

# Hypolipidemic Effect of Fermented Soymilk Related with Lactic Acid Bacteria in Feaces

Darimiyya Hidayati<sup>1,3</sup>, Soetjipto<sup>2</sup>, Annis Catur Adi<sup>1</sup>

<sup>1</sup>Faculty of Public Health, Airlangga University, Surabaya 60115, Indonesia, <sup>2</sup>Faculty of Medicine, Airlangga University, Surabaya 60132, Indonesia, <sup>3</sup>Faculty of Agriculture, Trunojoyo University, Bangkalan 69162, Indonesia

## Abstract

The problem of obesity is a global problem that is being faced in the world. One of the precautions taken is functional food. Fermented soy milk is a functional food that has a hypolipidemic effect. The purpose of this study was to analyze the relationship between the hypolipidemic effect of fermented soymilk and the content of lactic acid bacteria in animal experiments. The results showed that the provision of fermented soymilk and unfermented soymilk could increase the total content of lactic acid bacteria in rat feaces. This is in line with the decrease in total blood cholesterol levels in rats. Giving soy milk and fermented soymilk increases the total lactic acid bacteria in feaces thereby increasing cholesterol secretion in feaces

**Keyword :** hypolipidemic, fermented soymilk, lactic acid bacteria, total cholesterol, triglyceride

## Background

The problem of obesity is included in the third goal, namely in 2030 to reduce one third of premature deaths from non-communicable diseases through prevention and observation as well as promoting mental health and well-being in the concept of sustainable development (SDGs). Of the several risk factors that influence, obesity is one of the factors that is very considered because it increases the risk of several diseases, namely heart disease, diabetes, and high blood pressure. Around 38 million people in the world die from non-communicable diseases every year. Cardiovascular disease is the main cause (17.5 million people die each year), followed by cancer (8.2 million), respiratory disease (4 million) and diabetes (1.5 million) <sup>1</sup>.

The increase in obesity cases is also in line with the increase in cases of non-communicable diseases related to obesity. High cases of disease will be accompanied by an increase in health costs incurred for the treatment of diseases related to obesity. The high prevalence of diseases due to obesity will cause problems in the future because it increases health costs and decreases work productivity so that it can become an economic burden. Overweight and overweight children are related to health expenses for medicines, hospital stays, emergency rooms and US \$ 1.4 million annually. Improved hospital treatment with a diagnosis of obesity in children nearly doubled in 2005 when compared to data in 1999. The prevalence of diabetes doubled to 2% of the total population, hypertension increased to 9.5% of the total population and stroke increased to 1.2% of the total population. Based on the measurement of disease burden using Disability-adjusted life years (DALYs) in 2010 showed that stroke contributed the most in the overall burden of disease in Indonesia with a percentage of 8% <sup>2</sup>

Increased consumption of foods that are high in saturated fat, trans fatty acids, sugar and / or salt, combined with a lack of physical activity, contribute to obesity and diabetes, and other non-communicable diseases. The tendency to increase consumption of “junk food” which usually offers a larger portion of

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### Corresponding Author:

#### Darimiyya Hidayati

Faculty of Public Health, University of Airlangga  
Surabaya Indonesia, Campus C Mulyorejo Surabaya  
60115 East Java Indonesia

Faculty of Agriculture, Trunojoyo University, PO BOX  
2 Telang, Bangkalan, 69162, East Java Indonesia  
email : darimiyya@gmail.com

food, high in saturated fat and sugar is one of the dietary patterns that can trigger obesity. Improved economic status causes a shift in eating habits to a Western diet and a “comfort” lifestyle so as to increase calorie intake, decrease physical activity, and increase body weight<sup>3</sup>

Based on the factors of obesity, one of the things that affects is the food consumed. Food can affect body weight by controlling satiety or metabolic efficiency<sup>4</sup>. Several studies have shown that food sources have effects that can prevent obesity including mango leaf tea<sup>5</sup>, green coffee extract<sup>6</sup>, and fermented soymilk<sup>7-9</sup>

Several studies of fermented soymilk also show antiobesity or hypolipidemia<sup>7-9</sup>. Fermented soymilk has hypolipidemic and antiobesity effects because it can absorb fat and increase faecal fat excretion and function as an antioxidant<sup>9</sup> and increase leptin levels<sup>8</sup>. The effect of hypolipidemic is not followed by an increase in total fat in the liver as the results of a study by<sup>9</sup> which showed that the histology results of treated rats that did not experience fatty acid accumulation and decreased alaninamino transferase (ALT) and aspartate aminotransferase (AST) which is an indicator of liver damage. Whereas<sup>8</sup> showed that an increase in leptin in mice that received soy milk treatment so as to prevent insulin resistance.

One mechanism of fermented soymilk in reducing blood triglyceride and blood cholesterol levels is to increase the secretion of triglycerides and cholesterol in the fecal by lactic acid bacteria and soy isoflavones<sup>9,10</sup>. Therefore it is necessary to examine the relationship Between fermented soymilk feed, total lactic acid bacteria in faeces with hyperlipidemia.

## Method

### Preparation of soymilk

Soybeans soaked in water for 8 hours, washed and separated arinya skin. Then the soybean is ground with a ratio of soy: water is 1: 5. After the waste is separated, soy milk is sterilized at 115 °C for 10 minutes. Sterilized soymilk is divided into two parts, fermented soymilk and unfermented soymilk. Fermented soymilk inoculated with *Lactobacillus plantarum* bacterial concentration was 107 CFU / ml. Then soy milk was incubated for 18 hours at 37°C.

## Animal and treatment

The research sample was rat strain wistar with aged 8-9 weeks. The weight around 200-270 gram and obtained from Animal Center from Professor Nidhom Foundation (PNF). After being adapted for 2 weeks, the rats were divided into 4 groups (n=6) randomly : (1) control group; (2) HFD group, (3) HFD + soymilk (HFD + SM); (4) HFD + *L.plantarum* fermented soymilk (HFD+FSM). All rats had free access to water and were administrated intragastrically at 8.00 am daily. The study was conducted for 8 weeks and weight was measured weekly. Sampling faeces are taken every 2 weeks to measure the total lactic acid bacteria. Total lactic acid bacteria measured using the pour plate method. 1 gr of faeces diluted with sterile 0.85 % saline (w/v). The last dilution level was planted in MRS media and incubated for 48 hours. The growing colonies were counted and recorded as CFU per gram of faeces. The project was approved by the animal experimental ethics committee of Faculty of Nursing, Airlangga University. At the end of the study, rats were anesthetized using ketamine hydrochloride 300 mg/kg. Blood samples are collected through the heart arteries (intra cardiac). Blood is centrifuged for 10 minutes at 4 °C at a speed of 20,000 g. The serum was stored in an eppendorf tube at -20 °C before further analysis. Serum parameters including TC and TG levels were determined using analytical reagent kits.

## Statistic Analysis

Data is presented as means ± standard deviation (SD) and analyzed using SPSS software. Differences between groups were tested using ANNOVA with  $p < 0,005$  and Least Significant Difference (LSD) test with  $p < 0.05$ .

## Result and Discussion

The results showed that the total lactic acid bacteria for HFD+SM and HFD+FSM groups were higher than the control and HFD group. Differences in total lactic acid bacteria between groups occurred from the 2nd week. At the end of the study showed that the total lactic acid bacteria were lower when compared to HFD+SM and HFD + FSM groups. Total log lactic acid bacteria in faeces during experiment shows in Table 1.

**Table 1. Log total lactic acid bacteria in faeces during experiment**

Group	Mean	SD	P value
Week-0			
Control	5.87533 <sup>a</sup>	0.089536	0.459
HFD	5.29662 <sup>a</sup>	0.186323	
HFD+SM	5.29733 <sup>a</sup>	0.051391	
HFD+FSM	5.50133 <sup>a</sup>	0.051856	
Week-2			
Control	6.14633 <sup>a</sup>	0.017580	0.000
HFD	5.90367 <sup>b</sup>	0.083457	
HFD+SM	6.73800 <sup>c</sup>	0.048555	
HFD+FSM	6.76933 <sup>c</sup>	0.080401	
Week-4			
Control	7.37033 <sup>a</sup>	0.097465	0.000
HFD	6.47533 <sup>b</sup>	0.085184	
HFD+SM	7.46033 <sup>a</sup>	0.075176	
HFD+FSM	7.64133 <sup>c</sup>	0.045364	
Week-6			
Control	6.10633 <sup>a</sup>	0.036324	0.000
HFD	6.02367 <sup>a</sup>	0.097326	
HFD+SM	7.43200 <sup>b</sup>	0.090761	
HFD+FSM	7.98000 <sup>b</sup>	0.039930	
Week-8			
Control	6.60433 <sup>a</sup>	0.087952	0.000
HFD	6.51500 <sup>a</sup>	0.177482	
HFD+SM	8.04900 <sup>b</sup>	0.061527	
HFD+FSM	8.61267 <sup>b</sup>	0.092478	

Lactic acid bacteria can also provide anti obesity effect. Research <sup>8</sup> by shows that *L.plantarum* and *L. paracasei* can inhibit the differentiation of preadipocytes and reduce fat accumulation in 3T3-LI cells. Fermented soymilk with *B.bifidum*, *L.casei*, and *L.plantarum* can

significantly reduce weight, liver damage, fat index, and hyperlipidemia <sup>11</sup>.

Probiotics improve the microbial flora in the large intestine so that the composition of the beneficial bacteria

outweighs the harmful bacteria. Intestinal microbiota in NAFLD sufferers tends to have an increase in the bacteria Ruminococcus and Escherichia spp<sup>12</sup>. An increase in gram negative bacteria will increase the production of lipopolysaccharides (LPS) that enter the liver so that it can increase inflammation in the liver<sup>11</sup>. Provision of Lactobacilli, Bifidobacteria, and Streptococcus thermophilus probiotics can reduce the activity of jun N-terminal kinase (JNK) and reduce the DNA binding activity of the nuclear factor κB (NF-κB) so that TNF production decreases<sup>13</sup>

Lactic acid bacteria can improve fat metabolism by activating the 7-α hydroxylase cholesterol enzyme in the liver. Activation of this enzyme will increase faecal cholesterol secretion so that it can reduce cholesterol

in the blood<sup>10</sup>. Lactic acid bacteria also have the same role, namely increasing the secretion of cholesterol, triglycerides, and bile acids in faeces<sup>9</sup>. The supply of fermented soymilk provides probiotics and prebiotics so that it improves flora in the rat large intestine.

Rats triglyceride blood levels during the study ranged from 74.33 ± 24.8 - 130.17 ± 28.673. The highest triglyceride levels in the HFD group were 130.17 mg / dL, while the lowest triglyceride levels in the control group were 74.33 mg / dL. The total cholesterol level of rats during the study ranged from 50.83 ± 4.215 - 64.00 ± 7.823 mg / dL. The highest total cholesterol level in the HFD group was 64 mg / dL, while the lowest total cholesterol level in the HFD + FSM group was 50.83 mg / dL. Blood TG and TC levels are shown in Table 2.

**Table 2. triglyceride and cholesterol level in rat serum.**

Group	Triglycerida (mg/dL)		Total cholesterol (mg/dL)	
	Mean	SD	Mean	SD
Control	74.33 a	24.809	55.00a	8.877
HFD	130.17b	28.673	64.00a	7.823
HFD+SM	113.17b	23.302	51.00a	5.933
HFD+FSM	104.33ab	19.377	50.83a	4.215
P-value	0.006		0.012	

Fermented soy milk has a good effect for reducing total blood cholesterol. This is indicated by lower total blood cholesterol levels than the control group. While blood triglyceride levels for the HFD + FSM group were not different from the control group but were also not different from the HFD group. Unfermented soy milk also has a similar effect to unfermented soymilk.

Soybean has biogenic peptides which have the effect of improving blood lipid profile and liver steatosis<sup>14</sup>. The mechanism involved is soy and its bioactive peptide can stimulate bile acid secretion, change cholesterol metabolism in the liver, and regulate cholesterol

hormones and receptors. Research by<sup>15</sup>, soy protein intake of 31 - 47 grams per day can significantly reduce total cholesterol and LDL cholesterol, triglycerides, but not significantly to HDL values. Early cholesterol levels determine the effect of soy protein on cholesterol reduction

Soy protein can affect lipogenesis in the liver. Soy protein can reduce triglyceride concentrations in blood and in animal liver models. This effect is related to a decrease in the activity of lipogenic enzymes in the liver namely glucosa-6-phosphate dehydrogenase, malic enzyme, fatty acid synthetase, and acetyl-CoA

carboxylase (ACC). In addition, soy protein can activate PPARs, namely transcription factors involved in glucose homeostasis, fat metabolism and fatty acid oxidation <sup>16</sup>.

Research by <sup>17</sup> compared the administration of soy protein and cow's milk protein to liver steatosis in rats. The results showed that soy protein changed the microflora in the intestine so that it changed the metabolism of bile acids. Changes in microflora more lactic acid bacteria can reduce blood cholesterol levels because lactic acid bacteria have the enzyme bile acid hydrolase, thereby increasing the level of bile acids that are discharged into the faecal. Soy protein treatment can also increase ApoB-100 secretion so that it is thought to increase VLDL secretion from the liver. Soy protein treatment also increases the proportion of PUFA in the liver thereby reducing denovo lipogenesis in the liver.

Soy protein can improve insulin resistance and blood lipid profiles by increasing PPARs activation. According to <sup>18</sup> consumption of soy protein can increase PPAR $\alpha$  activation, and decrease SREBP1c activation. In addition, soy protein can reduce liver triglycerides and adipose tissue weight. This is related to the increased activity of enzymes involved in fatty acid oxidation, among others: CPT1. Soy protein is thought to increase the oxidation of fatty acids through the PPAR pathway because the administration of soy protein can increase PPAR $\alpha$  mRNA levels

The content of soy milk linolenic acid is also potential as a compound that can prevent obesity and liver steatosis. Omega 3 fatty acids can increase the oxidation of fatty acids in the liver and decrease TG synthesis by inhibiting the transcription activity of SREBP-1c and ChREBP factors in the liver. Omega 3 fatty acids can reduce the release of fatty acids from adipose tissue. In addition, omega-3 fatty acids can also inhibit macropage activation so that they are anti-inflammatory. Omega 3 fatty acids also increase the species of bacteria that can produce butyric acid in the intestine thereby reducing the production of lipopolysaccharides <sup>19</sup>.

Isoflavone supplementation can improve fat metabolism by activating the 7- $\alpha$  hydroxylase cholesterol enzyme in the liver. Activation of this enzyme will increase faecal cholesterol secretion so that it can reduce cholesterol in the blood <sup>10</sup>. Lactic acid bacteria also have the same role, namely increasing the secretion of

cholesterol, triglycerides, and bile acids in feces <sup>9</sup>

## Conclusion

Based on the results of the study showed that fermented soymilk has the effect of reducing blood cholesterol and triglyceride levels through an increase in total lactic acid bacteria in the large intestine.

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