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Hypolipidemic Effects of Modified Edamame Tempeh Flour on Lipid Profile Levels in Dyslipidemia Rats

Efek Hipolipidemia Tepung Tempe Edamame Modifikasi terhadap Profil Lipid Tikus Model Dislipidemia

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Dyslipidemia, Edamame, Fermentation, Saccharomyces cerevisiae, Tempeh

ABSTRACT

Background: Dyslipidemia is a lipid metabolism disorder that causes an increase or decrease in plasma lipid concentrations. Comprehensive management is an attempt to prevent and reduce dyslipidemia, one of which is nutritional therapy. Edamame contains nutrients such as protein, fat, fiber, and isoflavones that have the potential to improve lipid profiles. Edamame tempeh production is one way to improve product quality that is improving nutritional quality, digestibility, and bioavailability. Edamame tempeh modification is conducted by adding yeast *Saccharomyces cerevisiae*. This yeast plays a role in increasing the isoflavone levels and the product containing β-glucan.
Objectives: This research aims to analyze the effects of edamame tempeh flour (ET) and modified edamame tempeh flour (MET) on lipid profile levels in dyslipidemic rats.
Methods: This research was a true experimental with a randomized controlled group pretest-posttest design. Thirty-six Sprague Dawley male white rats that met the inclusion criteria were randomized and divided into six treatment groups consisting of negative control, positive control, the dose of ET flour was 2.7 g/200gBW and 5.4 g/200gBW, the dose of MET flour was 2.7 g/200gBW and 5.4 g/200gBW. The intervention was carried out once per day in the morning for 28 days. The parameters observed included total cholesterol, LDL-C, and HDL-C levels measured by the CHOP-PAP method and triglyceride levels using the GPO-PAP method with a spectrophotometer measuring instrument. Examination of lipid profile levels was carried out three times. All statistical tests used a 95% significance level.
Results: There was a significant difference in the decrease in total cholesterol, LDL-C, and triglyceride levels as well as an increase in HDL-C levels compared to before the intervention ($p<0.05$).
Conclusions: The administration of ET flour and MET flour could significantly reduce total cholesterol, LDL-C, triglyceride, and increased HDL-C, but the administration of MET flour, especially at a dose of 5.4 g/200gBW, showed a more effective improvement in lipid profile and approached the positive control group compared to ET flour.

ABSTRAK

Latar Belakang: *Dislipidemia merupakan kelainan metabolisme lipid yang menyebabkan peningkatan atau penurunan konsentrasi lipid dalam plasma. Pengendalian secara komprehensif sebagai upaya dalam mencegah dan menurunkan dislipidemia salah satunya dengan terapi gizi. Edamame memiliki kandungan gizi seperti protein, lemak, serat, dan isoflavon yang berpotensi dalam perbaikan profil lipid. Produksi tempe edamame merupakan salah satu cara meningkatkan mutu produk yaitu meningkatkan kualitas gizi, daya cerna, dan bioavailabilitas. Tempe edamame modifikasi dilakukan dengan menambahkan khamir Saccharomyces cerevisiae. Khamir ini ikut berperan dalam peningkatan kadar isoflavon dan produk yang hasilkan mengandung β-glukan.*
Tujuan: *Menganalisis pengaruh tepung tempe edamame (ET) dan tepung tempe edamame modifikasi (MET) terhadap kadar profil lipid pada tikus dislipidemia.*
Metode: *Penelitian ini merupakan true experimental dengan rancangan randomized controlled group pretest-posttest. Tiga puluh enam tikus jantan putih galur Sprague Dawley yang memenuhi kriteria inklusi diacak dan dibagi menjadi 6 kelompok perlakuan terdiri dari kontrol negatif, kontrol positif, pemberian tepung ET dosis 2,7 g/200gBB dan 5,4 g/200gBB,*

pemberian tepung MET dosis 2,7 g/200gBB dan 5,4 g/200gBB. Intervensi dilakukan satu kali perhari di pagi hari selama 28 hari. Parameter yang diamati meliputi kadar kolesterol total, LDL, dan HDL diukur dengan metode CHOP-PAP dan kadar trigliserida menggunakan metode GPO-PAP dengan alat ukur spektroforometer. Pemeriksaan kadar profil lipid dilakukan sebanyak 3 kali. Seluruh uji statistik menggunakan tingkat signifikansi 95%.

Hasil: *Penurunan kadar kolesterol total, LDL, dan trigliserida serta peningkatan kadar HDL terdapat perbedaan yang signifikan dibandingkan dengan sebelum intervensi ($p<0.05$).*

Kesimpulan: *Pemberian tepung ET dan tepung MET mampu menurunkan kadar kolesterol total, LDL, trigliserida dan meningkatkan kadar HDL secara signifikan, tetapi pada pemberian tepung MET terutama dosis 5,4 g/200gBB menunjukkan perbaikan profil lipid yang lebih efektif dan mendekati kelompok kontrol positif dibandingkan dengan tepung ET.*

Kata kunci: *Dislipidemia, Edamame, Fermentasi, Saccharomyces cerevisiae, Tempe*

INTRODUCTION

Dyslipidemia is an abnormal lipid metabolism that causes an increase or decrease in plasma lipid concentrations characterized by increased levels of total cholesterol, LDL-C, triglycerides, and decreased levels of HDL-C[1]. Dyslipidemia contributes to endothelial dysfunction which is a major factor in the pathogenesis of hypertension, thrombosis, and atherosclerosis.[2] Atherosclerosis causes blood flow to the heart muscle to be blocked and results in cardiovascular disease[1,3].

One of the management of dyslipidemia therapy is through food regulation. Nutritional therapy is an important supporting factor in the management of dyslipidemia. Nutrition therapy has shown effectiveness in efforts to reduce dyslipidemia including proper energy intake to maintain a normal weight, limiting saturated fat intake, limiting simple carbohydrate intake, consuming high fiber foods, limiting alcohol consumption, and implementing the consumption of functional food sources in the daily diet[4–6]. One of the functional foods that are closely related to antidyslipidemia is tempeh[7]. Tempeh has high nutritional values such as a source of protein, vitamin B_{12}, and bioactive compounds. The nutritional value of tempeh is recognized to support the improvement of dyslipidemia[8]. The main protein content in soybeans is globulin 7S (*β-conglycinin*) and globulin 11S (*glycinin*) which play a role in increasing bile salt secretion and inhibiting cholesterol absorption[9].

In general, tempeh is made from yellow soybeans or mature soybeans, but edamame can also be an alternative material for tempeh production[10]. Edamame or vegetable soybeans are rich in protein, vitamins, minerals, dietary fiber, calcium, iron, and bioactive compounds such as omega-3 fatty acids, sterols, isoflavones, and saponins, and high in essential amino acids[11–13]. Another advantage is the low content of anti-nutritional substances such as phytic acid, antitrypsin, phenolics, and tannins[12]. Edamame tempeh is a processed food from soybean derivatives as the attempts to increase the nutritional content and digestibility of the product[10]. The presence of enzymatic activity from the fungus *Rhizopus oligosporus* can reduce antinutrient compounds in beans and convert them from macromolecular substrates into simple forms such as amino acids, fatty acids, isoflavone aglycones, and other bioactive compounds so that they are more easily utilized by the body[14–17].

In addition, during the soybean fermentation process, other microorganisms can grow, such as the yeast *Saccharomyces cerevisiae*. Yeast *S. cerevisiae* is involved in the breakdown of glycosides into aglycones increasing the availability of isoflavone aglycones[18,19]. *S. cerevisiae* is one of the microorganisms that produce β-glucan[20]. β-glucan is effective in preventing an increase in blood cholesterol levels[21]. Therefore, tempeh produced with the addition of *S. cerevisiae* has the advantage of increasing the quality of tempeh with high β-glucan content[22].

Previous research has studied the health benefits of tempeh, while in this research the main material used is edamame. In addition, edamame tempeh in this research was modified by adding yeast S. cerevisiae. This modification process produces edamame tempeh with higher nutritional value. Based on the background, this research aimed to analyze the effect of edamame tempeh flour (ET) and modified edamame tempeh flour (MET) on lipid profile levels of dyslipidemic rats.

METHODS

Design, Time, Place of Research

This research was a true experimental with a randomized controlled group pretest-posttest design. The research was carried out in September-November 2021 at the Laboratory of the Center for Food and Nutrition Studies, Inter-university Center, Universitas Gadjah Mada, Yogyakarta. This research was declared ethically feasibly by the Research Ethics Committee of the Faculty of Medical Sciences, Universitas Sebelas Maret No. 49/UN27.06.6.1/KEP/EC/2021 on 7 June 2021.

Equipment and Materials

The equipment consisted of analytical digital scales, containers/basins, stoves, frying pans/pans, stirrers/spatulas, strainers, polyethylene (PE) plastic, freeze dryer, miller machine, measuring cups, mortar, and pestle, mixing spoon, experimental animals equipped with eating and drinking utensils, experimental animal scales, gloves, gastric probe, injection syringe, serum separator tube (SST), microhematocrit tube, micropipette, vortex mixer genie 2, centrifuge (Heraeus MegaFuge 1.0), spectrophotometer (SP- 300; Optima, Japan).

The materials used in this research included edamame from Pasirhalang, Sukaraja District, Sukabumi Regency, West Java Province, tempeh yeast *Rhizopus oligosporus* RAPRIMA brand, yeast *Saccharomyces cerevisiae* commercial Fermipan brand, male white rats

Sprague Dawley, aquades, standard feed comfeed, simvastatin drug, pork oil and duck egg yolk.

Research Material Preparation

The stages of making edamame tempeh and modifications consist of sorting, washing, and boiling for ± 30 minutes, peeling the outer skin and epidermis, dividing the edamame seeds into two parts, soaking for ± 18 hours, boiling again for ± 15 minutes, drying and adding 2 g/kg of tempeh yeast and adding 3% *S. cerevisiae* yeast, stirring, packaging, and fermentation at 28°C for 36 hours[23].

The process of making edamame tempeh and modification flour was conducted by slicing tempeh that has been aged for 36 hours, slicing with a thickness of 0,1 mm, steaming for ± 5 minutes, freezing in the freezer, drying using a freeze-drying method for 24 hours, milling with a miller machine for ± 30 seconds[23].

Research Sample

The sample used was a white rat (*Rattus norvegicus*) *Sprague Dawley* strain with inclusion criteria including male rats, body weight ranging from 150-200 g, 8 weeks old, and normal behavior. Samples that were not included in the research if they had the exclusion criteria, the rats experienced behavioral changes and diarrhea which was indicated by watery stools, and the dropout criteria were that the rats died during the research.

This research used 36 rats divided into six treatment groups. The calculation of the sample size for each group referred to the provisions of the World Health Organization in 2000, namely in each group a minimum of five rats and an estimated dropout of 20% were added, so that six rats were obtained in each treatment group. The determination of each group was conducted by randomization.

Before the intervention, the rats were adapted for seven days. Rats were placed in groups of 6 rats. Each group of cages has an individual cage, and each cage is equipped with a small hole. The cage is placed at an ambient temperature of 22-25°C and humidity of 30-70%. A light reception is divided into the light cycle and dark cycle, each cycle for 12 hours. During the adaptation, rats were given standard *comfeed* of 20g/200gBW/day, drinking water *ad libitum*, and induction of a high-fat diet (HFD) for 14 days. The composition of the HFD induction consisted of 2 ml/200gBW pork oil and 1 ml/200gBW duck egg yolk administered through a gastric probe[25]. The criteria for rats to experience dyslipidemia were if their total cholesterol levels were 107 mg/dl, LDL 82.54 mg/dl, HDL < 41.49 mg/dl, and triglycerides 60 mg/dl[26]. After HFD induction for 14 days, all samples of rats experienced dyslipidemia, then intervention was carried out for 28 days.

Research Design

Table 1. Experimental design

Treatment Group	Intervention
K-	Dyslipidemic rats
K+	Dyslipidemic rats + simvastatin 0.18 mg/200gBB
KP1	Dyslipidemic rats + ET flour dose 2.7 g/200gBB
KP2	Dyslipidemic rats + ET flour dose 5.4 g/200gBB
KP3	Dyslipidemic rats + MET flour dose 2.7 g/200gBB
KP4	Dyslipidemic rats + MET flour dose 5.4 g/200gBB

The treatment group consisted of negative control (K-), namely dyslipidemic rats that were not given the intervention, a positive control (K+) that was given the simvastatin drug, KP1 was the group that was given the intervention of ET flour at a dose of 2.7 g/200gBW, KP2 was the group that was given the intervention of ET at a dose of 5.4/200gBW, KP3 is the group that was given the intervention of MET flour at a dose of 2.7 g/200gBW, and KP4 is the group that was given the MET flour intervention at a dose of 5.4/200gBW.

Table 2. Research time

Time	Implementation
Day 1-Day 7	Experimental rats adaptation period
Day 8-Day 21	Induction HFD for all treatment groups
Day 22-Day 50	Intervention

Dosage Determination

The dose of simvastatin for dyslipidemic adults is 10 mg/day converted according to Laurence and Bacharach's table for *Rattus norvegicus* so that the dose of simvastatin is given to rats is 0.18 mg/200gBW[27]. The determination of dose was determined based on the consumption of tempeh per day according to the vegetable protein exchange unit equivalent to 150 g of tempeh[28]. The amount was converted so that the dose of ET flour was 2.7 g/200gBW (normal dose) and 5.4 g/200gBW (high dose) and MET flour 2.7 g/200gBB (normal dose) and 5.4 g/200gBB (high dose). The intervention was given once per day in the morning for 28 days[28].

Data Collection

Parameters observed in this research included levels of lipid profile (total cholesterol, LDL-C, HDL-C, and triglycerides). The sample used was rat' blood serum taken through the *vein retro-orbital*. Examination of total cholesterol, LDL-C, and HDL-C levels was obtained from the results of the enzymatic photometric

Cholesterol Oxidase-Peroxidase Aminoantipyrine Phenol (CHOP-PAP) test and triglyceride levels using the *Glycerol-3-Phosphate-Peroxidase Aminoantipyrine Phenol* (GPO-PAP) method. Then it was analyzed using a measure the spectrophotometer. Lipid profile levels in rats were measured three times after adaptation (before HFD induction), before intervention (after HFD induction), and after intervention.

Data Analysis

The data obtained were tested for normality using the *Shapiro-Wilk* test. Next, the data that were normally distributed were followed by a homogeneity test using *Levene's* Test. Data that were normally distributed and homogeneous can be continued with the *One-Way ANOVA* test. A *One-Way ANOVA* test was used to analyze the differences between each treatment group. Next, it was followed by the *Post Hoc Tukey High Significant Difference* (HSD) test, the data were normally distributed but not homogeneous. The next test was the *Games Howell* test. The data were not normally distributed but homogeneous so using the *Kruskal Wallis* non-parametric statistical test followed by the *Mann-Whitney* test. All statistical tests used a 95% significance level.

RESULTS AND DISCUSSION

Total Cholesterol

Based on Table 2, the average total cholesterol levels in all treatment groups decreased after being given the intervention. The results of statistical tests indicated significant differences in the four groups compared to before the intervention ($p<0.05$). The highest decrease occurred in the positive control group who were given simvastatin as much as 52.55% and the lowest decrease in cholesterol was in KP1 as much as 30.28%. The intervention group that experienced a decrease in cholesterol levels was closed to the positive control group that is the KP4 group (50.17%).

Table 3. Average changes in total cholesterol levels after intervention

Group	Average Total Cholesterol Levels (mg/dl)		Rate of Change	
	D1	D28	Δ Total Cholesterol (mg/dl)	%
K-	193.50 ± 3.02	195.08 ± 2.67	1.58 ± 0.35	0.82
K+	193.62 ± 2.87	91.88 ± 1.38	-101.74 ± 1.49	52.55
KP1	190.19 ± 5.17	132.60 ± 1.59	-57.59 ± 3.58	30.28
KP2	188.30 ± 4.68	108.49 ± 3.43	-79.81 ± 1.25	42.38
KP3	189.24 ± 5.78	101.23 ± 1.71	-88.01 ± 4.07	46.51
KP4	190.78 ± 2.05	95.07 ± 1.83	-95.71 ± 0.22	50.17
P	0.170[a]	0.001[a*]	0.004[b*]	

The number is mean ± deviation standard
KP1 = ET flour dose 2.7 g/200gBW; KP2 = ET flour dose 5.4 g/200gBW; KP3 = MET flour dose 2.7 g/200gBW; KP4 = flour dose MET 5.4 g/200gBW
Δ = The difference between D1 and D28
D1 = Before intervention (post HFD induction); D28 = after intervention
([a]) *One-Way ANOVA* test
([b]) *Kruskal Wallis* test
(*) There is a significant difference ($p<0.05$)

Table 3 is a *Mann-Whitney* test to determine the difference in the difference in total cholesterol levels in each treatment group before and after being given the intervention. The results of the analysis showed the difference in total cholesterol levels was significantly different between the treatment groups after being given the intervention for 28 days ($p<0.05$), but in KP2 and KP3 there was no significant difference ($p>0.05$). This showed that the administration of ET flour at a dose of 5.4 g/200gBW and MET flour at a dose of 2.7 g/200gBW has almost the same effect on lowering total cholesterol levels.

Table 4. *Post Hoc* analysis of differences in total cholesterol levels before and after intervention

Parameter		K-	K+	KP1	KP2	KP3
Δ Total Cholesterol	KP1	0.004*	0.004*		0.004*	0.004*
	KP2	0.004*	0.004*	0.004*		0.078
	KP3	0.004*	0.004*	0.004*	0.078	
	KP4	0.004*	0.006*	0.004*	0.004*	0,016*

(*) There is a significant difference ($p<0.05$)

Soybeans and their processed products were closely related to foods high in protein, essential fatty acids, as well as vitamins, and minerals. This had a direct effect on the body's metabolic processes[28]. Based on the results of statistical analysis, total cholesterol levels decreased significantly in the intervention group with ET flour and MET flour. The hypocholesterolemic effect on MET flour occurred because soy protein was a fundamental component in soybeans. After all, it had a beneficial effect on lipid metabolism[29]. The main protein content in soybeans are 7S globulin (*β-conglycinin*) and 11S globulin (*glycinin*)[9]. Lammi et al. (2015) found two hypolipidemic peptides namely YVVNPDNDEN and YVVNPDNNEN derived from soy protein globulin *β-conglycinin* which could modulate cholesterol. Peptide IAVPGEVA was derived from soy *glycinin*, this peptide

could work with bile acids to lower plasma cholesterol levels in the liver increasing plasma cholesterol levels[31]. In addition, the low content of methionine in soybeans is associated with hypocholesterol activity which has positive homocysteine levels and can induce hypercholesterolemia by increasing cholesterol synthesis in the liver, so that in an increase in plasma cholesterol levels[32]. The recommendation for soy protein intake in improving lipid profiles was 25 g/day[33].

In addition, the fiber content in soybeans played a role in reducing cholesterol levels caused by the ability of fiber to modulate intestinal microbiota to reduce cholesterol synthesis due to the formation of propionate which inhibits the action of *the HMG-CoA reductase* enzyme in decreasing the diffusion of bile salts[34,] .The recommended daily fiber intake for adults was 25-38 g[21]. Research by Huang et al., (2018) proved that hyperglycemic rats induced by HFD experienced a decrease in cholesterol levels after being given tempeh supplementation which was inoculated with *Lactobacillus plantarum*. Based on the results of research related to cholesterol levels, it was found that the MET flour group was more effective than ET flour due to the compounds contained in the two products. Edamame tempeh added *S. cerevisiae* contains β-glucan. *S. cerevisiae* was a source of β-glucan[20]. It was known that β-glucan could reduce bile salts and increase bile salt secretion[37,38]. These mechanisms caused cholesterol to decrease and prevented reabsorption back to the liver[38]. Higher isoflavone aglycones in MET flour compared to ET flour because *S. cerevisiae* helped in increasing isoflavone aglycones[19,23].

Soy isoflavones had an anticholesterol effect because they were able to bind to estrogen receptors on body cells, thereby affecting the function of estrogen in binding DNA sequences and inducing DNA transcription. Through this mechanism, isoflavones became ligands for lipid regulating proteins such as PPARs (*Peroxisome Proliferator-Activated Receptors*), liver X receptors, and farnesoid X receptors that carried out a leading role in lipid metabolism. Therefore, this mechanism could reduce lipid synthesis in the liver, bile salt synthesis, and cholesterol reabsorption[39]. Isoflavones in tempeh were isoflavone aglycones that were more easily absorbed by the body. During the fermentation process, microorganisms helped activate isoflavones from glycone forms (daidzin and genistin) to aglycones (daidzein and genistein) [1,7]

***Low-Density Lipoprotein Cholesterol* (LDL-C)**

Based on table 4, it can be seen that the average change in LDL-C levels in the rats group after the intervention decreased. Statistical analysis showed a significant difference between LDL-C levels before and after the intervention (p<0.05). The treatment group that experienced the highest decrease in LDL-C levels was the positive control group (simvastatin), which was 60.51%. The effect of giving the intervention of MET flour at a dose of 5.4 g/200gBW (KP4) could reduce LDL-C levels (57.27%) close to the positive control group and the lowest reduction in LDL-C levels is in KP1 of 33.82%.

Table 5. Average changes in LDL-C levels after intervention

Group	Average LDL-C Levels (mg/dl)		Rate of Change	
	D1	D28	Δ LDL-C (mg/dl)	%
K-	83.16 ± 2.41	84.39 ± 2.22	1.23 ± 0.19	1.48
K+	83.05 ± 1.27	32.80 ± 1.56	-50.25 ± 0.29	60.51
KP1	79.54 ± 2.59	52.64 ± 2.49	-26.90 ± 0.10	33.82
KP2	81.92 ± 5.35	41.00 ± 1.71	-40.92 ± 3.64	49.95
KP3	82.82 ± 3.07	38.62 ± 2.05	-44.20 ± 1.02	53.37
KP4	80.79 ± 1.79	34.52 ± 1.48	-46.27 ± 0.31	57.27
P	0.259	0.001*	0.001*	

The number is mean ± deviation standard
KP1 = ET flour dose 2.7 g/200gBW; KP2 = ET flour dose 5.4 g/200gBW; KP3 = MET flour dose 2.7 g/200gBW; KP4 = flour dose MET 5.4 g/200gBW
Δ = The difference between D1 and D28
D1 = Before intervention (post HFD induction); D28 = after intervention
(*) There is a significant difference (p<0.05)

The results of the follow-up test using the *Post Hoc Tukey HSD* test revealed that there was a significant difference in LDL-C levels between the treatment groups (p<0.05) except in the positive control group with KP2 and KP4, KP2 with KP3 and KP4 were not significantly different (P>0.05). This shows that ET flour at a dose of 5.4 g/200gBW, MET flour at a dose of 2.7 g/200gBW, and 5.4 g/200gBW had almost the same effect on lowering LDL-C levels (Table 5).

iTable 6. *Post Hoc* analysis of differences in LDL-C levels before and after intervention

Parameter		K-	K+	KP1	KP2	KP3
Δ LDL-C	KP_1	0.001*	0.001*		0.022*	0..001*
	KP_2	0.001*	0.125	0.022*		0.887
	KP_3	0.001*	0.041*	0.001*	0.887	
	KP_4	0.001*	0.188	0.001*	0.550	0.851

(*) There is a significant difference (p<0.05)

LDL-C was the main lipoprotein that had atherogenic properties so it become a target for improving the lipid profile[40]. Edamame had nutritional components and bioactive compounds in improving LDL-C levels including protein, unsaturated fatty acids, fiber, isoflavones, and vitamins[4]. Amino acids, fiber, and high isoflavones content in soybeans had the potential as cardioprotective in lowering LDL-C levels[41].

The results of the research related to LDL-C levels indicated that each treatment group that was given the intervention of ET flour and MET flour had a significant decrease. Based on the results of the analysis, it was recognized that the reduction in LDL-C levels was more effective in the group with MET flour compared to ET flour. This might happened because the main protein components in soybeans were globulin 7S (*β-conglycinin*) and globulin 11S (*glycinin*). This peptide had a physiological effect in reducing LDL-C levels by degrading LDL-C in the liver, resulting in a decrease in serum LDL-C levels[4,32]. In addition, the peptides obtained from soybean *glycinin* are IAVPGEVA, IAVPTGVA, and LPYP. These three peptides could increase LDL-C receptor activity and work in vitro as inhibitors of *HMG-CoA Reductase* activity to regulate cholesterol biosynthesis. This would decrease the synthesis of cholesterol in the intracellular which led to the activation of SREBP-2 and increased the absorption of LDL-C by Hep2 cells. Another peptide derived from *β-conglycinin*, namely FVVNATSN could increase the transcription of LDL-C receptors in hepatocytes and the small subunit of albumin 2S protein (*lunasin*) plays a role in improving LDL-C levels through the mechanism of decreasing *HMG-CoA reductase* expression and increasing LDL-C receptor expression[31].

The fiber content and unsaturated fatty acids in soy such as omega-3 and omega-9 fatty acids have been shown to reduce LDL-C levels in the blood[42]. The fat contained in soybeans was mostly unsaturated in the form of arachidonic fatty acids, linoleic fatty acid, and oleic fatty acid, and contained phospholipids such as lipopositol, sepalin, and lecithin[43]. This is in line with the research of Huang et al. (2018) reported that supplementation of tempeh co-inoculated with *Lactobacillus Plantarum* in induced hyperglycemic rats HFD significantly lowers LDL-C levels.

The decrease in LDL-C levels was more common in the group with MET flour administration also due to the presence of β-glucan compounds which played a role in increasing the number of LDL-C receptors in the liver, thereby eliminating LDL-C particles in plasma[44]. This was evidenced by the research of Grundy et al. (2018) stated that there was a decrease in LDL-C levels in hypercholesterolemic rats fed a diet containing β-glucan.

***High-Density Lipoprotein Cholesterol* (HDL-C)**

Table 6 shows that HDL-C levels in the rats treatment group increased with the administration of ET flour and MET flour interventions for 28 days and statistically the average HDL-C levels before and after the intervention were significantly different (p<0,05). The KP4 group was known to have the best HDL levels among the other intervention groups with a value of 71,55 mg/dl or an increase of 204.21% compared to HDL-C levels before the intervention. KP4 is the intervention group that is closest to the positive control HDL-C levels, which was 70.71 mg/dl (209.45%).

Table 7. Average changes in HDL-C levels after intervention

Group	Average HDL-C Levels (mg/dl)		Rate of Change	
	D1	D28	Δ HDL-C (mg/dl)	%
K-	22.63 ± 1.86	21.89 ± 1.93	-0.74 ± 0.07	3.27
K+	22.85 ± 1.96	70.71 ± 1.56	47.86 ± 0.40	209.45
KP1	23.63 ± 1.45	56.34 ± 2.16	32.71 ± 0.71	138.43
KP2	23.30 ± 1.96	63.87 ± 1.81	40.57 ± 0.15	174.12
KP3	23.30 ± 1.86	67.36 ± 1.57	44.06 ± 0.29	189.10
KP4	23.52 ± 1.55	71.55 ± 1.56	48.03 ± 0.01	204.21
P	0.918	0.001*	0.001*	

The number is mean ± deviation standard
KP1 = ET flour dose 2.7 g/200gBW; KP2 = ET flour dose 5.4 g/200gBW; KP3 = MET flour dose 2.7 g/200gBW; KP4 = flour dose MET 5.4 g/200gBW
Δ = The difference between D1 and D28
D1 = Before intervention (post HFD induction); D28 = after intervention
(*) There is a significant difference (p<0.05)

Post-Hoc Tukey HSD test in Table 7 showed that there was a significant difference between the treatment groups (p<0.05), but the positive control was not significantly different from KP3 and KP4, KP2 and KP3, KP3 and KP4 (p>0.05). Based on the results of the analysis, showed that MET flour was able to increase HDL-C levels close to positive control compared to ET flour. The group given ET flour at a dose of 5.4 g/200gBW had the effect of increasing HDL-C levels which was almost the same as the MET flour at a dose of 2.7 g/200gBW, while the group given TEM flour at a dose of 2.7 g/200gBW was able to increase HDL-C levels in rats not much different from the dose of 5.4 g/200gBW of TEM flour.

Table 8. *Post Hoc* analysis of differences in HDL-C levels before and after intervention

Parameter		K-	K+	KP1	KP2	KP3
Δ HDL-C	KP_1	0.001*	0.001*		0.001*	0.001*
	KP_2	0.001*	0.001*	0.001*		0.189
	KP_3	0.001*	0.127	0.001*	0.189	
	KP_4	0.001*	1.000	0.001*	0.001*	0.101

(*) There is a significant difference (p<0.05)

HDL-C was considered good cholesterol because it acted as a cleanser of excess cholesterol in the arteries by transporting LDL back to the liver and excreted by the body[46]. Table 6 shows that the HDL-C of rats with the effect of administration of the intervention of ET flour and MET flour a significant increase. This research was in line with the research of Astawan et al. (2015) which stated that rats fed tempeh rations experienced an increase in HDL-C levels compared to boiled soybean or casein rations. This was influenced by the presence of oleic, linoleic, and linolenic acids in tempeh which had the potential to increase HDL-C levels[48].

In addition, the results obtained showed that MET flour increased higher HDL-C levels in rats compared to ET flour. This was because the high content of isoflavone aglycones in MET flour has the potential to increase HDL-C levels[23,49]. The higher isoflavone content in the product had a greater effect on increasing HDL-C levels[49]. Soy isoflavone aglycones consisting of genistein, daidzein, and glycitein exhibit estrogenic effects which acted as a cardioprotective effect to maintain and increase HDL-C levels[50].

Edamame contained about 60% unsaturated fatty acids[42]. High levels of HDL-C in the blood could reduce the occurrence of deposition and plaque formation in the circulatory system[48]. Based on the results of HDL-C analysis obtained, tempeh could be used as a functional food to help reduce the risk of cardiovascular disease through various mechanisms such as removing cholesterol from macrophages, improving endothelial function, and having anti-inflammatory properties[51].

Triglycerides

The results of the analysis showed that the intervention of giving ET flour and MET flour for 28 days was able to reduce triglyceride levels in all treatment groups. This was evidenced by statistical tests showing a significant difference compared to before the intervention (p<0.05). The treatment group after being given the intervention had the highest triglyceride levels in the KP1 group (104.11 mg/dl) and the lowest was a positive control (81.50 mg/dl) followed by KP4 (84.02 mg/dl).

Table 9. Average changes in triglyceride levels after intervention

Group	Average Triglyceride Levels (mg/dl)		Rate of Change	
	D1	D28	Δ Triglyceride (mg/dL)	%
K-	122.54 ± 3.47	124.77 ± 3.17	2..23 ± -0.30	1.82
K+	127.66 ± 7.10	81.50 ± 2.77	-46.16 ± 4.33	36.16
KP1	117.84 ± 5.89	104.11 ± 2.12	-13.73 ± 3.77	11.65
KP2	126.14 ± 2.03	94.52 ± 1.79	-31.62 ± 0.24	25.07
KP3	125.03 ± 4.38	93.04 ± 2.19	-31.99 ± 2.19	25.59
KP4	123.51 ± 2.43	84.02 ± 2.31	-39.49 ± 0.12	31.97
P	0.122[a]	0.001[b*]	0.001[b*]	

The number is mean ± deviation standard
KP1 = ET flour dose 2.7 g/200gBW; KP2 = ET flour dose 5.4 g/200gBW; KP3 = MET flour dose 2.7 g/200gBW; KP4 = flour dose MET 5.4 g/200gBW
Δ = The difference between D1 and D28
D1 = Before intervention (post HFD induction); D28 = after intervention
([a]) *Kruskal Wallis* test
([b]) *One-Way ANOVA* test
(*) There is a significant difference (p<0.05)

Based on the *Post Hoc Tukey HSD* test, it was found that the treatment groups differed significantly (p<0.05) except that the positive control group did not differ significantly with KP4, KP2 with KP3, and KP4, and KP3 with KP4 (p>0.05). This research showed that the difference in decreasing triglyceride levels with MET flour for 28 days had a greater reduction effect than ET flour. The results of the analysis of the difference in triglyceride levels can be seen in Table 9

Table 10. *Post Hoc* analysis of differences in triglyceride levels before and after intervention

Parameter		K-	K+	KP1	KP2	KP3
Δ Triglyceride	KP_1	0.001*	0.001*		0.001*	0.001*
	KP_2	0.001*	0.001*	0.001*		1.000
	KP_3	0.001*	0.001*	0.001*	1.000	
	KP_4	0.001*	0.285	0.001*	0.141	0.178

(*) There is a significant difference (p<0.05)

The results of the research related to triglyceride parameters showed that the intervention treatment group with ET flour and MET flour decreased significantly. This is related to soy protein including *β-conglycinin* which had been shown to help in lipid metabolism and inhibit lipid synthesis in the liver and cause a decrease in serum triglycerides in rats[31,52]. Triglyceride levels in the intervention group with MET flour had a better lowering effect than ET flour with the same dose.

Besides, MET flour had a higher content of isoflavone aglycones so there was a greater decrease than the intervention group of ET flour[23]. Isoflavones found in soybeans significantly reduce triglyceride levels[53]. Soy isoflavones were able to modulate lipoproteins resulting in the transformation of several important enzymes regulating lipid metabolisms such as *lipoprotein lipase* (LPL), and *hepatic lipase* (HL), or *hepatic triglyceride lipase* (HTGL), and 7 *alpha-hydroxylase*[54]. According to the results of a meta-analysis by Nachvak et al. (2019) revealed that the concentration of triglycerides decreased significantly when individuals consumed soy protein containing soy isoflavones.

CONCLUSIONS

The administration of ET flour and MET flour gave significant results in reducing total cholesterol, LDL, and triglyceride levels as well as increasing HDL-C levels. Based on the results of the analysis, it was found that MET flour showed better effectiveness as a hypolipidemic functional food compared to ET flour, especially when given MET flour at a dose of 5.4 g/200gBW. This was indicated by the difference in the decrease in total cholesterol, LDL-C, and triglyceride levels as well as an increase in HDL-C levels approaching the positive control group.

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CONFLICT OF INTEREST

All authors have no conflict of interest in this article.

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