Anti-Obesity Effect of Yoghurt Synbiotic in High Fat Diet Induced Wistar Rats

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Abstract. The prevalence of obesity which tends to increase is also known to be one of the triggers for various degenerative diseases. The main reason for obesity is unbalanced lifestyle. One solution that can be applied in overcoming the problem of degenerative diseases is by consuming functional food such as probiotics. Probiotics are mostly familiar in fermented products, especially milk-based products as well as yoghurt. This study uses yoghurt added with sweet potatoes containing Lactococcus lactis subsp. cremoris and Streptococcus salivarius subsp thermophilus as a starter culture. This study aims to determine the effect of giving synbiotic yoghurt towards body weight’s decrease in wistar rats feed under a high-fat diet. In this study, 24 wistar rats were grouped into 4 treatment groups particularly P1 (control); P2 (by giving yoghurt with a dose of 1 ml per day); P3 (1.5 ml per day); and P4 (2.5 ml per day). The treatment is carried out for 3 weeks and weighing is done every week. The results showed that the treatment group had the ability to reduce body weight significantly (p<0.05) compared to the control group.

1. Introduction
Obesity is a common health condition today. Obesity is a condition where excessive or abnormal fat accumulation occurs, causing risk and disturbance for health [1]. The case of obesity often arises in countries with high socio-economic condition, but now it is starting to shift to countries with medium to low socio-economic conditions [2]. Daily lifestyle is very closely related to obesity. The shift in traditional diets that are high in carbohydrates, fiber and low in fat towards new diets that are high in calories, high in fat and low in fiber plays a major role in the current cases of obesity [3].

The prevalence of obesity in the world is constantly increasing. Almost half a billion people in the world are classified as overweight or obese. Whereas for Indonesia, the prevalence of obesity reaches 19.1% of the population aged 15 years and over, even for East Java the prevalence of obesity exceeds the national incidence of 20.4%. Obesity is very closely related to dyslipidaemia. One of the most common conditions of dyslipidaemia is hypercholesterolemia [4]. Hypercholesterolemia and obesity are the main risk factors for atherosclerosis. This was mentioned in previous studies, that even without the presence of other risk factors, hypercholesterolemia and obesity are able to stimulate the formation of lesions in the walls of blood vessels that result in atherosclerosis [5]. Atherosclerosis due to hypercholesterolemia has the potential to cause various cardiovascular diseases [6] which causes

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nearly 15 million deaths [7, 1]. If this condition continues, it is predicted that in 2020, cardiovascular disease, especially atherosclerosis, will become the main cause of non-accidental death [8].

The function of probiotics in reducing blood cholesterol levels has been widely proven in various studies [9, 10, 11]. Probiotics are beneficial microbes which improves the balance of microorganisms in the digestive tract. Indirectly, probiotics have an important role for health. Based on previous research, lactic acid bacteria (LAB) has the potential to reduce blood cholesterol level from fermentation results in organic acids such as lactic acid which acts as a cholesterol-lowering agent through the process of cholesterol assimilation and deconjugation of bile acids, where cholesterol will bind to the cell wall probiotics, which then form short chain fatty acids thus inhibiting the formation of cholesterol by the hepatic system [12, 13].

Based on the scientifically proven function of probiotics for overcoming metabolic diseases, especially cardiovascular diseases caused by dyslipidaemia, it is necessary to come up with an alternative fermentation product that is more accessible and preferred by the people in Indonesia. In this study the addition of sweet potatoes aimed to increase the effectiveness of lactic acid bacteria. Lactic acid bacteria can grow well on tubers media that are rich in oligosaccharides. Based on the reason above, this research is conducted to develop the functional aspects of probiotics by supplementing prebiotics from sweet potatoes in fermented milk products, therefore synbiotic yoghurt which have nutritional value and functional components for health is produced.

2. Materials and Methods
The ingredients used in this study include skimmed milk powder, yellow sweet potato, Lactococcus lactis subsp. cremoris and Streptococcus salivarius subsp thermophilus, mineral water, distilled water, 70% alcohol, material for testing viability, total acid, pH, and in vivo testing using experimental animals.

The tools used include pasteurizer, incubator, refrigerator, stirrer, analytical balance (Camry), sonde needle (force feeding need) for mice, syringe, micro tube, micro tip, micro tip, micro dropper, cage, food container, rat drinking bottles.

2.1. Preparations to make yoghurt synbiotic
Yoghurt synbiotic is made by pasteurizing skim milk 10% (w/v) and yellow sweet potato 5% (w/v) at 80 °C for 15 minutes. The milk’s temperature is then reduced to 40 °C and subjected to inoculation of the lactic acid bacteria culture starter of Lactococcus lactis subsp. cremoris and Streptococcus salivarius subsp thermophilus with a concentration of 3%. The milk is then incubated at 37°C for 20 hours. After the yoghurt fermentation process, the viability of lactic acid bacteria, pH, and total acid is then tested [14, 15, 16].

2.2. Preparation of Wistar white rat
The white rats used in this study were 24 Wistar strain male rats and were adapted for one weeks to its feed and the environment. The cage used is an individual cage with a size of 30x20x20 cm. The ingredients used to create overweight conditions in wistar rats are quail egg yolk and goat fat of 1 gr quail egg yolk / rat / day and 2 g goat fat / rat / day for one weeks.

2.3. Research design
The research design used was the Pre and Post Test with Control Group Design. This study uses a completely randomized design (CRD) research method under one factor, the dosage form. Each rat was given the same feed which was modified with AIN-93M standard feed. The rats were divided into 4 treatment groups, namely: P1 control group (rat given standard feed and aquades), P2 treatment group (rat given standard feed and 1 ml / day of synbiotic yoghurt), P3 treatment group (rat given standard food and synbiotic yoghurt as much as 1.5 ml / day), and the P4 treatment group (rat given standard feed and synbiotic yoghurt as much as 2.5 ml / day). The treatment was carried out for 21 days and the weight was monitored every week.
2.4. Data analysis
The data obtained were tabulated and analysed statistically by Analysis of Variants (ANOVA) using Microsoft Excel 2007 program. If a difference is indicated, then a further BNT test with a 5% level is performed to see the difference of each treatment.

3. Results and discussion
A chemical and physical test to determine the best treatment of skim milk yogurt fortified with yellow sweet potato was carried out. Data of physical testing for each additional concentration of sweet potatoes into liquid skim milk were obtained from previous studies. Chemical and physical tests used to determine the best treatment include viability of lactic acid bacteria, pH, total acid, and viscosity. Based on the weighing results, skim milk yogurt fortified with yellow sweet potato as much as 5% (w/v) as the best treatment. The test results are presented in Table 1.

| Parameter                     | Value       |
|-------------------------------|-------------|
| Viscosity                     | 3423 cp ± 6.65 |
| Total Acid                    | 1.54% ± 0.04 |
| pH                            | 3.9 ± 0.15  |
| Viability of Lactic Acid Bacteria | 7.15 x 10⁹ CFU/ml ± 0.09 |

Based on the results of physical and chemical testing, the addition of sweet potatoes can increase the viscosity of yogurt. The increase in the viscosity value is due to the addition of sweet potato starch which functions as a thickener. This is consistent with the statement made by Goncalves et al. [17] which states that the more concentration of thickener, the binding capacity of water will also increase.

The total acid produced from synbiotic yogurt is 1.54%. These results are still suitable with the requirements by SNI [18] where the standard amount of acidity in yogurt is 0.5-2%. Lactic acid is the largest acid component produced in yogurt fermentation process. According to Irvine and Hekmat (2011) [19], acids in yogurt comprise of 59% lactic acid; 28% citric acid; 5.3% acetic acid; 2.4% formic acid; 2.3% succinic acid and a number of other acids component. The pH produced from this study was 3.9. This pH value also comes close to the pH of cow milk yogurt which is 3.7 to 4.33 [20]. The conversion of lactose to lactic acid also decreases the pH and increases total acid, therefore more culture starter is added, the pH decreases and the total acid increases. The pH of yogurt correlates to the amount of acid. The increase in pH is due to the decrease in the number of H + ions which is triggered by a decrease in total acid [21].

This caspian starter contains Lactococcus lactis bacteria which is a probiotic that has the ability to produce lactic acid and producing sour taste in yogurt and support the coagulation of proteins. S. thermophilus which is also found in this starter helps lactose digestion by producing the enzyme lactase. S. thermophilus also ferment lactose homofermentatively [22]. This bacterium can convert more than 85% of lactose into lactic acid. Glucose, fructose and manosa can also be fermented, but galactose, maltose, and sucrose fermentation can only be carried out by certain strains. The content of this probiotic will cause the characteristic aroma of fermented product.

The viability of lactic acid bacteria produced from this test is 7.15 x 10⁹ CFU / ml. The minimum total LAB in probiotic food is 6 log CFU/g [22]. The recommended dosage to benefit from the probiotics is 8 log CFU / g per serving [23]. Oligosaccharides in sweet potatoes are one of the ingredients fermented by lactic acid bacteria, therefore if more sweet potatoes are added, the levels of lactic acid increases and the pH gets smaller. These acidic properties provide an optimal environment to support the survival of probiotics.

Synbiotic yogurt is then in vivo tested on animals to find out the effect of giving synbiotic yogurt towards the reduction in body weight of male white rats. Feed intake was seen from the remaining feed that was weighed every day during the study suited with the amount of feed given (15 g/head /day) with the rest of the rat feed during the treatment period every day. Based on the analysis of
ANOVA variance, the treatment given to the rat group had no significant effect ($p > 0.05$) towards feed consumption. The average amount of feed intake consumed by rats in each treatment group can be seen in Figure 1.

![Graph of changes in feed consumption during treatment](image1)

**Figure 1.** Graph of changes in feed consumption during treatment

Weighing rats was carried out for each group of rats which aims to identify the increase or decrease in rat weight. The body weight of the rats body were weighed on days 0, 7, 14, and 21. The graphs of rats body weight change patterns in the five experimental groups can be seen in Figure 2.

![Graph of changes in body weight during treatment](image2)

**Figure 2.** Graph of changes in body weight during treatment

Based on the graphs, during 3 weeks of treatment at all doses of yoghurt given namely treatment groups P2 treatment group (rat given standard feed and 1 ml / day of synbiotic yoghurt), P3 treatment group (rat given standard food and synbiotic yoghurt as much as 1.5 ml / day), and the P4 treatment group (rat given standard feed and synbiotic yoghurt as much as 2.5 ml / day) all have the ability to lose weight ($p < 0.05$) when compared with controls given aquades alone. Probiotics in yogurt can improve blood lipid profile by increasing HDL due to the formation of spingolipids on yogurt and the probiotic cell membrane itself [12]. An increase in HDL and a decrease in blood cholesterol levels will reduce fat deposits in the form of adipose cells which has the potential to cause obesity that occurs in people without co-morbidities or with type 2 diabetes mellitus [5]. In addition, lactic acid bacteria such as the culture stater used in making yogurt, namely Lactobacillus and Streptococcus thermopillus groups have been known to be able to form conjugated linoleic acid (CLA), which is a component that is formed from a mixture of linoleic acid with double bond isomers which has the activity of reducing
body fat thus functions as an anti-obesity [24]. Similar to Lactobacillus plantarum which is able to produce trans-10, cis-12-CLA during fermentation process will survive in the digestive tract and will continue to produce CLA which acts as an anti-obesity [25, 26, 27].

4. Conclusion
Based on the results of the study, it can be concluded that giving symbiotic yoghurt can reduce body weight in experimental animals compared with those not given symbiotic yoghurt.

References
[1] World Health Organization 2018 Obesity and Overweight Available from https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
[2] Gropper S, Smith J L and Groff J L 2005 Advanced Nutrition and Human Metabolism (4th Ed.) (USA: Wardsworth, a Division of Thomson Learning) pp 129-161
[3] Almatsier S 2004 Penuntun Diet (Jakarta: PT. Gramedia Pustaka Utama)
[4] Bigazzi R, Bianchi S, Batini V, Guzzo D and Campese V M 2006 Metabolic risk factors and markers of cardiovascular and renal damage in overweight subjects Am J Hypertens. 19(4) 426-431
[5] Ejtahed H S 2011 Effect of probiotic yogurt containing Lactobacillus acidophilus and Bifidobacterium lactis on lipid profile in individuals with type 2 diabetes mellitus J. Dairy Sci. 94 3288–3294
[6] Packard R R and Libby P 2008 Inflammation in atherosclerosis: From vascular biology to biomarker discovery and risk prediction Clinical Chemistry 54(1) 24-38
[7] Omole J O and Ighodaro O M 2013 Comparative studies of the effects of egg yolk, oats, apple, and wheat bran on serum lipid profile of Wistar rat ISRN Nutrition 2013 1-4
[8] Lattimer J M and Mark D H 2010 Effects of dietary fiber and its components on metabolic health Nutrients 2 1266-1289
[9] Baroutkoub A, Mehdi Z R, Belgarian, Hassan J, Zahra S, Mohammad S M, et al. 2010 Effects of probiotic yoghurt consumption on the serum cholesterol levels in hypercholesteromic cases in Shiraz Southern Iran Scientific Research and Essays 5(16) 2206-9
[10] Vodjani A 2003 How Probiotics Help Lower Cholesterol Available from www.natren.com
[11] Varady K A and Jones P J H 2005 Combination diet and exercise interventions for the treatment of dyslipidemia: an effective preliminary strategy to lower cholesterol levels J.Nutr. 135(5) 1829-1835
[12] Ooi L G and Liong M 2010 Cholesterol-lowering effects of probiotics and prebiotics: A review of in vivo and in vitro findings International Journal of Molecular Sciences 11 2499–2522
[13] Ishmayana S, Juanda A, Suprijana O, Djajasoepona S and Rachman I S D R 2015 Pengaruh konsumsi yoghurt yang dibuat dengan kultur dua bakteri (Streptococcus thermophilus dan Lactobacillus bulgaricus) dan tiga kultur bakteri (Streptococcus thermophilus, Lactobacillus acidophilus dan Lactobacillus bulgaricus) terhadap kadar kolesterol serum darah tikus Jurnal Chimica et Natura Acta 3(3) 94-99
[14] Peleazar M J and Chan E C S 2007 Dasar-Dasar Mikrobiologi Jilid I. Terjemahan Hadioetomo, R.S., Imas, T., Tjirosomo, S.S., Angka, S.L (Jakarta: Indonesia Press)
[15] Nielsen S S 2003 Food Analysis (3rd Ed.) (New York: Kluwer Academic/Plenum Publishers)
[16] AOAC 2005 Official Methods of Analysis 2005 (USA: Association of Official Analytical Chemist)
[17] Goncalves D, Perez C, Reolon G, Segura N, Lema P, Gambaro A, Varela P and Ares G 2005 Effect of thickener on the texture of stirred yoghurt Alim. Nutr. Araraquara. 16(3) 207-211
[18] SNI 2009 SNI 01-2981-2009 Yoghurt (Jakarta: Badan Standarisasi Nasional)
[19] Irvine S L and Hekmat S 2011 Evaluation of sensory properties of probiotic yogurt containing food products with prebiotic fibres in Mwanza, Tanzania Food and Nutrition Sciences 2 434-439
[20] Olugbuyiro J A O. and Oseh J E 2011 Physico-chemical and sensory evaluation of market yoghurt in Nigeria *Pakistan Journal of Nutrition* **10**(10) 914-918

[21] Anjum R R and Zahoor T 2007 Comparative study of yoghurt by using local isolated and commercial imported culture *Journal of Research Science* **18**(1) 35-41

[22] Tamime A Y, Saarela M, Sondergaard A K, Mistry V V and Shah N P 2005 Production and maintenance of viability of probiotic microorganisms in dairy product *Probiotic Dairy Products* **3** 39-63

[23] Rahayu E S 2009 Perkembangan terkini penggunaan probiotik dalam industri susu *Food Review* **4** 30-33

[24] Park Y, Albright K J, Liu W, Storkson J M, Cook M E and Pariza M W 1997 Effect of conjugated linoleic acid on body composition in mice *Lipids* **32** 853–858

[25] Lee K 2007 Antiobesity effect of trans-10, cis-12-conjugated linoleic acid-producing *Lactobacillus plantarum* PL62 on diet-induced obese mice *Journal of Applied Microbiology* **103**(4) 1140-6

[26] Dong-Cho K, Chan-Kyu H and Bog-Hieu L 2013 Loss of body weight and fat and improved lipid profiles in obese rats fed apple pomace or apple juice concentrate *Journal of Medicinal Food* **16**(9) 823–830

[27] Lee S O, Kim C S, Cho S K, Choi H J, Ji G E and Oh D K 2003 Bioconversion of linoleic acid into conjugated linoleic acid during fermentation and by washed cells of *Lactobacillus reuteri* *Biotechnol Lett* **25** 935–938