Administration of *Lactobacillus acidophilus* FNCC 0051 in *Rattus norvegicus* with Type-1 Diabetes Mellitus: Glucose and Lipid Metabolism

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Abstract. The imbalance of gut microbiota contributes in development of type 1 diabetes melitus (DM), which is a chronic autoimmune disease characterized by immune-mediated destruction of the insulin-producing pancreatic beta (ß) cells. Around 7–12% of all diabetics were type 1 DM with the most cases occurred in children and adolescents. *Lactobacillus acidophilus* probiotics play important role in reducing proinflammatory cytokines (TNF-α, IL-1β) and modulating the metabolism of the intestinal microbiota. This research aimed to examine the effects of *L. acidophilus* probiotics FNCC 0051 strain on glucose and lipid metabolism in type 1 DM *Rattus norvegicus* induced by streptozotocin. *L. acidophilus* FNCC 0051 were obtained from the collection of Center for Food and Nutrition Studies, Universitas Gadjah Mada. Bacteria were cultured on de Man-Rogosa Sharpe broth media, incubated at 37 °C for 48 h under carbon dioxide enriched condition, then measured the suspension doses for 1.5×10^9 CFU/ml and 1.5×10^8 CFU/ml. The rats were initially induced with streptozotocin, followed by administration of probiotic at the dose of 1.5×10^8 and 1.5×10^9 CFU/mL/day for 3 weeks. Twenty one days of *L. acidophilus* treatment at the dose of 1.5×10^9 CFU/mL/day reduced blood glucose levels and had significant effect towards lipid profile.

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The mean reduction of blood glucose levels was 82.25 ± 31.12 mg/dL, and total cholesterol, triglyceride, HDL, and LDL levels mean reduction were 61.5 ± 6.24, 56.75 ± 10.69, 32.75 ± 3.86, 16.25 ± 3.40 mg/dL respectively. In conclusion, the administration of *L. acidophilus* probiotics at a dose of 1.5×10^9 CFU/ml/day for 3 weeks improved glycemic control and lipid profile.

Keywords: Blood glucose · *Lactobacillus acidophilus* · lipid profile · and type 1 diabetes mellitus

1 Introduction

The worldwide prevalence of diabetes mellitus (DM) prevalence is 463 million people, and around 7–12% of all diabetics were type-1 DM with the most cases occurred in children and adolescents [1] Indonesia's national registry data states that the number of children with type-1 DM in 2014 was 1,021 cases, while more than 50% of patients with newly diagnosed type 1 DM were >20 years old [2]. Based on these data, it shows a significant increase in the incidence and prevalence of type-1 DM in the world. Thus, it is very important to analyze the effect of probiotics as an effort to prevent type-1 DM, including studies on the effect of probiotics administration on glucose and lipid metabolism. Our study aimed to analyze the effectiveness of *Lactobacillus acidophilus* probiotics to control the blood glucose level and improve the lipid profile in type-1 DM rat induced by streptozotocin (STZ).

Type-1 DM is a chronic autoimmune disease characterized by immune-mediated destruction of the insulin-producing pancreatic beta (ß) cells. Reduction of insulin secretion and ß-cells death is the early pathogenesis of type-1 DM, which caused by the rise of inflammation in pancreas (insulitis) and the surge of autoantibodies production against ß-cell antigens. Gut microbiota associated with development of type 1 DM due to the influence on the gut mucosal immune cells functions and initiate abnormal immune cell functioning such as autoimmunity against ß-cells [3, 4]. Bacteroidetes and Firmicutes are the two major phyla that dominate gut microbiota population. The ratio between these two phyla are associated with type 1 DM development. Several factors may impact the difference of gut microbiota, such as gastrointestinal environment or diet which impact the varying glucose levels in host body fluids. Moreover, mode of birth by vaginal delivery or Cesarean section may also contributes to the microbiota composition. Hence, this factor might be associated with the development of type-1 DM in newborns via Cesarean section delivery, because they are not exposed to probiotics in maternal vagina [5].

Lactobacillus acidophilus is one of the predominant Firmicutes bacteria in vagina. Wang *et al*. showed that supplementation of *L. acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 combination modulated microbiota composition and function, regulated host metabolism and gut microbiota metabolism in weaned rats [6]. An in-vivo study of *L. acidophilus* NS1 conducted by Song *et al*. resulted in alleviation of cholesterol levels more than 50% [7], while the effectiveness of *L. acidophilus* bacteria in controlling the blood glucose and improving the lipid profile in rat with type-1 DM is still not clear. In

this study, we induced STZ into rats, which cause toxic effects by penetrating pancreatic ß cells through the glucose transporter GLUT 2 in the plasma membrane. It causes damage to the DNA structure that leads to a dysfunctional ß-cell and immune response such as necrosis or apoptosis [8].

2 Materials and Methods

2.1 Probiotic Strain

Lactobacillus acidophilus probiotics FNCC 0051 strain were obtained from the collection of Center for Food and Nutrition Studies, Universitas Gadjah Mada (Yogyakarta, Indonesia), were cultured on de Man-Rogosa Sharpe broth media (MRS; Merck™), incubated at 37 °C for 48 h under CO_2-enriched condition. Furthermore, the optical density at 600 nm wavelength were measured using spectrophotometer to determine the 1.5×10^9 CFU/ml and 1.5×10^8 CFU/ml doses of *L. acidophilus* suspension.

2.2 Experimental Animals

A total of 16 *Rattus norvegicus* of Sprague-Dawley strain aged 10–12 weeks with a body weight of 250–300 g were adapted for 1 week before streptozotocin (STZ) induction. Prior to the acclimatization period, each experimental animal was weighed. They were kept in $30 \times 30 \times 25$ cm^3 individual cages. The cages were placed in a dry, ventilated environment, and kept in 12 h of darkness and 12 h of brightness cycle during the duration of experiment. The temperature were always maintained in the range of 28–30 °C and the animals were fed with Rat Bio and drinking water *ad libit*. The research protocol using *R. norvegicus* as animal model had been approved by Research Ethic Committee of Faculty of Medicine and Health Sciences, University of Bengkulu. The protocol applied the principle outlined in the Declaration of Helsinki 2008 and the 3Rs (replacement, reduction, dan refinement) principal.

2.3 Experimental Model and Groups

The research procedure diagram can be seen in Fig. 1. Rats were induced with STZ after fasted for 12 h, then blood glucose levels were checked to confirm diabetes mellitus rats with criteria of blood glucose level >200 mg/dL were selected as samples. A total of 16 rats were divided into four groups of rats, namely:

- P0 as a normal control group;
- P1 as DM rats control group with two injections of STZ 50 mg/kgBW;
- P2 as DM rats group with *L. acidophilus* probiotics at the dose of 1.5×10^8 CFU/mL/day administration for 3 weeks;

P3 as DM rats group with *L. acidophilus* probiotics at a dose of 1.5×10^9 CFU/mL/day administration for 3 weeks.

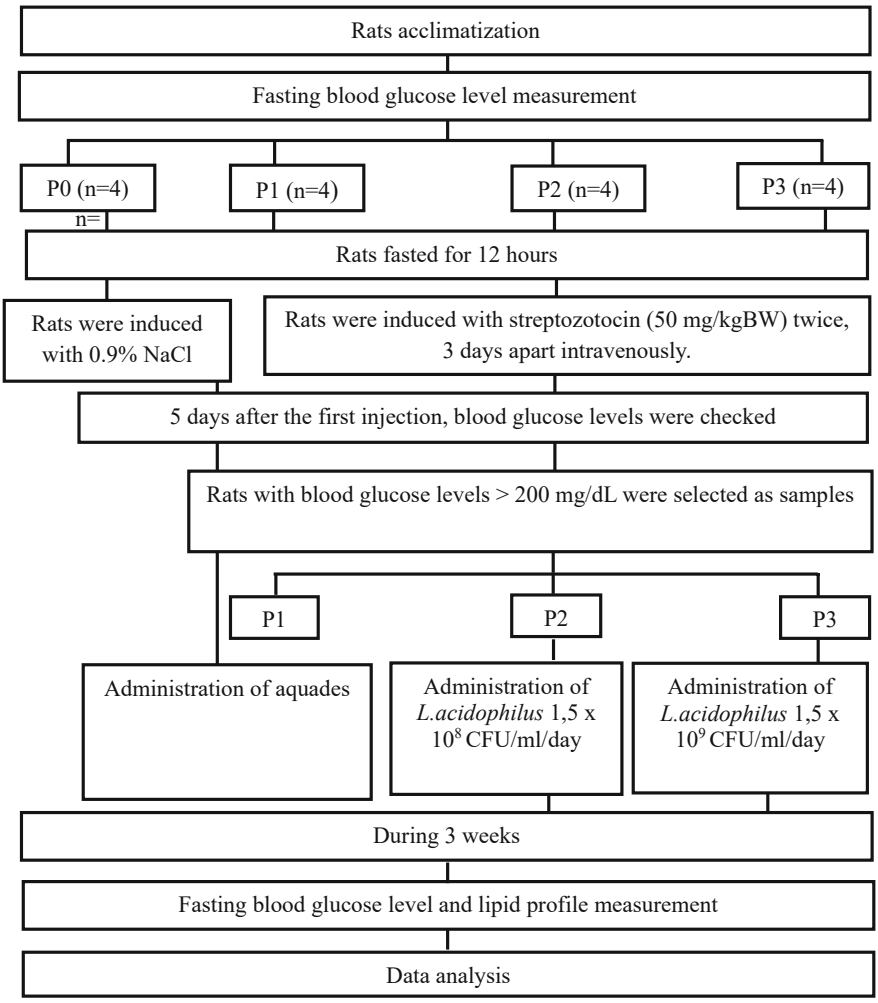

Fig. 1. The research procedure diagram

2.4 Glucose and Lipid Measurement

Blood were drawn from the retroorbital sinus using microhematocrit tubes. Blood glucose examinations were carried out three times; the 7th day of study (after acclimatization), the 13th day (after streptozotocin induction), and the 34th day (after probiotic treatment). About 1 mL of each blood samples were centrifuged for 40 s at a speed of 2,000 rpm and 300 μl of supernatant were taken for glucose examination. The measurements of total cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL) and triglyceride levels in white rats only performed after administration of the probiotic *Lactobacillus acidophilus*. Examination of glucose and lipid levels was carried out using the BT-1500 automatic biochemistry analyzer machine.

2.5 Statistical Analysis

Statistical analysis was performed using the Statistical Program for Social Science (SPSS) for windows version 23. Data from different groups were presented as mean ± standard deviation (SD). The data that has been obtained was carried out in a comparative test using a paired *t*-test to assess the significance of weight loss and to compare the blood glucose levels before and after treatment. To determine the significance of differences in lipid profiles, One-Way ANOVA analysis was performed. Furthermore, Kruskal-Wallis and *post hoc* Tukey test was carried out to compare between the variables.

3 Results

Based on *t*-test paired analysis, there was significant weight loss following the STZ treatment (between BW1 and BW2) in the P1 ($p = 0{,}000$), P2 ($p = 0{,}000$), and P3 ($p = 0{,}002$) group of rats, while in the P0 group there was no significant difference in weight loss before and after STZ treatment, with p value of 0.127 (Table 1). There was a significant differences of weight loss between groups (P0, P1, P2, and P3) using Kruskal-Wallis test ($p = 0.019$). Furthermore, there was also significant weight loss between the STZ-induced groups (P1, P2, and P3) compare with P0 control groups (Table 1).

The mean of fasting blood glucose (FBG) level on the 7th day in each group were below 100 mg/dL with an insignificant difference for P0 group in all test say ($p = 0.923$). Meanwhile P1 group showed a significant increased between day 7 and day 13 ($p = 0.02$). Along with the significant increased seen in the P1 group, the P2 ($p = 0.000$) and P3 group ($p = 0.001$) also demonstrate the significant increased result (Fig. 2). The highest level of FBG on day 13 after STZ induction was found in the P3 group with a glucose level of 512.25 ± 55.72 mg/dL, while the lowest was seen in the P0 group with a glucose level of 95 ± 5.59 mg/dL. These results indicate STZ induction elevated FBG level. The highest FBG level on the day 34 was found in the P1 group with a glucose level of 512 ± 81.51 mg/dL, while the lowest was seen in the P0 group with a glucose level of 91.25 ± 9.91 mg/dL. There was no significant differences between day 13 and day 34 in mean of FBG levels in P0 ($p = 0.551$), P1 ($p = 0.262$), and P2 ($p = 0.058$)

Table 1. Rat body weight before and after treatment

Group	n	BW1	BW2	BW loss (kg)	*p*
P0	4	270.50 ± 7.59	267.25 ± 7.50	3.25 ± 3.09	0.127
P1	4	248.25 ± 10.50	187.50 ± 7.14	60.75 ± 6.94[a]	0.000
P2	4	255.00 ± 8.04	198.00 ± 2.58	57.00 ± 6.16[a]	0.000
P3	4	276.75 ± 15.58	206.50 ± 9.60	70.25 ± 13.52[a]	0.002

BW1 = Body weight before STZ treatment; BW2 = Body weight after STZ treatment; [a] = Significantly difference to P0 ($p < 0.05$) using post hoc Mann-Whitney test; n = number of sample.

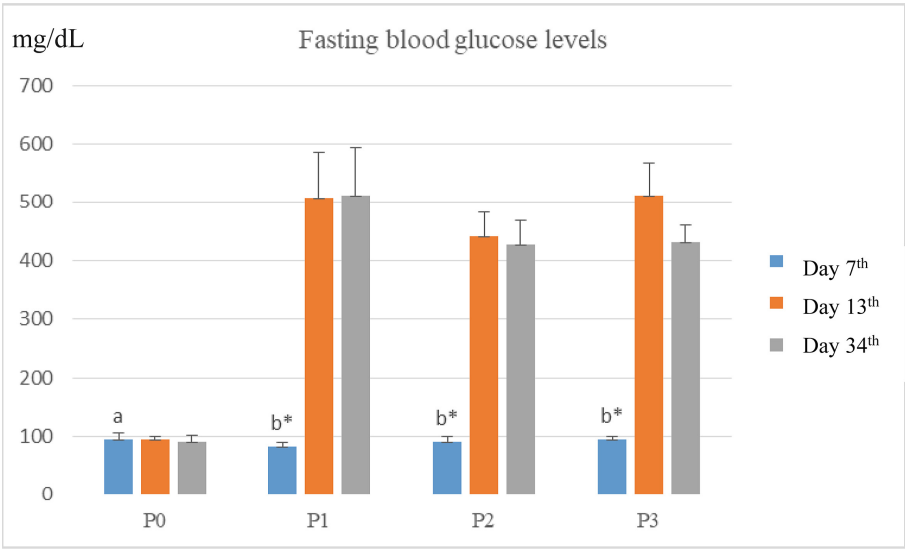

Fig. 2. Fasting blood glucose levels. a = unsignificantly different with day 13 using paired *t*-test; b = significantly different with day 13 using paired t-test

groups. Meanwhile, in the P3 group was found a significant difference with FBG level decreasing of 82.25 ± 31.12 mg/dL ($p = 0.013$) (Fig. 2). These results show that the administration of *L. acidophilus* probiotics of dose 1.5×10^9 CFU/mL/day diminish FBG level.

Post hoc Tukey's post hoc test showed that the administration of STZ with a double dose of 50 mg/kgBW can increase total cholesterol levels, which indicated by significantly difference in the mean of total cholesterol levels ($p = 0.01$) between the P1 group and P0 group. The administration of *Lactobacilus acidophilus* 1.5×10^9 CFU/mL/day (P3 group) could improve lipid profiles in the form of a decrease in total cholesterol ($p = 0.001$), triglycerides ($p = 0.000$), and LDL levels ($p = 0.038$) as well as a significant alleviation in HDL levels compared to the P1 group ($p = 0.000$) and P2 group ($p = 0.001$). This increased HDL level was not significantly different to P0 group ($p = 0.999$). The administration of *L. acidophilus* 1.5×10^8 CFU/mL/day had not been able to significantly improve the lipid profile (Fig. 3).

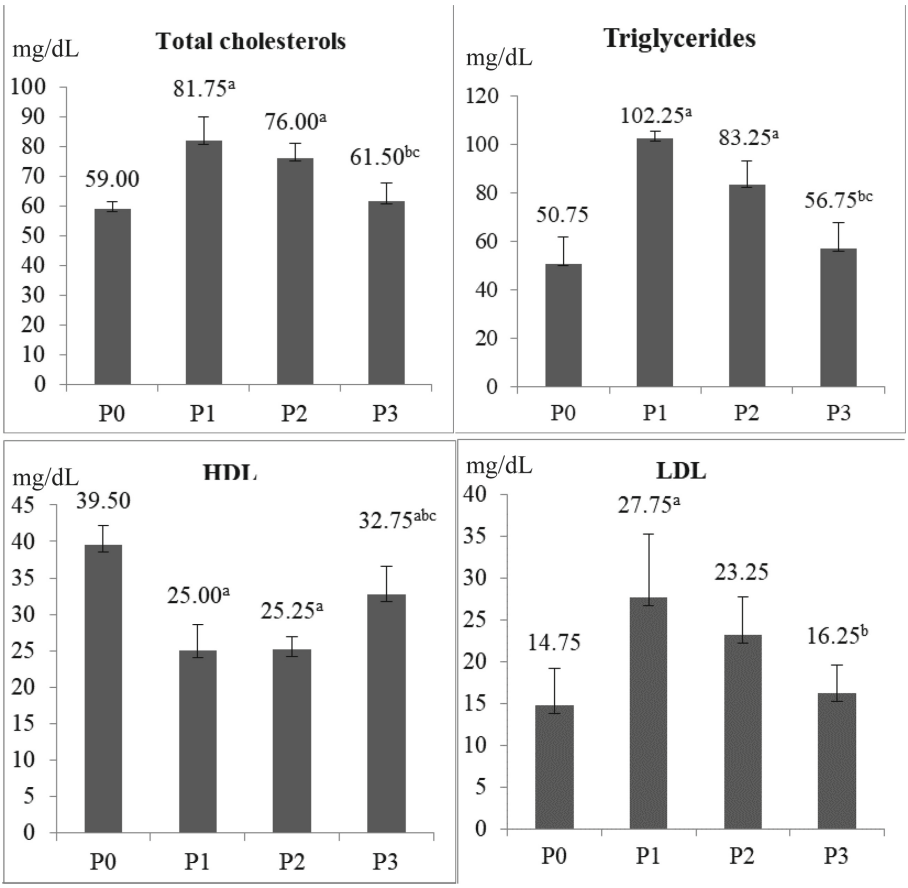

Fig. 3. Lipid profile analysis. a = significantly different with P0; b = significantly different with P1; c = significantly different with P2

4 Discussion

Our study showed the results as preliminary data on the potential development of *Lactobacillus acidophilus* probiotics for the type 1 diabetes mellitus alternative treatment. The administration of the *L. acidophilus* probiotics at a dose of 1.5×10^9 CFU/ml/day for 21 days significantly reduce fasting blood glucose levels and improve the lipid profile of DM rats. Currently, it is known that gut microbiota contributes in pathogenesis of type 1 DM related to autoimmunity disease. Metagenomic studies explained that the human gut microbiota in early postnatal environmental exposures plays a crucial role in determining the composition of adult human gut microbiota. *Lactobacillus acidophilus* is the major probiotic which could modulate gut microbiota via cellular component production, short-chain fatty acids (SCFAs) from the polysaccharide fermentation in the intestine [9]. The predominant SCAFs (acetate, propionate, and butyrate) are present in the intestinal lumen of humans and mice which are able to stimulate glucagon like

peptide-1 (GLP-1) secretion through free fatty acid receptor 2 (FFAR2) and free fatty acid receptor 3 (FFAR3) [10].

The effect of *Lactobacilli* on GLP-1 secretion in hyperglycemic states is able to reprogram intestinal cells into insulin-secreting cells that are responsive to glucose so as to improve the state of hyperglycemia in diabetes [11]. In addition, GLP-1 secretion is also able to stimulate insulin gene transcription, islet cell growth, neogenesis, pancreatic cell proliferation in mice and pancreatic cell DNA synthesis in mice to repair damage to pancreatic β-cells, thereby lowering blood glucose levels [12]. The effect of SCFAs is also related to the decrease in inflammatory status which reduces insulin resistance and increases glucose uptake in tissues [10]. Inflammation of the pancreas caused by STZ induction causes the release of the amylase enzyme which functions to break down carbohydrates into the bloodstream, resulting in high levels of amylase. Giving probiotics can reduce the activity of the amylase enzyme, thereby limiting the hydrolysis process and carbohydrate absorption which will reduce blood glucose levels [13].

The research of Bharti *et al*. showed that the administration of both strains of *Lactobacillus acidophilus* and *Lactobacillus plantarum* at 10^8 CFU/mL/day for 21 days were able to reduce blood glucose levels in alloxan-induced rats, but decreased blood glucose levels with 1.5×10^8 CFU/mL/day dose of probiotic were found to be insignificant [14]. The possibility of hyperglycemia achieved with a dose of alloxan of 40 mg/kgBW in this study was not sufficient, because administration of alloxan at a dose below 140 mg/kgBW would make the blood glucose levels of rats return to normal within a week [15]. Meanwhile in our study, double dose of STZ (50 mg/kgBW each time) were used as an inducer of DM which were able to maintain a state of hyperglycemia for up to 40 weeks [16]. Another possible cause of the absence of a significant decrease in blood glucose levels at a probiotic dose of 1.5×10^8 CFU/mL/day in the our study is the shorter treatment duration and smaller dose than the study by Mahajan *et al*. with the administration of probiotics at a dose of 2×10^8 CFU/mL/day for 8 weeks, which can reduce blood glucose levels significantly [17].

Gut microbiota contributes to the lipid metabolism through the regulation of the farnesoid X receptor (FXR), the bile acid receptor responsible for the regulation of bile acid synthesis and hepatic triglyceride accumulation. Activation of FXR affects two other receptors related to lipid metabolism, namely liver X receptor (LXR) and sterol regulatory element binding protein 1C (SREBP-1C), which inhibit triglyceride synthesis, trigger triglyceride degradation and fatty acid oxidation by activating lipoprotein lipase, PPARα. The activation of LXR triggers lipid synthesis by inducing the expression of the SREBP-1C gene and influencing fatty acid synthetase to increase FAS mRNA levels [18]. This is supported by research from Huang and Zheng which stated that *L. acidophilus* can act as LXR agonist and inhibit cholesterol absorption. In addition, *L. acidophilus* can downregulate Niemann-Pick C1-Like 1 (NPC1L1) in the duodenum and jejunum of rats fed with a high-fat diet which causes a decrease in cholesterol absorption [19].

The normal value of total cholesterol and triglyceride (TG) of *Rattus norvegicus* Sprague Dawley male strain has not been established, but in the study of Otto *et al.*, the normal range of total cholesterols and TG values are, 119 ± 51.3 mg/dL and 266 ± 121.4 mg/dL, respectively [20]. The results of this study showed a significant difference in the total cholesterol and TG between P1 and P0 group, meaning that there was an

increase in cholesterol and TG levels after STZ induction. The average of cholesterol and TG level in P1 group were 81.75 ± 7.93 mg/dL and 102.25 ± 2.98 mg/dL. In comparison with normal value, the cholesterol level in P1 group cannot be classified as hypercholesterolemic because these levels are still below normal level. The induction with STZ also had not been able to cause hypertriglyceridemia eventhough there had been a significant increase in TG levels. It is possible that due to the relatively short duration of this study, STZ induction with a double dose of 50 mg/kgBW could not cause hypertriglyceridemia.

According to the data obtained, the administration of the *L. acidophilus* probiotics at a dose of 1.5×10^9 CFU/ml/day for 21 days significantly reduce fasting blood glucose levels and improve the lipid profile of DM rats, but did not significantly at a dose of 1.5×10^8 CFU/ml/day for 21 days. Thus, further research need to observe the effect of probiotic *L. acidophilus* at a dose of 1.5×10^8 CFU/ml/day with a longer duration. This study needs to be continued to analyze the immune response (inflammatory cytokine profile) involved in the pathogenesis of type-1 DM associated with intestinal microbiota dysbiosis.

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