

Functional Effect of Lesser Yam Symbiotic Yoghurt on Hypercholesterolemia Wistar Rats

Rosida^{1*} and S S Santi²

¹Food Technology Department, Engineering Faculty, UPN Veteran Jawa Timur Indonesia

²Chemical Engineering Department, Engineering Faculty, UPN Veteran Jawa Timur Indonesia

Abstract. Synbiotic lesser yam yoghurt is fermented drink made from the mixture of cow's milk and lesser yam extract which has functional benefits. Lesser yam yoghurt contains lactic acid bacteria, lactic acid, inulin, and soluble fiber which can improve the lipid profile of hypercholesterolemia sufferers. This study aims to determine the effect of the intake of lesser yam yoghurt on the lipid profile of hypercholesterolemia wistar rats. This study used nested randomized design with two factors, the first factor was a feeding group (0,1,2,3,4 ml/day, while the second factor was the time of blood collection (0, week 1 and week 2). The data obtained were analyzed by using ANAVA and further test DMRT (Duncan Multiple Range Test) with a level of 5%. The results showed that giving lesser yam yoghurt for 2 weeks had a significant effect on the lipid profile improvement. Rats from 4 ml of yoghurt treatment experienced a decrease in total serum cholesterol by 48.4%, triglyceride levels by 35.8% and LDL levels by 65,5% and an increase in serum HDL levels by 150.3%. So it can be concluded that the intake of synbiotik lesser yam yoghurt has functional benefits in improving the lipid profile of hypercholesterolemia rats.

Keywords: hypercholesterolemia rats, synbiotic yoghurt, lesser yam tubers, lipid profile

1 Introduction

Hypercholesterolemia is a condition in which the concentration of cholesterol in the blood exceeds the normal value [1]. The mechanism of hypercholesterolemia is that fats from food will undergo digestive process in the intestines to become free fatty acids, triglycerides, phospholipids and cholesterol. Then it is absorbed into the form of chylomicrons. The rest of the breakdown of chylomicrons circulates to the liver and is broken down into cholesterol. Some of this cholesterol is excreted in the bile as bile acids and partly together with triglycerides will combine with certain proteins (apoproteins) and form Very Low Density Lipoprotein (VLDL), which is further broken down by lipoprotein enzymes into non-insoluble Intermediate Density Lipoprotein (IDL). can last 2-6 hours because it will directly be converted into Low Density Lipoprotein (LDL) [2].

One alternative way to lower total blood cholesterol levels is by modifying the diet. Diet modification can be done by consuming foods that contain probiotic and prebiotic components, otherwise known as synbiotics. In this study lesser yam together with fermented milk were developed into synbiotic yogurt. Lesser yam contains prebiotic components, such as inulin, FOS, GOS and dietary fiber. Meanwhile, milk fermented with

lactic acid bacteria contains probiotic components. The two components will form a synbiotic yogurt.

Lesser yam is a species of *Dioscorea* spp. This yam has the prospect to be developed as a functional food because it contains a lot of inulin and dietary fiber, or is often called a prebiotic component. Previous research has shown that lesser yam contains high levels of dietary fiber (3.05%) and inulin (1.20%) [3]. Inulin is a soluble dietary fiber which is beneficial for digestion and body health [4]. Lesser yam tubers also contain water soluble dietary fiber in the form of Water Soluble Polysaccharides (PLA) which are viscous and form gel that can inhibit macronutrient absorption and reduce postprandial glucose response, so that it has hypoglycemic effect [5].

Previous in-vitro studies have evaluated a number of proposed mechanisms for the cholesterol-lowering effect of probiotics and prebiotics. One recognized mechanism includes the enzymatic deconjugation of bile acids by probiotic bile salt hydrolases. Bile, the water-soluble end product of cholesterol in the liver, is stored and concentrated in the gallbladder, and is released into the duodenum when food is swallowed. It consists of cholesterol, phospholipids, conjugated bile acids, bile pigments and electrolytes. Once deconjugated, bile acids are less soluble and are absorbed by the intestines, leading to their elimination in the feces. Cholesterol is used to synthesize new bile

* Corresponding author: rosidaupnjatim@gmail.com

acids in a homeostatic response, resulting in a decrease in serum cholesterol [6].

The decrease in blood cholesterol levels due to consuming synbiotic yogurt is thought to be due to the content of inulin and lactic acid bacteria contained in synbiotic yogurt. The mechanism is that inulin is known to increase viscosity in the gastrointestinal tract and increase the thickness of the lining of the small intestine, thereby preventing the absorption of cholesterol and increasing its excretion through feces. This causes cholesterol catabolism and has a hypocholesterolemic effect [7]. In addition, inulin is fermented in the colon by LAB to produce short chain fatty acids, such as butyric acid and propionic acid. These short chain fatty acids can affect cholesterol metabolism in the liver. Propionic acid was identified to reduce cholesterol levels by inhibiting the action of the enzyme hydroxy- β methyl glutamyl CoA (HMG-CoA) reductase which plays a role in cholesterol synthesis. Propionic acid can also inhibit the incorporation of acetate into plasma cholesterol by competing with acetic acid transporters to the hepatocyte cells. This will result in a decrease in cholesterol synthesis because acetate is a precursor in the formation of cholesterol [8].

Previous research has shown that rats fed modified white cassava flour can reduce total cholesterol, LDL and blood triglycerides because they contain relatively high dietary fiber (13.8%) [9].

2 Materials and Methods

The materials used in this study were lesser yam obtained from Sopyonyo Market, Rungkut, Surabaya, cow's milk, sugar, skim milk and yogurt starter (*Lactobacillus bulgaricus*, *Streptococcus thermophilus*, and *Bifidobacterium bifidum*) obtained from the Biology Laboratory, Faculty of Science and Technology, Airlangga University, Surabaya and chemical reagents for analysis

2.1 Lesser Yam Yoghurt Production

Fresh cow's milk was pasteurized at 70°C for 15 minutes and added with 5% (w/v) skim milk and 8% (w/v) sucrose. Cow's milk which is being heated is mixed with lesser yam tuber filtrate in a ratio of 50:50 and then cooled. Cold cow's milk was inoculated with 5% starter (v/v) and incubated at 37°C for 18 hours.

2.2 Bioassays

A total of 30 white Wistar rats (aged 2-3 months, average weight 200 g) were put in closed individual cages with the following cage conditions: light is not controlled, air ventilation in the cage is sufficient, and the temperature of the cage uses room temperature. The experimental rats fed high cholesterol diet (from cow brain) for 7 days to make the rats become hypercholesterolemia. Then the rats were fasted for 12 hours and taken blood through the eyes (plexus retroorbitalis) to measure blood cholesterol levels and ensure that the rats were positive for

hypercholesterolemia. Then the rats were divided into 5 (five) feed groups, each of which consisted of 6 (six) rats, namely the control group which was given water, and the group that was given lesser yam synbiotic yogurt as much as 1, 2, 3, 4 , ml per day besides standard diet. The making of standard diet referred to the AIN-93 standard diet formula [10]. The rat blood serum was analyzed for total cholesterol, HDL, LDL, and serum triglycerides level.

This study used a nested randomized design with two factors, the first factor was a feeding group (0,1,2,3,4 ml/day, while the second factor was the time of blood collection (0, week 1 and week 2). The data obtained were analyzed by using ANAVA and further test DMRT (Duncan Multiple Range Test) with a level of 5%.

3 Equations and Mathematics

The preliminary study revealed that lesser yam yogurt from proportion of milk : lesser yam filtrate (50:50) and 5% starter concentration was the best treatment which in relatively high levels of total BAL (7.24 log cfu/ml), dietary fiber (3.05%), and inulin (1.20%) [3]. This sinbiotic yogurt was further analyzed its hypocholesterolemic effect using experimental rats.

3.1 Serum Cholesterol Total and Triglyceride

At the beginning, after hypercholesterolemia diet intervention, cholesterol total, triglyceride, LDL level of all rats were very high. The initial cholesterol total and triglyceride level were 188,2-196.4 mmol/dL and 127.2-133.6 mmol/dl, respectively. Total cholesterol and triglyserida level of experimental rats can be seen on Fig. 1 and Fig. 2

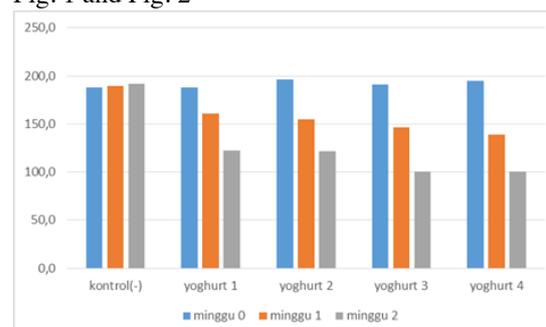


Fig. 1. Serum cholesterol total of hypercholesterolemia rats after two week feeding treatment

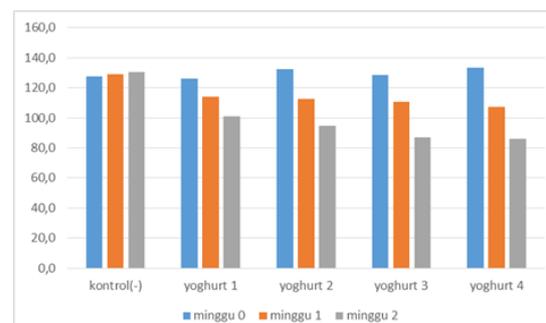


Fig. 2. Serum triglyceride level of hypercholesterolemia rats after two week feeding treatment

After 14 day intervention, serum cholesterol total and triglyceride level of lesser yam group decreased by 48.40% and 35.80%, respectively. However, those of control group were constant (188.2 – 191.2 mg/dL) and (127,7-130,4 mg/dL), respectively. The data proved that consuming lesser yam yoghurt had potency to reduce blood cholesterol due to its inulin and dietary fiber content.

The reduction of cholesterol (Fig 1) was in accordance with triglyceride (Fig 2). Dietary fiber and resistant starch diet could inhibit cholesterol absorption in the small intestine and finally reduce cholesterol level in the blood plasma and increase cholesterol production in the liver, bile acid production and cholesterol excretion in the feces [11].

The hypocholesterolemic effect of probiotics is also associated with their ability to bind cholesterol in the small intestine. Usman [12] previously reported that *Lactobacillus gasseri* strains can remove cholesterol from laboratory media by binding to cellular surfaces. Cholesterol binding ability appears to be growth and strain specific.

Cholesterol can also be converted in the intestine into coprostanol, which is directly excreted in the feces. This decreases the amount of cholesterol absorbed, causing a decrease in the concentration in the physiological cholesterol pool. The possibility of conversion of cholesterol to coprostanol by bacteria has been evaluated. In their study, the authors found that cholesterol dehydrogenase/isomerase produced by bacteria such as: *sterolibacterium denitrificans* is responsible for catalyzing the transformation of cholesterol to cholest-4-en-3-one, an intermediate cofactor in the conversion of cholesterol to coprostanol. This serves as the basis for further evaluation using probiotic bacterial strains [13].

3.2 Serum LDL and HDL level

Serum LDL and HDL level of experimental rats can be seen on Fig. 3 and Fig. 4

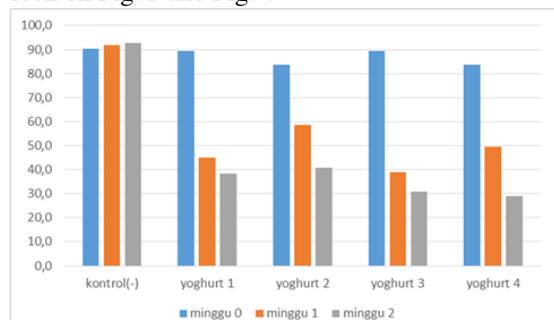


Fig. 3. Serum LDL level of hypercholesterolemia rats after two week feeding treatment

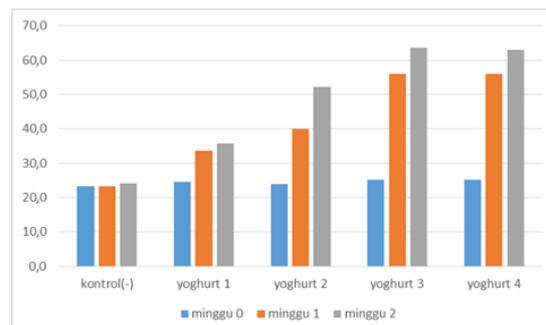


Fig. 4. Serum HDL level of hypercholesterolemia rats after two week feeding treatment

After hypercholesterolemia diet intervention, all rats had high LDL serum and low HDL serum. However, after feeding treatment, the LDL level of lesser yam yoghurt groups were lower than those of control, while the HDL level of treatment groups were higher than those of control (non treatment group). The reduction LDL level (Fig 3) was in accordance with cholesterol total reduction (Fig 1). It indicated that lesser yam yoghurt had potency in lowering blood LDL and increasing HDL level.

Donkor et al. [14] stated that yoghurt is a probiotic drink that is useful in lowering cholesterol, protecting against intestinal infections, colon cancer, anticarcinogenic, antihypertensive and increasing HDL cholesterol. According to Kai [15] total cholesterol and triglycerided level was reduced and HDL cholesterol level was increased in the rats of administration group. The lipid metabolism is modulated trough promoting lipid oxidation and cholesterol homeostasis. Beside thta, the high fiber and high resistant starch diet could increase LDL receptor activity in the liver. This activity fulfilled the availability of tissue cholesterol, so more blood cholesterol was used that reduced blood cholesterol level [11].

4 Conclusion

The hipercholesterolemia rats, after two week feeding treatment had lower cholesterol total, TG, and LDL level, but higher HDL level than control grup, especially those which were feed 4 ml yoghurt per day. The research revealed that lesser yam yoghurt had hypocholesterolemic effect due to its inulin and dietary fiber content.

References

- [1]. Guyton, A.C., dan Hall, J.E. 2008. Buku Ajar Fisiologi Kedokteran. Edisi 11. Jakarta: EGC.
- [2]. Soeharto, I. 2004. Lemak dan Kolesterol Edisi Kedua. Jakarta : PT Gramedia Pustaka. Utama.
- [3]. Rosida, S. S.Santi, F.R.Rohman. 2021. Study of Proportion of Milk with Lesser Yam Filtrate and Starter Concentration for Producing Synbiotic Yoghurt. International Journal of Eco-Innovation in Science and Engineering Vol 2 (02) 2021 (20-25).

- [4]. Sardesai V.M. 2003. Introduction to Clinical Nutrition. 2nd ed. Marcel Dekker, Inc, USA
- [5]. Prabowo, A.Y., Estiasih, T. & Purwantiningrum, I. 2014. Umbi gembili (*Dioscorea esculenta* L.) sebagai bahan pangan mengandung senyawa bioaktif: Kajian pustaka. *Jurnal Pangan dan Agroindustri*, **2** (3), 129–135
- [6]. Begley, M.; Hill, C.; Gahan, C.G.M. Bile Salt Hydrolase Activity in Probiotics. *Appl. Environ. Microbiol.* 2006, **72**, 1729-1738.
- [7]. Lay, G.O dan Min, T.L. 2010. Cholesterol-Lowering Effects of Probiotics and Prebiotics: A Review of in Vivo and in Vitro Findings. *Int. J. Mol. Sci.* **11**, 2499-2522.
- [8]. Beylot M. 2005. Effects of Inulin-Type Fructans On Lipid Metabolism In Man And In Animal Models. *British Journal of Nutrition.* **93**, Suppl. 1, S163–S168
- [9]. Rosida, Purnawati, A, Susilowati, E.K. 2018 Hypocholesterolemic and hypoglycemic effects of autoclaved-cooled water yam (*Dioscorea alata*) on hypercholesterolemia rats, *IFRJ* **25** suppl 2
- [10]. Reeves, P. G. 1997. Components of AIN-93 diets as improvements in the AIN-76A diet. *Journal of Nutrition* 127: 838S-841S.
- [11]. Anderson, J. W., Baird, P., Davis, R. H. Jr., Ferreri, S., Knudtson, M. and Korarym, A. 2009. Health benefits of dietary fiber. *Nutrition Review* **67**(4): 188-205.
- [12]. Usman, H.A. 1999. Bile Tolerance, Taurocholate Deconjugation, and Binding of Cholesterol by *Lactobacillus gasseri* Strains. *J. Dairy Sci.* 1999, **82**, 243-248.
- [13]. Chiang, Y.R.; Ismail, W.; Heintz, D.; Schaeffer, C.; van Dorsselaer, A.; Fuchs, G. Study of Anoxic and Oxic Cholesterol Metabolism by *Sterolibacterium denitrificans*. *J. Bacteriol.* 2008, **190**, 905-914.
- [14]. Donkor, O., Nilmini, S., Stolic, P., and Vasiljevic, T., 2007. Survival and activity of selected probiotic organisms in set-type yoghurt during cold storage. *International Dairy Journal* (17): 657–665.
- [15]. Kai, Z. and Blanchard, Z. C. 2015. Resistant starch manipulated hyperglycemia / hyperlipidemia and related genes expression in diabetic rats. *Int. J. of Biological Macromolecules* **75**: 316-321.