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RESEARCH ARTICLE

The Effects of *Tempeh Gembus*, an Indonesian Fermented Food, on Lipid Profiles in Women with Hyperlipidemia

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ARTICLE HISTORY

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Abstract: ***Background*****:** Hyperlipidemia is the major precursor of lipid-related diseases. Consumption of high fiber foods may decrease lipid profiles. The fiber content in *tempeh gembus* is three times higher than regular *tempeh*.

Objective**:** This study was conducted to investigate the effect of tempeh *gembus* on lipid profiles in women with hyperlipidemia.

Methods**:** This research used the quasi-experimental design with nonequivalent control group design. Subjects were 41 women with hyperlipidemia, classified into 3 groups: control group, treatment group 1 given 103 g/day *tempeh gembus*, and treatment group 2 given 206 g/day *tempeh gembus* for 14 days. All of the groups received nutrition education. Total cholesterol and HDL-C were determined by *CHOD-PAP* method, triglyceride determined by enzymatic GPO-PAP method after subjects had been fasting for approximately 10 hours. LDL-C was calculated by Friedewald equation.

Results**:** These results showed that consumption of *tempeh gembus* 103 g/day and 206 g/day decreased LDL-C 27.9% and 30.9% as well as decreased total cholesterol 17.7% and 19.8% respectively. However, HDL-C increased 3.91% and 8.79% and triglyceride increased 2.3% and 3.1%. *Tempeh gembus* given 206 g/day was more effective to decrease total cholesterol and LDL-C than 103 g/day.

Conclusion**:** Increasing *tempeh gembus* consumption in women with hyperlipidemia should be addressed to decrease LDL-C and total cholesterol.

Keywords: Fiber, HDL-C, hyperlipidemia, LDL-C, *Tempeh Gembus*, total cholesterol, triglyceride.

1. INTRODUCTION

Coronary heart disease was the leading cause of death in the world according to World Health Organization (WHO). A total of 17.3 million deaths each year is due to this disease. The number of patients is expected to continue to grow by more than 23.6 million by 2030 [1]. Riset Kesehatan Dasar (Basic Health Research) 2013 mentioned that the incidence of coronary heart disease in Indonesia reached 1.5% of the total Indonesian population, where cases in women (1.6%) are higher than men (1.3%) [2]. The incidence in Central Java Province amount to 1.4%, close to the national average of coronary heart disease cases (1.5%) [2]. The number of coronary heart disease cases in Semarang city in 2015 was 1756 people according to the 2015 Health Profile of Semarang City [3].

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Although there are many risk factors for coronary heart disease, hyperlipidemia is a major determinant of coronary heart disease [4]. Hyperlipidemia is a condition of lipid metabolism disorder characterized by an increase of one or all lipid profiles in the blood.

The major lipid fraction disorder is an increase in fasting concentration of total cholesterol which may be followed by elevated triglyceride levels [4, 5].

Hyperlipidemia is a common risk factor for diseases associated with blood and heart lipids such as atherosclerosis and coronary heart disease. Common causes of hyperlipidemia are genetic and lifestyle [4, 6]. It has been studied that 39.6% of 19513 woman participants with coronary heart disease had hyperlipidemia (total cholesterol >200 mg/dL and LDL-C >100 mg/dL) [7].

The risk of hyperlipidemia is linear to age [8]. This is due to the decreasing function of LDL receptors in charge of eliminating bad cholesterol along age [9]. Minnesota Heart Survey (MHS) 2002 mentioned that 46.5% of adult women aged 25- 84 years old had hypercholesterolemia. Meanwhile, according to NHANES 2004, 29.6% of adult women>20 years had hypertriglyceridemia [10, 11]. Additionally, a cross-sectional study in Thailand suggested that hyperlipidemic women were predominantly in the 30-49 years age group (50.3%) [12].

Implementation of medical nutrition therapy (dietary regulation and diet modification) has more significant effect on modulating lipid profile [9]. The intervention in dietary intake such as increasing unsaturated fat by eating *Arbutus unedo* L. [13], reducing saturated fat intake, cholesterol and increasing fiber source foods such as soybeans can decrease total cholesterol for hypercholesterolemia subjects [14, 15]. Fermented soy products such as tempeh are known to have positive effects on blood cholesterol levels by consistently inhibiting the enzyme that is responsible for cholesterol biosynthesis and preventing LDL oxidation. The content of protein and fiber in tempeh can affect blood cholesterol levels [16]. One of the fermented products of tempeh that is well known by the public with low price is tempeh *gembus*.

Tempeh *gembus* is a fermented product based on soy pulp as a substrate and added with tempeh fungus (*Rhizopus oligosporus*) as microorganism. In Japan, there is similar product of tempeh gembus with the same way of making, Okara. The differences lies in on the substrate, Okara is the waste product from making soy milk or tofu [17, 18].

Based on the previous study, tempeh *gembus* had health effects such as anti-inflammation [19-23], anti-microbial [24], and some important nutrient content [25]. The nutrient contents of 100 grams tempeh *gembus* were 77.70 kcals of energy, 75.75% of water, 6.7% of protein, 3.04% of lipid, 13.73% of carbohydrate, 0.78% of ash, 232.09 mg of calcium, 751.42 ppm of phosphorus, 20.04 ppm of iron, 11.69 mg of sodium, and 28.73 mg of potassium [25]. Tempeh *gembus* (4.69 grams) has triple fiber than soybean tempeh (1.40 gr) [26].

The mechanism of lowering blood cholesterol levels with high fiber foods is through the binding of bile acids which increases the excretion of cholesterol, causing the liver to use cholesterol to make new bile acids. In addition, the bacteria from the production of fermented fiber in the colon inhibit cholesterol synthesis in the liver. The fermented dietary fiber is converted by intestinal bacteria into short chain fatty acids that can lower blood cholesterol [26-28].

Consumption of dietary fiber as much as 2-10 g/day had a significant effect on the decrease of total cholesterol [29, 30]. In the previous study, giving 8% per 100 grams of tempeh gembus to hypercholesterolemic mice showed the best result in decreasing total cholesterol and LDL-C, as well as significantly increasing HDL/LDL ratio [26].

2. MATERIALS AND METHODS

2.1. Materials

Grobogan-grown Soybeans were processed into tempeh *gembus* in a tofu and tempeh production house at Mrican, Semarang, Central Java, Indonesia. The tempeh *gembus* was cooked in two ways: *bacem* (sweetly marinated with spices) and stir-fried, at the Integrated Laboratory of Food Science Technology, Diponegoro University.

2.2. Preparation of Tempeh *gembus* Stir-fry

2 g of shallots, 1.5 g of garlic, and some chili were all sliced and sautéd with 1 tbsp of oil, and then 100 g of tempeh *gembus* and 1 g of salt were later added and left to cook.

2.3. Preparation of *bacem* (Sweet Marinated Food with Spices)

1.5 g of garlic, 1 g of candlenut, 0.1 g of coriander, 1 sheet of bay leaf, 5 g of soy sauce, 3 g of palm sugar and 1 g of salt were mashed and cooked together with 100 ml of water and 100 g of tempeh gembus.

2.4. Participants

This was a quasi-experimental study using the randomized pre-posttest control group design. This study was conducted for a month, from December 2016 to January 2017, in Semarang City Hall Office, the Regional Employment Board of Central Java, and the Labor and Transmigration Office of Central Java. The ethical clearance of this research is obtained under the approval of Health Research Ethical Committee of Medical Faculty, Diponegoro University, and Dr. Kariadi General Regional Hospital, Semarang (962/EC/FK-RSDK/IX/2016). The independent variable of this study was the administration of tempeh *gembus* at the dose of either 103 g or 206 g, while the dependent variable was the LDL-C and HDL-C levels in hyperlipidemic women.

Inclusion criteria included women aged 30 years or older, fully consented in becoming a participant by completing the informed consent form, having total cholesterol levels > 200 mg/dL and/or LDL> 100 mg/dL, does not smoke or drink alcohol, not prescribed on any cholesterol-lowering medications, and in a good condition or currently not on a heart disease-related therapy.

In the experimental period of 14 days, forty-five subjects were divided into three groups; control group (C) was given 10 mg of statin (Simvastatin, PT Kimia Farma, Indonesia), treatment group 1 (T-1) was given 103 g of tempeh *gembus*, and treatment group (T-2) was given 206 g of tempeh *gembus*.

C, T-1 and T-2 all received prior information on the recommended diet for patients with hyperlipidemia. Dietary intakes of energy, protein, fat, carbohydrates, fiber and cholesterol were obtained using 3x24 hour recall form. Participant's compliance in consuming tempeh *gembus* was controlled using the compliance form. Physical activity was measured by International Physical Activity Questionnaire (IPAQ).

2.5. Lipid Levels Determination

Blood samples were taken through veins in the morning after fasting for ±10 hours, before being brought for testing in Sarana Medika and Medista, Semarang. Total cholesterol and HDL-C levels were analyzed by Phenazone Cholesterol Amino Phenol Oxidase (CHODPAP-PAP) and triglycerides were analyzed by Glycerol Oxide-Phenyl Phosphate Aminopyrazolone (GPO-PAP). LDL-C levels were calculated with the Friedewald formula.

Table 1. Subjects characteristics.

Characteristic	Treatment 1	Treatment 2	Control
	Mean±SD	Mean±SD	Mean±SD
Gender (women)	13	13	15
Age (y)	46.38 ± 7.44	48.31 ± 6.03	45.13 ± 6.65
Weight (kg)	68.07 ± 11.75	66.65 ± 7.81	61.39 ± 7.35
Height (cm)	155.91 ± 4.41	155.28 ± 5.13	152.84 ± 4.42
IMT (kg/m$^{2]]}$)	27.74 ± 3.92	27.56 ± 2.49	26.58 ± 3.65
Energy (kkal)	1291.2 ± 295.12	1049.0 ± 286.13	1395.8 ± 276.63
Carbohidrat	178.92 ± 42.00	175.00 ± 89.10	181.4 ± 48.45
Protein	58.57 ± 17.53	28.69 ± 16.07	57.30 ± 22.44
Lipid	38.46 ± 21.72	47.94 ± 19.14	44.49 ± 16.13
Fiber	9.87 ±4.59	11.20 ± 6.45	9.32 ± 3.71
Cholesterol	192.42 ± 124.33	152.52 ± 94.99	174.43 ± 86.79
Physical Activity	2190 ± 1079.4	1932 ± 417.2	2180 ± 1740.0

Table 2. Total cholesterol levels before and after the intervention.

Group	Total Cholesterol		Difference		p
	Before (mg/dL)	After (mg/dL)	(mg/dL)	%	
Control	221.47 ± 26.12	192.40 ± 26.68	-29.06 ± 27.60	13.12	0.001[a]
Treatment 1	218.23 ± 33.62	179.46 ± 27.18	-38.76 ± 14.70	17.76	0.000[a]
Treatment 2	233.62 ± 22.39	187.23 ± 23.55	-46.38 ± 23.25	19.85	0.000[a]
P	-	-	0.145[c]	-	-

[a]*Paired t test* [b]*Wilcoxon* test [c]*One way ANOVA test*

2.6. Statistical Analysis

Dietary intake was analyzed using Nutrisurvey Program. The significant difference of the means of lipid profile among groups was analyzed by ANOVA test ($p<0.05$).

3. RESULTS

A total of 90 women employees were screened, revealing that 45 of them had hyperlipidemia (Table **1**). The decrease in total cholesterol levels occurred in all groups, though the largest decrease was found in the T-2 group. Analysis of variance showed that there was no difference in the decrease of total cholesterol levels among the three groups ($p>0.05$) (Table **2**).

Triglyceride levels in the T-1 and T-2 groups surprisingly increased, whereas the opposite was observed in the C group. Moreover, ANOVA test showed that there was no difference among the three groups ($p>0.05$) (Table **3**).

HDL-C levels improved in the T-1 and T-2 groups but declined in the C group. The largest increase in HDL-C levels was found in the T-2 group. ANOVA test indicated that there was no difference in the increase of HDL-C levels among the three groups ($p>0.05$) (Table **4**).

Our study managed to demonstrate that tempeh *gembus* could lower LDL-C levels. Participants from all three groups experienced a substantial drop in LDL-C levels, although the reduction in the T-2 group was evidently larger than the other groups. Analysis of variance showed that there was a prominent difference in the decrease of LDL-C levels among three groups ($p<0.05$) (Table **5**).

Post-hoc analysis revealed a significant decrease of LDL-C levels in the C group to the T-2 group. The tempeh *gembus* diet exhibited the largest effect in the T-2 group (206 g), proven by the higher mean difference in the T-2 group in comparison with the C group (Table **6**).

Table **7** showed the means of dietary intake of energy, carbohydrate, protein, lipid, fiber, and cholesterol among three groups without additional tempeh *gembus* intake. Neither dietary intake nor physical activity presented any effect on the levels of all examined indicators of lipid profile (Table **8**).

Table 3. Serum triglyceride levels before and after the intervention.

Group	Triglyceride		Difference		p
	Before (mg/dL)	After (mg/dL)	(mg/dL)	%	
Control	147.20 ± 29.31	136.87 ± 35.54	-10.33 ± 45.11	7.01	0.390[a]
Treatment 1	147.85 ± 50.70	151.31 ± 21.79	3.46 ± 50.76	2,34	0.810[a]
Treatment 2	196.00 ± 110.80	202.15 ± 77.17	6.15 ± 48.50	3.13	0.442[b]
P	-	-	0.621[b]	-	-

[a]*Paired t test* [b]*Wilcoxon* test [c]*One way ANOVA test*

Table 4. Serum HDL-C levels before and after the intervention.

Group	HDL-C		Difference		p
	Before (mg/dL)	After (mg/dL)	(mg/dL)	%	
Control	48.06±9.14	48.00±8.30	-0.06± 9.26	1.24	0.978[a]
Treatment 1	41.15±5.20	42.76±7.72	1.61± 8.12	3.91	0.487[a]
Treatment 2	39.76 ±9.07	43.23 ±7.13	3.46 ±10.91	8.70	0.275[a]
P	-	-	0.622[c]	-	-

[a]*Paired t test* [b]*Wilcoxon* test [c]*One way ANOVA test*

Table 5. Serum LDL-C levels before and after the intervention.

Group	LDL-C		Difference		p
	Before (mg/dL)	After (mg/dL)	(mg/dL)	%	
Control	143.96±25.01	117.03±29.30	-26.93± 22.39	18.70	0.002 [b]
Treatment 1	147.51±36.51	106.43±23.35	-41.07 ± 26.66	27.84	0.000 [a]
Treatment 2	154.65 ±20.52	103.57 ±21.06	-51.07 ± 22.07	33.02	0.000 [a]
P	-	-	0.035[c]	-	-

[a]*Paired t test* [b]*Wilcoxon* test [c]*One way ANOVA test*

Table 6. Effects of different doses of tempeh *gembus* on LDL-C levels.

Groups		Means Difference	P
Control	Treatment 1	14.14359	0.124 [d]
	Treatment 2	24.14359*	0.011 [d]
Treatment 1	Control	-14.14359	0.124 [d]
	Treatment 2	10.00000	0.289 [d]
Treatment 2	Control	-24.14359*	0.011 [d]
	Treatment 1	-10.00000	0.289 [d]

[d]Pos Hoc test

Table 7. Effects of dietary intake during the intervention.

Variable	Control (n=15) Mean±SD	Treatment 1 (n=13) Mean±SD	Treatment 2 (n=13) Mean±SD
Energy (kkal)	1395.8 ± 276.63	1291.2 ± 295.12	1049.0 ± 286.13
carbohydrate (g)	181.43 ± 48.57	178.92 ± 42.00	175.00 ± 89.10
Protein (g)	57.30 ± 22.44	58.57 ± 17.53	28.69 ± 16.07
Lipid(g)	44.49 ± 16.13	38.46 ± 21.72	47.94 ± 19.14
Fiber (g)	9.32 ± 3.71	9.87 ± 4.59	11.20 ± 6.45
cholesterol (mg)	174.43 ± 86.79	192.42 ± 124.33	152.57 ± 94.99

Table 8. Effects of Confounding variables.

Variable	*p*chol. Total	*p*Triglyceride	*p*HDL-C	*p*LDL-C
Physical activity	0.780	0.918	0.569	0.632
Energy	0.165	0.130	0.637	0.568
Carbohidrat	0.103	0.509	0.950	0.168
Protein	0.525	0.288	0.826	0.915
Lipid	0.168	0.080	0.789	0.585
Fiber	0.420	0.071	0.701	0.097
Cholesterol	0.670	0.569	0.824	0.772

[c]Ancova test

4. DISCUSSION

Our study showed that there was no significant difference between the mean differences of total cholesterol levels across all three groups. However, tempeh *gembus* appeared to have an effect on total cholesterol levels at a dose of 206 g /day.

Total serum cholesterol may rise due to consuming food which contains saturated fats and trans fats. Cholesterol synthesis within the body declines with adequate intake [31, 32]. Our study showed that the total cholesterol levels decreased in all groups including the control group, with the largest reduction observed in both treatment groups. The T-2 group, which was given a tempeh *gembus* diet at a dose of 206 g /day, experienced changes in total cholesterol levels with a mean decrease of 19.8%. An earlier study by Mohammad Sulchan and MG Isworo Rukmi mentioned that administrating tempeh *gembus* at 8% and 12% per 100 g ameliorated serum lipid profiles by lowering total cholesterol in hyperlipidemic rats [26].

Depleted total cholesterol levels may be attributable to increased fiber intake from consuming tempeh *gembus* at a dose of 103 g/day and 206 g/day in both treatment groups. The subjects of the T-1 group showed less reduction in total cholesterol levels as they were provided with a lower dose of tempeh *gembus* and consequently, less additional fiber in comparison with the T-2 group.

Furthermore, this study indicated that there was a mean difference in LDL-C levels between the three groups after the tempeh *gembus* intervention, where consuming 206 g of tempeh *gembus* for 14 days had a greater effect on lowering LDL-C than the control group. The dose of 206 g contains 9.6 g of fiber, accounting for 32% of daily fiber requirement. On the other hand, there was no difference in the increase of HDL-C levels.

A previous study has suggested that the administration of nata de coco containing 2.7 g/day of fiber in hyperlipidemic patients for 20 weeks successfully decreased LDL-C levels by 2.3% (3.9 mg/dL) and increased HDL-C by 7.7% (3.9 mg/dL) [29]. Another study mentioned the administration of nata de coco with 4.8 g or 9.6 g of fiber for 14 days, had an effect on cholesterol levels by reducing LDL-C levels up to 6.3% (9.31 mg/dL) and enhancing HDL-C by 12.1% (5.61 mg/dL) [33].

Studies in mice proved that tempeh *gembus* at a dose of at least 8% managed to increase triglyceride levels; lower total cholesterol, LDL-C and HDL-C levels; as well as improve HDL / LDL ratio [34]. Another study showed that the group given soy fiber-biscuits with a total fiber of 27.5 g / day, exhibited significant changes in LDL-C and total cholesterol after 8 weeks and 12 weeks of intervention. The group that consumed soy-fiber biscuits for breakfast was reported to be less hungry than the control group [35]. Similarly, a study showed that lipid profiles in a group given a high-fat diet with an additional 20% okara (6.46 g fiber) for 12 weeks showed a significant decrease in LDL-C and total cholesterol [36].

The mechanism of fiber in lowering blood cholesterol is still undefined. Evidence suggested that some soluble fiber (polysaccharides) bound bile acids *in vitro*, decreases cholesterol absorption in the gastrointestinal tract, and also increases cholesterol or liver fatty acids excretion during intraluminal formation of micelles, so the liver would use cholesterol to form new bile acids [27, 37]. Moreover, fiber changes the concentration of hormones / short-chain fatty acids that may affect lipid metabolism, inhibits the synthesis of liver fatty acids with intestinal fermentation products (short-chain fatty acid production such as acetate, butyrate, propionate), and alters bowel motility. High viscosity fiber improves insulin sensitivity by slowing macronutrient uptake and increases satiety in order to reduce energy intake [38]. Sedaghat, *et al*. demonstrated that consumption of 60 grams of soybeans for 8 weeks had significant effects on total cholesterol (18.6%) and LDL-C (13.9%) reduction [39].

In the same way, the association of the effects of fiber intake on HDL-C levels is also still controversial. Some studies showed only a small, inconsistent, or inexistent decrease in HDL-C levels as an effect of fiber intake [38, 40, 41]. Compared with dietary intake, exercise has a larger influence on HDL-C levels. A year-long sports training program recorded the largest increase in HDL-C. The idea of HDL-C improving with exercise is supported by several theories, particularly how fatty acids are a major energy source during exercise and how high HDL-C levels are associated with increased postprandial triglyceride clearance [42].

Studies on Okara showed that not only fiber but also other components such as proteins may be involved in decreasing serum lipids. The consumption of soy protein is beneficial to serum lipid concentrations in animal studies [43, 44]. Many potential mechanisms may explain the cholesterol reducing-effects of hydrolyzed soy protein. Among these mechanisms, three of them are the most plausible, namely inhibition of bile acids and/or cholesterol absorption, inhibition of cholesterol synthesis, and promotion of the transcription process of LDL receptors [44]. Additionally, soy protein contains isoflavones that may significantly decrease total cholesterol, LDL-C, and triglyceride, as well as increase HDL-C [45-47].

Yanwen Wang *et al*. showed that the consumption of soy protein at a relatively high level of 25 grams/day for 6 weeks could decrease total cholesterol and LDL-C in hyperlipidemic subjects [38, 48]. Soy protein along with fiber were shown to be capable of lowering intestinal cholesterol absorption and increasing bile acid secretion. The bioactive component in soy protein are hypolipidemic. In addition, isoflavones, such as genistein and daidzein, function as phytoestrogens (exhibit estrogen-like activities) that supports the effect of soy protein by increasing the activity of LDL receptors [26].

The results showed that there were no significant difference in triglyceride change between 3 groups ($p > 0.05$). The T-1 group and the T-2 group experienced a non-significant increase (2.3% and 3.1%) in triglyceride levels. The studies of tempeh *gembus* on cholesterol profiles in hyperlipidemic rats also showed an insignificant increase in triglyceride levels in the three treatment groups (4%, 8%, and 12%) [26]. Elevated triglyceride levels in the treatment group were not in line with theory and some previous studies on dietary fiber whereas dietary fiber intake has a decreasing effect on triglyceride levels. Depleted triglyceride levels in the control group may be associated with consumption of 10 mg statin drug, lower intake of energy, carbohydrates, fat, cholesterol and higher physical activity than the two treatment groups.

The tendency of elevated triglyceride levels in the T-1 group and the T-2 group may be caused by the sensitivity of serum triglycerides and its susceptibility into external food intake from outside (exogenous) than from synthesis of the body (endogenous) compared with total cholesterol. Triglycerides from diet are 100% absorbed into the bloodstream and transported by chylomicrons. Therefore, triglyceride levels are very easily influenced by the food. Postprandial uptake of chylomicrons from the gastrointestinal tract after 30-60 minutes of food consumption may lead to an increase in serum triglycerides for 3-10 hours [37].

Parks E J *et al* stated that the condition of Body Mass Index can make individuals more sensitive to the changes of lipids and lipoproteins when carbohydrate consumption increases. Subjects that are obese in that study had experienced a 30% increase in serum triglycerides [49]. This finding suggests that the condition of subject BMI (Obese 1) in this study may cause elevated triglycerides along with excessive carbohydrate consumption. The increase of triglyceride levels in this study can be related to the fat intake by subjects and lack of compliance to the nutritional education (daily diet control) given.

Hyperlipidemia is associated with body mass index (BMI) and weight gain [17, 50]. Obesity and overweight associated with lipid levels in plasma, one of them are triglyceride serum [51, 52]. In subjects with obesity, adipose tissue increased in tissue volume, because of the accumulation of triglycerides and excessive cholesterol [53]. Adipose tissue stores excess energy from food consumption in the form of triglycerides. Adipose tissue lipolysis and the release of uncontrolled fatty acids that increase the synthesis of VLDL from liver so it will inhibit lipolysis chylomicron and cause hypertriglyceridemia [9].

Food intake and physical activity may also contribute to elevated triglyceride levels in this study [54]. The average fat intake during the intervention in the control group and treatment group did not exceed the recommended fat intake by NCEP ATP III, ie 25% -35% of total energy and saturated fat is less than 10% of total energy [4, 55, 56]. Excessive saturated fat intake is associated with increased LDL-C levels in the body. Saturated fatty acids can lower LDL receptors and cause decreased catabolic rates of LDL-C. Trans fat in the body, converts blood cholesterol to the same as saturated fat, which increases LDL-C and decreases HDL-C. While unsaturated fatty acids can lower cholesterol by increasing the number of LDL receptors in the liver [34, 55].

Hyperlipidemic conditions increase when fat intake is more than 40% of total energy [5]. High fat diet can increase postprandial lipemia and chylomicron remnants in blood. Besides, fat intake >25% of total calories effect on elevated triglyceride levels and decreased HDL-C levels [38].

Cholesterol intake in all three groups had met the NCEP ATP III recommendations (< 200 mg/day). The cholesterol response varies for each individual. Some of them were hypo-responsive individuals where their cholesterol levels did not increase after cholesterol consumption while the others were hyper-responders where their cholesterol levels responded stronger than expected to cholesterol intake. The hypo-responders have apo E-4 and low cholesterol conversion rates to bile acids [32]. A person is at risk of hyperlipidemia if their daily intake of cholesterol is greater than 300 mg/day.

Energy intake in the control and treatment groups were in the lower category according to Nutritional Score. Carbohydrate intake in the control and treatment groups hadn't met the NCEP ATP III recommendation that is 50% -60% of total energy. This was related to the consumption of foods with high sugar content (sucrose) such as sweet tea, sponges, and cakes that contribute to the average intake of energy and carbohydrate. Sucrose is positively associated with elevated triglyceride levels. Sucrose helps and promotes increased secretion of bile acids and decreases the secretion of neutral steroids. A study showed that with a diet of sucrose, women with 32 percent body fat may experience increased triglyceride levels up to 29 mg / dL [41, 46, 47].

High carbohydrate intake was significantly associated with diabetes mellitus and low HDL-C in women. The molecular mechanisms that underlie the decline of HDL-C as a result of excessive carbohydrate intake are not fully understood. HDL-C is known to be affected by BMI, physical activity, and many other intake factors. High fat intake was associated with high total cholesterol and HDL-C [57, 58]. Based on meta-analysis research, low carbohydrate intake was significantly associated with decreased LDL-C and increased HDL-C [59].

High intake of fat and low intake of carbohydrates for 12 weeks on obese subjects led to a 23% decrease in triglycerides and 11% increase in HDL-C but no effect was found in low intake of fat and high intake of carbohydrate with the same amount of calories. It should that the composition of macronutrients, rather than the total calories that affect blood lipid parameters [60].

The average of fiber intake after intervention in the T-2 group was higher than the C group. This suggest an increase of fiber intake in the treatment group compared with the control group after consumption of tempeh *gembus*. As many as 80% of Indonesia's population consume only 15 grams of fiber per day. The average fiber intake of subjects in the three groups was still not consistent with the fiber intake requirements based on NCEP ATP III recommendation, which are 20-30 g / day [55, 56]. Tempeh *gembus* with the dose of 103 g / day and 206 g / day respectively contributed 16% and 32% of total fiber intake after intervention, showing that the subjects fiber consumption before the intervention still did not meet the recommended daily fiber intake. The result of depleted cholesterol in liver cells led to an increase in LDL receptor regulation and then increased the clearance of LDL-C [10]. Fermented fiber is converted by intestinal bacteria into short chain fatty acids. Short-chain fatty acids (acetate, butyrate, and propionate) play a role in inhibiting the synthesis of HMG-CoA reductase so that cholesterol synthesis in the liver also decreases [27, 28, 38, 61].

Physical activity increases the energy expenditure and burns body fat that accumulates in individuals who consume more energy intake than required. Studies showed that regular physical activity does not significantly lower total cholesterol or LDL-C, but the effects of physical activity are more influential on apolipoprotein B, HDL-C, and triglycerides [62]. Subjects' physical activity values were classified into the low to moderate category based on IPAQ, making it possible for the occurrence of elevated triglyceride levels. Physical activity such as walking or jogging for 24-32 minutes per week with high and low intensity can decrease triglyceride successively by 30% and ± 10% [32].

CONCLUSION

Consumption of tempeh *gembus* with a dose of 103 g / day and 206 g / day in hyperlipidemic women for 14 days was found to significantly lower total cholesterol and LDL-C, in addition to raising HDL-C. However, the increase of triglyceride levels was not achieved because of the inappropriate subject consumption (high carbohydrate and fat) and the lack of compliance to the education. Tempeh *gembus* with a dose 206 g / day had a better effect to lower LDL-C.

Further research can be done to investigate the effects in hyperlipidamic men, so the role of fiber from tempeh *gembus* in lowering cholesterol can be used in general. In addition, research on the effect of tempeh gembus protein and polyphenolic / antioxidant properties needs to be done, considering the protein content in tempeh *gembus* is not much different from soybean tempeh. Tempeh *gembus* can be a low priced alternative fiber rich food that has an effect in decreasing blood lipid profile levels.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The ethical clearance of this research is obtained under the approval of Health Research Ethical Committee of Medical Faculty, Diponegoro University, and Dr. Kariadi General Regional Hospital, Semarang, Indonesia with certificate number 962/EC/FK-RSDK/IX/2016.

HUMAN AND ANIMAL RIGHTS

No animals were used in the study. All reported human subjects were experimented in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the *Helsniki Declaration* of 1975, as revised in 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/).

CONSENT FOR PUBLICATION

Written informed consent was taken from the participants of this study.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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The first author DNA as principal investigation, responsible for ethical clearance, conduct research according to ethical clearance, and reports the results of the study in accordance with relevant rules. The second and third author execute research, collects data, and analyzes data. All authors review this manuscript in advance of publication.

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