoflavones in reducing lipid accumulation in the early stages of

adipogenesis via inhibition and attenuation of the PI3K/Akt signaling pathway.⁶ This effect was also associated with *L. rhamnosus* administration to tempeh. Previous studies have shown that *L. rhamnosus* can reduce lipid accumulation and inhibit adipocyte differentiation by downregulating adipogenic transcription factors in white adipocytes 3T3-L1 and browning white adipose tissue through the expression of the PPAR gene.¹⁹⁻²⁰ *Lactobacillus rhamnosus* also improves leptin response by decreasing leptin resistance in obesity by reducing the ratio of bacterial phyla in the crypt, decreasing the proportion of proteobacteria and Firmicutes/Bacteroidetes ratio.²¹

All groups treated with HFHS diet has higher hs-CRP, triglyceride, total cholesterol, and LDL and decreased HDL compare to normal control group. This condition is related to increased lipotoxicity caused by chronic HFHS administration. Lipotoxicity triggers adipocytes to increase macrophage secretion, which stimulates an increase in the inhibitory enzyme nuclear factor kappa beta Kinase B (NF- κ B) and the release of pro-inflammatory cytokines from adipocytes such as adipocyte TNF α , IL1, and acute phase proteins.^{17,22} These pro-inflammatory cytokines cause high levels of CRP in the blood, which can cause cell damage.²³ Conversely, in obese conditions, cholesterol absorption is mediated by NPC1L1.²⁴ Lipotoxicity due to HFHS inhibiting lipoprotein lipase activity, which increases triglyceride levels in the blood.²² This HFHS-induced lipotoxicity is indicated by a decrease in HDL levels which is associated with an increase in HDL absorption by adipocytes and an increase in apolipoprotein A-I catabolism in HDL particles.²⁵ An increase in free fatty acids in adipocytes also increases the absorption and accumulation of fat in the liver, which increases VLDL and its release into the bloodstream.²¹ Nonetheless, treatment of high-dose tLGG showed significant benefits in improving the inflammatory status and lipid profile in rats given HFHS. Among all HFHS treatment groups, the high-dose tLGG group showed the lowest value of hs-CRP, triglycerides, total cholesterol, and LDL, while the highest value of HDL. These results are in accordance with Huang et al., which showed that combining *Rhizopus oligosporus* with *Lactobacillus* bacteria can achieve a synergistic effect in improving the lipid profile.²⁶ The low value of hs-CRP in the high dose tLGG was associated with the ability of aglycone isoflavones to reduce the expression of proteins PPAR α , PPAR γ , adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK), malonaldehyde (MDA), TNF- α , IL-1, NFIL3, and NF- κ B in white adipose tissue and liver.⁶ This benefit was also derived from the ability of *L. rhamnosus* to decrease LPS levels and the inflammatory parameters PPAR γ , TNF- α , and IL-1 in serum and mRNA, forming linoleic acid and improving several biomarkers of obesity through the acetyl Co-A and fatty acid synthase pathways.²⁷

CONCLUSION

co-fermented tempeh tLGG diet showed significantly higher Lee Index depletion compared to negative control and tS groups. Moreover, the high dose tLGG diet showed lower the hs-CRP, triglyceride, total cholesterol, LDL, and higher HDL compared to negative control and

tS group. Diet containing the co-fermentation tempeh with *L.rhamnosus* GG has better effect on obese rats; most likely due to its higher flavonoid and genistein content.

Research Limitations

Random sampling analysis of rats' total genistein from time to time and fat mass needs to be done for more specific data. Optimization co-fermented tempeh to increase isoflavones needs to be done to get more optimal benefits from the product.

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