

## The Effect of Gembili Yogurt (*Dioscorea esculenta L.*) Intake on Lipid Profile of Hypercholesterolemic Rats

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### ABSTRACT

A disorder known as hypercholesterolemia occurs when the blood's cholesterol concentration is higher than it should be. Food products like yogurt have been demonstrated to reduce blood pressure. The cholesterol-lowering properties of yogurt may be strengthened by the inclusion of inulin. Gembili (*Dioscorea esculenta L.*) is a food component with a high inulin content. The objective of this study is to ascertain how providing rats with high cholesterol affects their lipid profiles (total cholesterol, LDL cholesterol, and triglycerides). A straightforward randomized approach was used to divide the 30 male hypercholesterolemic Wistar rats into 1 control group and 4 intervention groups for the investigation. For 14 days, subjects received 1, 2, 3, and 4 ml of gembili yogurt every day. The CHOD-PAP method was used to calculate triglyceride levels, while the GPO-PAP method was used to calculate total cholesterol, LDL, and both. The lipid profile of hypercholesterolemic rats could be significantly reduced by administering gembili yogurt in doses of 1, 2, 3, and 4 ml/day over the course of two weeks. The group receiving the dose of 4 ml/day of yogurt experienced the greatest reduction, with total cholesterol levels of 100.68 mg/dl, LDL levels of 28.87, and triglyceride levels of 85.80 mg/dl.

**Keywords:** gembili yogurt; inulin; lipid profile; hypercholesterolemia

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### INTRODUCTION

Based on 2018 data, it showed an increase in the total cholesterol of the Indonesian population by 43%, an increase in triglycerides by 26% and increase in LDL by 83% (Anonim, 2019). One alternative that is safe to reduce total blood cholesterol levels is to modify the diet by consuming foods that are hypocholesterolemic. One of the products that have hypercholesterolemic properties is yogurt. Yogurt is a product obtained from pasteurized milk, then fermented with bacteria from Lactic Acid Bacteria (LAB) as a starter.

Consumption of yogurt from increases by years this can be seen from the value of increase in consumption of yogurt, in 2002-2005 the volume of yogurt increased by 70% from 1,039,279 L to 1,765,831 L (Central Bureau of Statistics, 2011). With increased consumption of yoghurt, it is necessary to improve the quality of yogurt. One of the improved in the quality of yogurt is the manufacture of synbiotic yogurt. Synbiotic yogurt is a combination of probiotics and prebiotics. There is a synergistic effect between probiotics and prebiotics that significantly lower cholesterol, so it can be an alternative food for hypocholesterolemic foods for people with hypercholesterolemia (Hendrati, 2014). There are many types of prebiotics, one of them is inulin. In Indonesia, there are many plants that are a source of inulin, namely from tubers. Gembili tuber (*Dioscorea esculenta L.*) is one of the plants that grows in Indonesia and contains high inulin. Istianah (2010) stated that inulin levels in gembili were 14.77%.

A disorder known as hypercholesterolemia occurs when the blood's cholesterol concentration is higher than it should be. Food products like yogurt have been demonstrated to reduce blood pressure. Yogurt's ability to decrease cholesterol may be enhanced by the inclusion of inulin (Rachman, 2015). Researched by Hai-Qing Ye (2016), consumption of oat-based frozen yogurt containing fermented oats, probiotics, and inulin can reduce total

cholesterol and tryglycerides in rats. Research on giving gembili yogurt to lipid profiles has never been done. Based on this description, this study was conducted to determine how the effect of giving gembili yogurt on the reduction of lipid profile in hypercholesterolemic Wistar rats.

## **METHODS**

### ***Material***

The ingredients used in this study to make gembili yogurt included gembili tubers bought at the Sopoonyo Market, fresh cow's milk bought at milk vendors on Jl. Raya Jemursari in Surabaya, and *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum* bought at the biology lab of the Faculty of Science and Technology, Airlangga University, Surabaya. Skim milk and sugar were added components. 30 Wistar rats were utilized in the testing with experimental rats, and they were fed a high-cholesterol diet made up entirely of cholesterol.

### ***Tool***

The tools used in this research were scales, blender, stove, autoclave, incubator, inoculation container, colony counter, test tube, other glassware, sonde, centrifuge, cholesterol reagent kit, LDL reagent kit, triglyceride reagent kit and others.

### ***Methods***

Make gembili yogurt begins with making gembili tuber filtrate. Gembili is peeled using a knife and made sure there is no more skin attached to the tuber. The peeled bulbs are washed with clean running water until the dirt is gone. Cleaned gembili is cut into small pieces using a knife to facilitate the next process. The bulbs are mashed using a blender with the addition of 1:3 water until smooth. A filter cloth is used to filter the fine gembili. The obtained filtrate from the gembili tuber was precipitated for an hour at room temperature before filtering. The gembili tuber filtrate was chilled after 15 minutes of pasteurization at 70°C. Fresh cow's milk was pasteurized at 70°C for 15 minutes to make yoghurt, and then 5% (by volume) skim milk and 8% (by volume) sugar were added. In a 50:50 mixture, the heated cow's milk and gembili tuber filtrate were added, and the mixture was then chilled. 5% (v/v) starters of *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum* were added to the chilled cow's milk before it was incubated at 27°C for 18 hours.

Tests with lab rats were conducted with 30 Wistar rats were kept in individual cage for adaptation period. Rats were fed a diet high in cholesterol in the form of pure cholesterol for 1 week. Rats that have been given for 1 week (week 0) checked for total cholesterol, low density lipoprotein, and triglyceride levels. The 30 rats divided into 5 groups were given a different diet consisting of 24% casein, 0.3% DL-Methionie, 61% cornstarch, 1% vitamin mix, 3.5% mineral mix, 0.2% choline chloride, alpha cell 5%, and corn oil 5% with 1 control group, and 4 treatments with doses of yogurt gembili 1 ml, 2 ml, 3 ml, and 4 ml. Mice fed with gembili yogurt and feed were checked for cholesterol levels at weeks 1 and 2 (total cholesterol, low density lipoprotein, and triglyceride).

In this study, a nested completely randomized design (CRD) was used with groups: feeding time (0,1, 2 weeks) and yogurt treatment factor at 4 levels (0,1, 2, 3, 4 ml) with 6 replications. The data obtained were tested by ANOVA.

The method for measuring total cholesterol and LDL levels was determined enzymatically colorimetrically using the CHOD-PAP (Cholesterol Oxidase – Para Aminophenazone)

method, while serum triglyceride or TG levels were determined enzymatically colorimetrically using the GPO-PAP (Glycerol Phosphate Oxidase  $\pm$  Para Aminophenazone) method.

## RESULT AND DISCUSSIONS

### **Total cholesterol**

Blood sampling was carried out in all treatment groups after the acclimatization stage, the high-cholesterol feeding stage, and the intervention stage. Relationship between intake yogurt and total cholesterol levels in hypercholesterolemic rats can be seen on Figure 1.

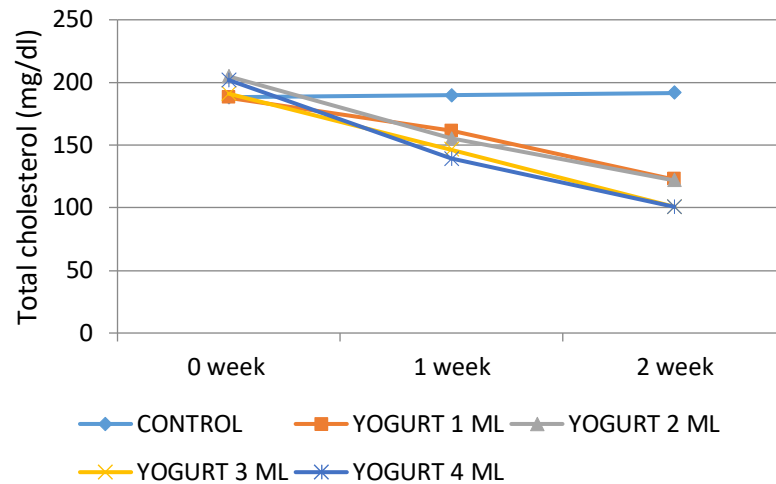


Figure 1. The Relationship Between Intake Yogurt And Total Cholesterol Levels in Hypercholesterolemic Rats

Figure 1. shows that in the control there was an increase in total blood cholesterol, while with 1, 2, 3, and 4 ml yogurt there was decrease in total blood cholesterol. Yogurt is a probiotic which in its fermentation use lactic acid bacteria. The consumption of probiotics which contain lactic acid bacteria become a natural way for lowering cholesterol levels (Baroutkoub et al, 2010). The addition of the bacteria *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and inulin contained in gembili yogurt contributed to the reduction in total cholesterol levels. Because inulin increases viscosity and thickens the lining of the small intestine, cholesterol is less likely to be absorbed and is more likely to be eliminated through stools. This causes cholesterol to degrade and has a hypocholesterolemic effect. (Lay, 2010).

In addition, yogurt can bind cholesterol. The CHOD-PAP method was used to calculate triglyceride levels, while the GPO-PAP method was used to calculate total cholesterol, LDL, and both. The lipid profile of hypercholesterolemic rats could be significantly reduced by administering gembili yogurt in doses of 1, 2, 3, and 4 ml/day over the course of two weeks. The group receiving the dose of 4 ml/day of yogurt experienced the greatest reduction, with total cholesterol levels of 100.68 mg/dl, LDL levels of 28.87, and triglyceride levels of 85.80 mg/dl. (Beylot, 2005).

The processes by which cholesterol is absorbed and converted to coprostanol are part of LAB's mechanism for lowering cholesterol. Through the process of cholesterol assimilation, lactic acid bacteria take up cholesterol, which is subsequently integrated into the bacterial cell membrane and prevents cholesterol from being absorbed. Because lactic acid bacteria produce the cholesterol reductase enzyme, they can also convert cholesterol into coprostanol

molecules. The small intestine does not absorb coprostanol; instead, it is ejected along with feces. (Baroutkoub et al, 2010).

### **LDL (Low Density Lipoprotein)**

Intake yogurt gembili on hypercholesterolemic rats has a significant effect on LDL level. Relationship between intake yogurt and LDL level in hypercholesterolemic rats can be seen on Figure 2.

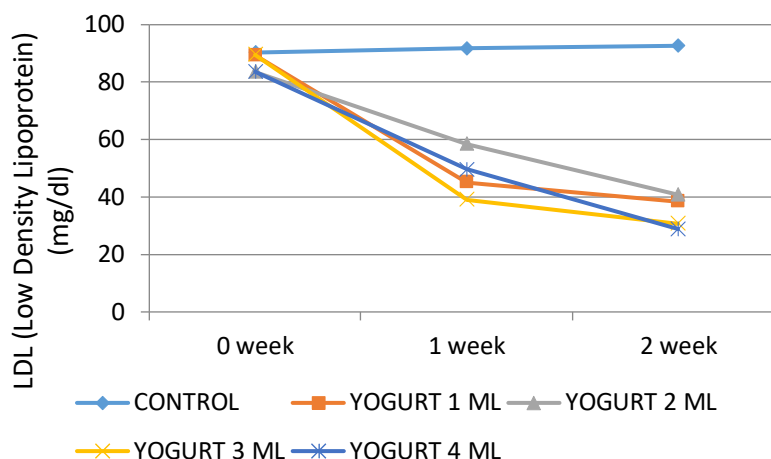


Figure 2. The Relationship Between Intake Yogurt and LDL Level in Hypercholesterolemic Rats

Figure 2. shows an increase in LDL in the control, whereas with 1, 2, 3, and 4 ml yogurt there was a decrease in LDL. Inulin as a prebiotic and lactic acid bacteria *Streptococcus thermophiles* and *Lactobacillus bulgaricus* found in synbiotic yogurt affect the reduction of LDL cholesterol level (Zainudin et al, 2008). The hypotriglyceride impact of inulin through suppression of lipogenic enzyme activity in the liver, leading to reduced triglyceride production, is the mechanism of LDL cholesterol lowering. Reduced triglyceride synthesis results in less VLDL secretion. Because triglycerides are broken down during the production and secretion of VLDL, IDL (intermediate density lipoprotein) and residual VLDL are formed. This results in a decrease in blood LDL cholesterol levels. Through LDL receptors, IDL or residual VLDL can either be absorbed by the liver directly or transformed into LDL. (Karlina, 2013).

Yogurt is a probiotic that uses lactic acid bacteria in its fermentation. Consumption of probiotics containing lactic acid bacteria will be a natural method to reduce blood cholesterol levels (Karlina, 2013). The mechanism of probiotics in reducing LDL is that inulin undergoes a fermentation process by probiotics to create propionic acid, a short-chain fatty acid. By preventing the HMGCoA reductase enzyme from working, propionic acid lowers the amount of cholesterol produced in the liver. The enzyme HMGCoA reductase is involved in the liver's production of cholesterol. The synthesis and secretion of VLDL are inhibited when cholesterol biosynthesis is reduced. Because LDL is a byproduct of VLDL metabolism, VLDL production and secretion can lower LDL levels. (Akoma et al, 2000).

### **Triglyceride**

Intake yogurt gembili on hypercholesterolemic rats has a significant effect on triglyceride level. Relationship between intake yogurt and triglyceride level in hypercholesterolemic rats can be seen on Figure 3.

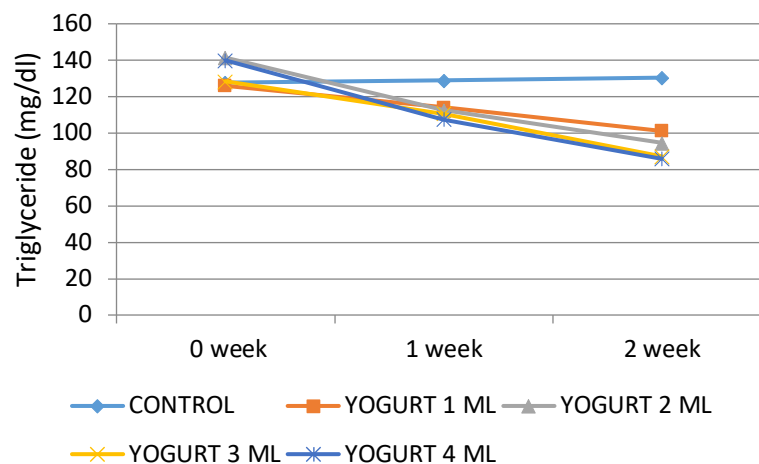


Figure 3. The Relationship Between Intake Yogurt and Triglyceride Level in Hypercholesterolemic Rats.

Figure 3. shows that in the control there was an increase in triglycerides, while with the administration of 1, 2, 3, and 4 ml yogurt there was a decrease in triglycerides. The decrease in triglyceride occurs due to the presence of inulin and lactic acid bacteria found in gembili yogurt. Another study conducted on rats stated that the influence of inulin can inhibit lipogenic enzymes that synthesize fatty acids in the liver so can reducing trygliceride levels (Kaur, 2012). The mechanism of reducing triglycerides by inulin as a prebiotic is by inhibiting the activity of lipogenic enzymes in synthesizing triglycerides in the liver. Lipogenic enzymes consist of acetyl coenzyme A (coA), malic enzyme, ATP citrate lyase, and fatty acid synthase. In fatty acid synthase, inulin inhibits the expression of mRNA genes in regulating the activity of the fatty acid synthase enzyme so that it can inhibit the formation of triglycerides in the liver (Nasar et al, 2013).

Previous research states that the administration of 4 ml of synbiotic yogurt for 2 weeks in rats can significantly reduce triglycerides by 70.10 mg/dl (Regie, 2014). The mechanism of reducing triglyceride levels by probiotics is that lactic acid bacteria (LAB) ferment inulin into short-chain fatty acids such as butyric acid and propionate. Furthermore, propionate competes with acetic acid transporters to hepatocyte cells. Propionate has a role in inhibiting the process of lipogenesis in the liver while acetate acts as a substrate for lipogenesis. By inhibiting the process of lipogenesis, the triglyceride level can decrease (Letexier D, 2002). In addition to propionate, probiotics are also able to modify the gene expression of peroxisome proliferator receptor (PPAR) in regulating triglyceride balance in adipose tissue. ppar is one of the receptors of nuclear receptor and is also a target gene of hemeostatic energy and adipogenesis (Zhang and Zhang, 2013).

In two studies that used either oligofructose (20 g/d) or inulin (14 g/d), there was no difference in fasting total, LDL, or HDL cholesterol or blood triglycerides. Two other studies that provided inulin either in a morning cereal (9 g/d) or as a powdered supplement to beverages and meals (10 g/d) also revealed similar reductions in fasting triglycerides (27 and 19%). In one of these investigations, the levels of total and LDL cholesterol were also somewhat reduced (5 and 7%, respectively). Because inulin's ability to decrease triglycerides in animals relies mostly on inhibiting hepatic fatty acid production, and because this pathway is largely inactive in humans unless they consume a lot of carbohydrates, Future studies

showing inulin's ability to decrease cholesterol should take into account the background diet as a predictor of response. (Christine, 2009).

## CONCLUSION

In conclusion, there is a significant interaction between total cholesterol, LDL, and triglyceride levels on intake of yogurt with different doses in hypercholesterolemic rats based on research on the effect of intake yogurt gembili with doses of 1 ml, 2 ml, 3 ml, and 4 ml/day for 2 weeks on lipid profiles in hypercholesterolemic rats. With total cholesterol levels of 100.68 mg/dl, LDL levels of 28.87 mg/dl, and triglyceride levels of 85.80 mg/dl, the best treatment group experienced the greatest reduction.. It is necessary to conduct clinical trials of gembili yogurt consumption on humans to see if it has the same effect as the rat study.

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