FOOD
RESEARCH

The effect of *tempeh* flour on LDL (low-density lipoprotein)-level lowering in *Rattus norvegicus* fed a high-fat diet

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Abstract

Tempeh is an antioxidant-rich food that can prevent LDL cholesterol oxidation in the arteries. The primary antioxidants from *tempeh* are isoflavone aglycones, which potently lower cholesterol levels in the blood. This research aimed to determine the effect of *tempeh* flour on the LDL levels of rats fed a high-fat diet. The experimental design included a completely randomized design (CRD) and pretest-posttest control group design. In this study, twenty-four male *Rattus Norvegicus* rats were divided into four groups. The rats were acclimatized for seven days and then supplied with a high-fat diet in the form of pork oil for 14 days. The pork oil was administered at 2 mL/kg body weight/day. In addition, *tempeh* flour was also administered at three doses as an intervention for 14 days. The dosage groups were as follows: P1 with 50 g, P2 with 75 g, and P3 with 100 g. All the dosages were converted with a dose conversion table for rats so that the final dosages were P1 1.9 g, P2 1.35 g, and P3 1.8 g. The positive control group was given 0.18 mg simvastatin. The LDL level was measured by using the CHOD-PAP method. In addition, the data were analyzed by using one-way ANOVA. The results indicated that the implementation of *Tempeh* flour intervention significantly altered the LDL levels of rats. There was a significant decrease in LDL levels after the administration of *Tempeh* flour at all three (0.9 g, 1.35 g, and 1.8 g) doses for 14 days. Moreover, the most effective dosage compared to the control group was the 1.35 g dosage.

1. Introduction

Cardiovascular disease is still a global threat. It is also the leading cause of death worldwide, with 17.9 million deaths every year or 30% of the total number of deaths worldwide (WHO, 2021). It is known that atherosclerosis with dyslipidemia is the main risk factor for cardiovascular disease. Dyslipidemia is a lipid metabolism disorder that is associated with alterations in the lipid fraction levels in plasma. The main alterations to lipid fractions that are associated with this disorder are an increase in total cholesterol levels, low-density lipoprotein (LDL) levels, and triglyceride (TG) levels and a decrease in high-density lipoprotein (HDL) levels (Sulistijo *et al*., 2017).

LDL is the most atherogenic lipoprotein and the main target of dyslipidemia therapy. The oxidation process enables high LDL levels to block the artery and form plaques (Sulistijo *et al*., 2017). The blocked artery is also known as atherosclerosis. Based on the LDL reference value in humans, the LDL level is categorized as normal when it is 100-129 mg/dL, borderline high when it is 130-159 mg/dL, high when it is 160-189 mg/dL and very high when it is ≥190 mg/dL (Kementrian Kesehatan, 2018). Some controllable risk factors for atherosclerosis are lifestyle, diet patterns, physical activity, and smoking habits. Meanwhile, uncontrollable risk factors are genetic factors (Lau, 2009).

Nonpharmacological therapy or lifestyle modifications could be implemented to decrease the risk of dyslipidemia, especially by modifying diet patterns. Plant protein and dietary fibre are believed to have an antidyslipidemic effect. For example, soybeans are a source of plant protein that is also high in dietary fibre. Intake of approximately 25 g of soy protein leads to a significant reduction in total cholesterol and LDL cholesterol levels. Genistein, daidzein and glycitin (isoflavonoids) are included in soy products (Koutelidakis and Dimou, 2016).

Isoflavone is the phytoestrogen found in soybeans and is related to the process of making soybeans *tempeh*. It is known that this process improves the digestibility of the nutritional content and improves the isoflavone

bioavailability of soybeans. Thus, *tempeh* is a potential source of isoflavones. The isoflavones contained in *tempeh* are free isoflavones, which include isoflavone aglycones, genistein, daidzein, and glycitein. These isoflavones result from the hydrolysis process during fermentation, with the assistance of a β-glucosidase enzyme from *Rhizopus oligosporus* mould (Kustyawati, 2012). Aglycone isoflavones in *tempeh* have higher bioavailability than glycoside isoflavones in soy milk (Kwon *et al.*, 2006). Isoflavones are absorbed by the body in the form of aglycones. Fermented soybean preparation (*tempeh* flour) shows better antidyslipidemic activity than unfermented soybean preparation (soy milk). This means that the fermentation process of tempeh improves the bioavailability of various bioactive compounds in soybeans, which is beneficial for improving lipid profile levels (Nugraheni and Bintari, 2017).

Based on 'Tabel Komposisi Pangan Indonesia (2018)', the nutrition content of 100 g of raw *tempeh* consists of 20.8 g of protein, 8.8 g of fat, 13.5 g of carbohydrate, 1.4 g of dietary fibre, 155 mg of calcium, 55.3 g of water, 201 calories energy, 326 mg of phosphor, 4.0 mg of iron, 9 mg of sodium, 234.0 mg of potassium, 0.57 mg of copper, 0.57 mg of zinc, 0.19 mg of thiamine, 0.59 mg of riboflavin, and 4.9 mg of niacin (Kementrian Kesehatan Republik Indonesia, 2018).

Antioxidants in *tempeh* effectively prevent LDL cholesterol oxidation in the artery and further lower cholesterol levels (Loaloka and Pantaleon, 2020). In addition, the isoflavone-rich protein from soybeans could improve the activity of LDL cholesterol receptors (Syakri *et al.*, 2014). Isoflavones in *tempeh* could also reduce free radicals, such as hydroxyl radicals, superoxide radicals, and lipid peroxyl radicals. It was also shown in 2017 that there was a significant relationship between isoflavone-rich food intake and total blood cholesterol levels (Bintanah *et al.*, 2017).

In 2013, it was reported that black soybean consumption could lower LDL levels by 6.1±16.45 mg/dL (Priastiti and Puruhita, 2013). Moreover, the consumption of yellow soybean could lower LDL cholesterol by 4.9±9.91 mg/dL after 14 days of intervention. This is in line with research done by Agung (2013), which showed that *tempeh* intake within 20 g/kg body weight/day significantly lowered LDL levels in rats (p<0.05) (Ari-Agung *et al.*, 2013). Based on this background, this research aimed to determine the effect of *tempeh* flour on the LDL levels of rats fed a high-fat diet.

2. Materials and methods

This research used a pretest-posttest control group design as the research method. In this study, 24 male *Rattus Norvegicus* rats were divided into four groups. The intervention used in this research was *tempeh* flour. *Tempeh* flour was commercially purchased from the available *tempeh* in the community of Semarang city with a composition of 100% pure *tempeh* and was labelled as homemade tempeh flour. The *Rattus Norvegicus* rats for this research were housed in the Animal Experimental Lab of the Department of Biology in Mathematics and Natural Sciences Faculty Universitas Negeri Semarang (UNNES). In addition, the LDL level test was performed in the Provincial Health Laboratory (LABKESDA) of Central Java.

The high-fat diet used in this research was pork oil, and the pork oil was administered at 2 mL/day for 14 days. The LDL level was measured after being given the high-fat diet and after the intervention of *tempeh* flour. For the LDL level measurement process, blood was obtained from the orbital sinus using a hematocrit/TLC tube and collected in an Eppendorf tube at as much as 2 mL. Then, it was centrifuged for 10 mins to obtain plasma for the enzymatic LDL level test by using the CHOD-PAP method. Therefore, the dosages obtained were P1 1.9 g, P2 1.35 g, and P3 1.8 g. Additionally, the positive control group was given 0.18 mg simvastatin. The intervention was administered through nasogastric intubation for 14 days.

The research data were analyzed using a computer program and then analyzed using the parametric statistical one-way ANOVA test ($p<0.05$). The research was performed based on proper research ethics, and the letter of statement from the Ethics Committee of Public Health Faculty Universitas Muhammadiyah Semarang is number 458/KEPK-FKM/UNIMUS/2020.

3. Results and discussion

3.1 Phenotypic characteristics

Food intake affected the bodyweight of the rats during the research. Weight was regularly monitored before and after high-fat diet implementation. The mean body weight before and after high-fat diet implementation is presented in Table 1. Based on the results of one-way ANOVA, it was found that there was a significant difference in the mean body weight before the implementation of a high-fat diet (p = 0.000). The difference in body weight before and after the high-fat diet was not significant (p = 0.919). Meanwhile, the intervention group with the highest weight gain was the P1 group, with a difference of 40 g/rat body weight

Table 1. The distribution of body weight for the rats during the research period

Intervention Group	Mean body weight		Δ weight	P value
	Before high-fat diet day-1	After high-fat diet day-14		
Control	212.33±18.06	212.00±109.72	-0.333±91.69	0.994
P1	166.00±12.77	206.00±22.83	40.00±10.06	0.028
P2	190.83±14.89	224.17±42.65	33.34±27.76	0.039
P3	169.00±21.64	200.17±31.85	31.17±10.21	0.078
P value	0	0.919	0.919	

between the start and termination of the high-fat diet implementation. Based on the findings of a paired t-test on the control group and P3 intervention group, no significant differences in body weight before (p = 0.994) and after (p = 0.078) the implementation of the high-fat diet were found. However, the mean rat body weight in the control group decreased, as some rats died due to stress from the intake of pork oil through nasogastric intubation. In the P1 and P2 groups, there was a significant difference between the body weights before and after the implementation of a high-fat diet; the bodyweight of these rats increased (p = 0.028 and p = 0.039, respectively). This is in line with research by Gustomi *et al.* (2017) that found a significant increase in rat body weight after an intervention of 0.3 mL of lard for 3 weeks (*p*<0.05). In this case, the mean value of body weight before the implementation of lard was 31.76 g, and after the implementation of lard, it was 33.05 g (Gustomi *et al.*, 2017). According to Cani *et al.* (2008), high-fat diet feeding in rats could increase body weight through gut microbiota modulation, in which gut permeability and absorption ability are improved. In addition to an increase in body weight, the implementation of a high-fat diet also significantly increased LDL levels in rats (Cani *et al.*, 2008). This was in line with research by Harsa *et al*. (2014) that showed that the implementation of a high-fat diet in white rats in the form of lard within 3 g/200 g body weight/day and duck egg yolk within 2 g/200 bodyweights could increase the mean LDL level. The mean LDL level before the implementation of the high-fat diet was 27.67 mg/dL, and after the implementation of the high-fat diet, it was 41.33 mg/dL (Harsa, 2014).

3.2 LDL level analysis before and after the intervention of tempeh flour

As shown in Table 2, the P2 (1.35 g) group demonstrated the greatest decrease in LDL levels before and after the intervention; in this group, LDL levels were reduced by 8.76 mg/dL. A significant difference was found (p = 0.047) by one-way ANOVA for the LDL levels of rats after the intervention of *tempeh* flour for 14 days. This is in line with other research, which showed that the implementation of *tempeh* flour at 20 g/kg body weight/day could significantly lower LDL levels in rats (p<0.05) after 13 weeks of intervention (Ari-Agung *et al*., 2013). However, *tempeh* flour at 1.9 g/200 g of body weight per day for 21 days significantly (p<0.004) affected LDL levels (Fadhilah, 2019). Research in 2017 showed that LDL levels could be significantly (p<0.004) decreased in rats with a *tempeh* flour intake of 1.8 g of in four weeks.

The isoflavones contained in tempeh flour are antioxidants from the flavonoid group 1,2-diarylpropane, and they have the potential to reduce cholesterol levels. The cholesterol reduction mechanism acts through the catabolism of fatty cells for energy generation, which increases LDL clearance from the bloodstream and reduces the cholesterol level in the blood (Bintanah et al., 2017). The isoflavones also consist of genistein, daidzein, and glycitein, which are capable of reducing the risk of cardiovascular diseases by binding to blood lipids (Koutelidakis and Dimou, 2016).

However, a previous paired t-test found that there was no significant difference (p>0.05) in LDL levels before and after the implementation of *tempeh* flour (Nugraheni and Bintari, 2017). Consistent with these results, there was no significant difference (p>0.05) in

Table 2. LDL levels before and after an intervention with *tempeh* flour

Intervention Group	Mean LDL level		Δ Delta	P value
	Before intervention with *tempeh* flour	After intervention with *tempeh* flour		
Control	50.33±28.50	41.48±22.26	-8.85±6.24	0.073
P1	66.90±8.47	58.14±8.31	-8.76±8.39	0.173
P2	68.90±14.76	63.62±10.48	-5.28±12.62	0.547
P3	61.78±3.56	59.28±6.50	-2.50±5.03	0.452
P value	0.247	0.047	0.200	

LDL levels before and after treatment in any group (control: 0.07, P1: 0.173, P2: 0.547, P3: 0.452).

3.3 The most significant tempeh flour dosage for lowering the LDL level in rats

Based on the post hoc test (Table 3), a significant difference was found in the control group (p<0.05). Thus, the tempeh flour dosage intervention was not as effective as the simvastatin intervention in lowering LDL levels. Moreover, the *tempeh* flour intervention group demonstrated a greater decrease in LDL levels (p: 0.01) than the control Group P2 (1.35 g). Statins reduce cholesterol formation in the liver by competitively inhibiting HMG-CoA enzyme reductase. The intracellular cholesterol concentration increases LDL receptor expression on the hepatocyte surface, further increasing LDL removal from the blood and reducing its concentration in the blood (Sulistijo *et al*., 2017). However, the P1 (0.9 g), P2 (1.35 g), and P3 (1.8 g) dosages showed no significant difference (p: 0.486, p: 0.884) compared to the intervention group based only on the post hoc test. However, it showed a significant difference between the control and the intervention group (P1: 0.043, P2: 0.010, P3: 0.032). This finding is consistent with research reported by Dara *et al*. (2011), which demonstrated that an intervention with a moderate and high amount of *tempeh* from the traditional market did not significantly affect total cholesterol levels in the blood of rats. Total cholesterol and LDL cholesterol are highly correlated. An increase in total cholesterol levels is followed by LDL cholesterol. This is because cholesterol and saturated fat from food is able to increase LDL cholesterol serum levels (Dara *et al*., 2011). Moreover, Haron *et al*. (2009) showed that the total isoflavone content from 100 g of raw *tempeh* based on its dry weight was 205±56 and was significantly reduced to 113±41 mg after the frying process. The comparison of total isoflavone content in raw and cooked *tempeh* is based on the dry weight, as the water content contributes to the isoflavone content variability (Haron *et al*., 2009). A finding from research by Utari *et al*. (2010) indicated that the steaming process is the least isoflavone-reducing process for *tempeh* (13.3%) compared to the frying process (39.15%) and boiling process (18.2%). Isoflavone is relatively susceptible to high heat. The higher the exposure to heat, the less remaining isoflavone compound is found in *tempeh* due to isoflavone transfer from the basic ingredients (Utari, 2010). Fadhilah (2019) showed that the duration of the intervention affected LDL level-lowering in rats. Soybean *tempeh* flour extract could better lower LDL blood serum levels after 30 days of intervention than after 10 and 20 days of intervention (Fadhilah, 2019). Therefore, it could be concluded that the longer the

intervention duration of *tempeh* flour intervention is, the greater the decrease in LDL levels.

Table 3. Post Hoc Test

Intervention group	Intervention group			
	Control	P1	P2	P3
Control	-	0.043	0.010	0.032
P1	0.043	-	0.486	0.884
P2	0.010	0.486	-	0.0581
P3	0.032	0.884	0.561	-

4. Conclusion

There was a significant decrease in LDL levels after an intervention of *tempeh* flour in all of the P1 (0.9 g), P2 (1.35 g), and P3 (1.8 g) dosage variations that were administered for 14 days. Moreover, the greatest LDL level decrease before and after the intervention was demonstrated by the P2 (1.35 g) dosage, which reduced LDL levels by 8.76 mg/dL.

Conflicts interest

There are no conflicts of interest in this research article.

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