Probiotic potential of the Indonesian local variety of fermented parboiled rice (tape) improved the metabolic syndrome of diabetic rats

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Abstract

The number of people with diabetes mellitus (DM) at the global level and in Indonesia continues to increase every year. Consumption of probiotic foods can improve symptoms of metabolic syndrome in DM. The research objective was to determine the effect of probiotic parboiled rice tape (fermented parboiled rice) on glucose, insulin, lipid profile, number of lactic acid bacteria (LAB), pH, and short-chain fatty acids (SCFA) in diabetic rats. The probiotic parboiled rice tape was made with the addition of Lactobacillus plantarum Dad-13. The steamed rice was mixed with CMC as a thickener (2% of rice). The mixture was cooled to room temperature (for 5-10 mins) before being further mixed with the NKL yeast and powdered sugar (each of them was 0.2% of rice). Then, the mixture was inoculated with $1 \times 10^8$ CFU/g of L. plantarum Dad-13 and fermented at room temperature for 48 hrs. The tape was then given to diabetic Wistar rats in the form of a wet tape product at 1.8 g per head per day. Treatment diets were given for 28 days to 4 groups of rats, namely SFHR (Standard Feed in Healthy Rats), SFDR (Standard Feed in Diabetic Rats), PTDR (Probiotic rice Tape in Diabetic Rats), PPTDR (Probiotic Parboiled rice Tape in Diabetic Rats). The data obtained were analyzed statistically using one-way ANOVA. The results showed that, in the PTDR and PPTDR groups, their glucose levels decreased significantly (p<0.05) from 257.47 mg/dL to 155.78 mg/dL (39.49%), and from 260.32 mg/dL to 109.88 mg/dL (57.79%), respectively. For insulin levels, there was a significant increase (p<0.05) from 418.35 ug/dL to 485.00 ug/dL (15.86%) and from 414.84 ug/dL to 532.85 ug/dL (26.52%) in the PTDR and PPTDR groups respectively. In the PPTDR group, cholesterol, triglyceride, and LDL levels decreased significantly (p<0.05) by 37.65%, 34.50%, and 59.29%, respectively, and the percentage of reduction was greater than the other diet groups. HDL levels increased significantly (p<0.05) from 24.94 mg/dL to 67.64 mg/dL (171.21%) in the PPTDR group. Meanwhile, the amount of LAB, levels of SCFA (acetic acid, propionate and butyrate), and pH in rats faeces did not show any significant (p>0.05) changes. The provision of probiotic tape improved the metabolic synthesis of diabetic rats and treatment with PPTDR was better than that of PTDR.

1. Introduction

Diabetes mellitus (DM), pre-diabetes, obesity, and cardiovascular disease need serious treatment because their prevalence has continued to increase dramatically over the past decade, both globally and in Indonesia. The prevalence of pre-diabetes at the global level (world) is 25% higher than that of type 2 diabetes mellitus (Kassaian et al., 2019). The number of people living with diabetes in Indonesia ranks 7th highest in the world after China, India, the USA, Pakistan, Brazil and Mexico (IDF, 2019). Results of Basic Health Research conducted in 2018 by the Indonesian Ministry of Health (Indonesian Ministry of Health, 2020) showed that the prevalence of DM in people over 15 years of age has increased from 1.5% in 2013 to 2% in 2018 and based on the results of blood sugar tests, it has increased from 6.9% in 2013 to 8.5% in 2018. The increased risk of type 2 diabetes can be caused by metabolic syndrome, which is present in about 20-25% of adults in the world (Mazidi
et al., 2016). Signs of metabolic syndrome are indicated by the following 3 or more parameters: high fasting blood sugar levels (>1100 mg/L) and high triglycerides (≥1500 mg/L), high density lipoprotein (HDL) - low cholesterol (<400 mg/L for men and <500 mg/L for women), visceral obesity (waist circumference >1.02 m for men and >0.88 m for women), and hypertension (≥130/85 mm Hg). The high number of diabetics in the human population can be caused by an unhealthy lifestyle which results in an imbalance of the intestinal microbiota leading to metabolic disorders (Anderson, 2008; He and Shi, 2017). In addition to using medical drugs, efforts to deal with DM sufferers can be done by consuming probiotic foods.

Probiotic foods can improve fasting blood sugar levels and lipid profiles (Razmpoosh et al., 2018) as well as improve insulin resistance in patients with type 2 diabetes mellitus (Kobyliak et al., 2018). Giving foods that contain prebiotics and probiotics can reduce fasting blood sugar levels, total cholesterol, and triglycerides (Tabrizi et al., 2018). One of the fermented food products in Indonesia that can be used as probiotic food is a tape, which is usually made from fermented glutinous rice. Purwandhani et al. (2008) reported that fermented glutinous rice (tape) inoculated with Lactobacillus acidophilus SNP2 can act as a probiotic. Rice (not glutinous) which is cheaper in price can be made into tape by adding carboxymethyl cellulose (CMC).

Meanwhile, to provide a prebiotic substrate, parboiled rice can be used which is known to have high resistant starch (RS). Yulianto et al. (2018) and Gopalsamy et al. (2019) reported that parboiled rice contains 3.75-10.56% of RS and can act as a prebiotic. To increase the potential of parboiled rice as a functional food, it can be inoculated with lactic acid bacteria that have been tested as probiotics, such as Lactobacillus sake, L. casei, L. acidophilus SNP2, or L. plantarum Dad-13. Thus, parboiled rice which is rich in prebiotics can be used as a raw material for making tape by inoculating lactic acid bacteria (probiotics) to produce the parboiled synbiotic tape.

Research on the use of probiotic-rich parboiled rice against the metabolic syndrome of rats is still rare. Therefore, this research aimed to evaluate the effect of feeding probiotic rice tape and probiotic parboiled rice tape on blood glucose, insulin, lipid profile (total cholesterol, triglycerides, HDL, and LDL), and microbial digesta (total LAB, pH, and SCFA) in diabetic rats.

2. Materials and methods

2.1 Materials

The main materials used in this study were probiotic fermented rice (probiotic rice tape) and probiotic fermented parboiled rice (probiotic parboiled rice tape) made from prime quality local Ciherang rice (CIHERANG SS, Tani Rejo Seed, Yogyakarta, Indonesia). Other materials used included carboxymethyl cellulose (CMC Food Grade, Lansida, Yogyakarta, Indonesia) which served to increase rice stickiness, yeast (Ragi Tape NKL, Surakarta, Indonesia), and lactic acid bacteria (LAB) (L. plantarum Dad-13, Center for Food and Nutrition Studies of Gadjah Mada University, Yogyakarta, Indonesia). Male Wistar rats aged 2 months and weighing 150-200 g were obtained from the Center for Food and Nutrition Studies of Gadjah Mada University laboratory for in vivo biological testings. MRS Agar (Oxoid, UK) was used to enumerate LAB.

2.2 Probiotic rice tape preparation

The probiotic tape was prepared from rice and parboiled rice with a thickening agent in the form of CMC. The RS content of parboiled rice used in this study was 5%, while rice (without parboiling) had an RS content of 1%. Parboiled rice was prepared using the method of Yulianto et al. (2018) with a slight modification, namely a cooling temperature of 0°C for 12 hrs and without the addition of chromium. In the first step, the rice was washed and then soaked for 12 hrs. After soaking, it was steamed for 20 mins (half-cooked). After which, the rice was removed and mixed with water at 50°C, at a ratio of 2 (rice): 1 (water) and then steamed again for 15 mins. Furthermore, 2% CMC thickener was added to Ciherang rice and 1.5% parboiled rice and was evenly stirred. After that, chilled at room temperature for 5-10 mins, and then each was inoculated with NKL yeast and powdered sugar at 0.2% of the rice. After stirring, it was inoculated with 1×10⁸ CFU/g of L. plantarum Dad-13 and fermented for 48 hrs at room temperature in a hollow container (woven bamboo basket). The addition of LAB was carried out in a sterile room to prevent contamination. The finished tape was then given to diabetic Wistar rats in the form of a wet tape product at 1.8 g per head per day.

2.3 Testing of probiotic tape on rats

The protocol for using experimental animals was approved by the Health Research Ethics Committee (No.354.3/FIKES/PL/XI/2019). Twenty-four (24) male Wistar rats of 2 months old and 180-200 g body weight were grouped into 4 groups. Each group consisted of 6 rats. Each group was given either SFHR (standard feed-in healthy rats), SFDR (standard feed-in diabetic rats),
PTDR (probiotic rice tape in diabetic rats + standard feed), and PPTDR (probiotic parboiled rice tape in diabetic rats + standard feed). Analyses of glucose, insulin, cholesterol, triglycerides, HDL, and LDL were carried out on days 7 and 11 to confirm that the rats had diabetes and as data before treatment, and on day 40 for data after 28 days of treatment. Digesta microbial analysis was carried out after 28 days of treatment. The standard rat diet used refers to was American Institute of Nutrition 1993 (AIN93) (Reeves et al., 1993) and drinking water was provided ad libitum. AIN93 standard feed composition for a total weight of 1000 g consisted of 620.7 g corn starch, 140 g casein, 100 g sucrose, 40 g soybean oil, 50 g fibre, 35 g mineral mix, 10 g vitamin, 1.8 g L-cystine, 2.5 g of choline bitarate, and 2603.63 kcal as the energy level. All rats were adapted for 7 days with standard AIN93 feed and drinking water was given ad libitum, then on the 8th day, three groups of rats (SFD, PTDR and PPTDR) were induced with diabetes by intraperitoneal injection of streptozotocin (STZ) (45 mg/kg body weight) which is dissolved in 0.1 mol/L sodium nitrate with a pH of 4.5. On days 8-11, the rats were also given standard AIN93 feed and drinking water ad libitum. The feeding trial according to the experimental group started on day 1 after the rats were proven to have diabetes by checking their blood sugar levels which showed hyperglycemia, namely ≥200 mg/dL (WHO, 2006). Probiotic tape administration to rats was based on dose conversion for humans with a serving of 100 g for a bodyweight of 70 kg. The weight of the tape given was 1.8 g per rat per day containing LAB 9.2×10⁸ CFU/g of probiotic rice tape and 1.9×10⁹ CFU/g of probiotic parboiled rice tape.

2.4 Glucose and insulin analyses

The procedures for glucose and insulin analyses were done by following the manufacturer’s procedures listed on the respective kits (DiaSys diagnostic systems GmbH, Alte Strasse 9 Holzheim, Germany and Sigma-Aldrich, USA). Blood was drawn through retro-orbital plexus using a microhematocrit and then centrifuged at a speed of 2600 rpm for 20 min to obtain serum. The serum was analyzed for glucose level using glucose-oxidase peroxidase-aminopantypyrine-phenol (GOD-PAP). Briefly, 10 µL serum was mixed with 1000 µL of reagent (containing phosphate buffer, phenol, 4-aminoantipyrine, glucose oxidase, peroxidase). The solution was then incubated at 37°C for 10 mins. After that, it was measured the absorbance within 60 mins at a wavelength of 500 nm.

Insulin analysis was performed using an enzyme-linked immunosorbent assay (ELISA). The blood sample was centrifuged for 10 min at 3000×g with a temperature of 4°C then reacted with monoclonal anti-mouse insulin in the mouse insulin ELISA kit (the reagent is phosphate buffer saline containing sodium azide). After that measured with a microplate reader at a wavelength of 450 nm.

2.5 Lipid profile analysis

Lipid profile analysis (cholesterol, triglycerides, HDL, and LDL) was done using DiaSys diagnostic systems GmbH and Alte Strasse 9 Holzheim kits, Germany by following the manufacturer’s instructions. Lipid profile analysis (Cholesterol, HDL, and LDL) was done using cholesterol-oxidase peroxidase-aminopantypyrine-phenol (CHOD-PAP), while triglyceride analysis was done using glycerol-phosphate-oxidase peroxidase-aminopantypyrine-phenol (GPOP-PAP). Blood was drawn through retro-orbital plexus using microhematocrit and then centrifuged at 3600 rpm for 10 mins to obtain serum.

Cholesterol was analyzed by taking 10 µL of the sample or standard and mixed with 1000 µL of reagent kit (containing good’s buffer, phenol, 4-Aminoantipyrine, cholesterol esterase, cholesterol oxidase, peroxidase) then incubated at 37°C for 10 mins and within 60 mins read the absorbance at a wavelength of 500 nm.

In triglyceride analysis, 10 µL of sample or standard were taken and mixed with 1000 µL of the reagent kit (containing good’s buffer, 4-chlorophenol, ATP, Mg²⁺, glycerol kinase, peroxidase, lipoprotein lipase, 4-aminoantipyrine, glicerol-3-phosphate-oxidase) then incubated at 20-25°C at 20 mins and read the absorbance within 60 mins at a wavelength of 500 nm.

HDL measurement was used was the supernatant by mixed 200 µL sample or standard and 200 µL reagent kit (containing phosphotungstic acid 1.4 mM/L, magnesium chloride 8.6 mmol/L) and incubated for 15 min at room temperature, then centrifugated for 20 min at 2500×g. Within 2 hrs after centrifugation transfer 0.1 mL of the clear supernatant to the reaction solution for the determination of cholesterol. Mixed 100 µL supernatants with 1000 µL cholesterol reagent and incubated for 10 mins at room temperature then measure the absorbance within 45 mins at a wavelength of 500 nm.

LDL measurement was used the supernatant by mixing 100 µL sample with 1000 µL reagent kit (containing heparin 100000 U/L, sodium citrate 64 mmol/L) and incubated for 15 min at room temperature then centrifugated for 20 mins at 2500×g. Within an hour after centrifuging, transfer 100 µL of clear supernatant to the reaction solution for the determination of cholesterol.
Mixed 100 µL supernatant with 1000 µL cholesterol reagent and incubated for 10 mins at room temperature, then read absorbance within 45 mins at a wavelength of 500 nm.

2.5 Digesta microbial analysis

The digesta microbial profile analysis was done by determining the amount of LAB, pH, and SCFA levels. LAB analysis was done by plating on de Man Rogosa and Sharpe media (Hartemink and Rombouts, 1999) that is, 1 g of faeces was diluted with 9 mL of 0.85% NaCl solution with various dilutions. The last three dilutions were plated on the media and incubated at 37°C for 48 hrs. SCFA were analyzed by gas chromatography method using Shimadzu GC-2010 with FID (Flame Ionization Detector) and GC Capillary Column Stabilwax-MS 30 (length), 0.25 mm (inner diameter), and 0.25 µm (film thickness) - (Kyoto, Japan), and helium gas as the carrier compounds. Faecal homogenates were prepared by suspending 200 mg of faeces in 1 mL ultrapure water. Faecal suspension was homogenated by vortex and sonication for 20 mins, then centrifuged at 10000×g for 5 mins and clear brownish supernatant was transferred to different tubes. After that, 1 µL was taken using microsyringe and injected into the GC injector. The sample will move through the column to the detector to be identified and then displayed in the form of a chromatogram in the software. The detection retention time for acetic acid was 4.7 mins, propionic acid was 5.4 mins, and butyric acid was 7 mins. pH was determined by mixing faeces with 2% eosin solution and then measured using an electronic pH meter (Mettler-Toledo, USA) by dipping the cathode tip in the sample. The displayed values were read on the pH meter.

2.6 Statistical analysis

This research belongs to an experimental research design. A completely randomized design method was used by implementing the design of pre and post-test control group design. To determine the difference between the treatment group and the control group (standard feed), an Analysis of Variance (ANOVA) was carried out at a 95% confidence level. Significant differences (p<0.05) were determined using Duncan Multiple Range Test. Paired Sample T-Test was used to determine the difference between before and after treatment for each group. The analysis used SPSS version 25.0 software.

3. Results and discussion

3.1 Glucose and insulin levels

Glucose levels of rats in all groups before STZ injection were in the normal range is<200 mg/dL, then three days after the STZ injection, glucose levels were analysed, the results of which can be seen in Table 1. The table shows that in the group injected with STZ, blood glucose levels increased significantly (p<0.05), reaching a hyperglycaemic rate where SFDR was 262.50 mg/dL, PTDR was 257.47 mg/dL, and PPTDR was 260.32 mg/dL. This proves that the rats had diabetes and are ready to be treated. Similar research conducted by Li et al. (2016) found that after injecting rats with STZ they experienced an increase in sugar levels to ≥200 mg/dL. In this study, after the rats had diabetes, they were given treatment for 28 days. The results of the analysis of glucose levels after 28 days of treatment showed that the glucose levels in the diet group with probiotic rice tape and probiotic parboiled rice tape showed a significant difference (p<0.05) with the control group (standard feed) (p<0.05). The PTDR and PPTDR treatment groups showed a decrease in glucose by 39.49% and 57.79%, while in the standard feed group SFHR and SFDR experienced an increase in glucose respectively by 3.99% and 0.02%.

Insulin levels of rats before and after treatment can be seen in Table 2, which shows a significant difference (p<0.05) between the probiotic tape diet group and the control group (standard feed). The PPTDR group had the highest increase in insulin levels (26.52%), followed by the PTDR group (15.86%). In the SFDR and SFHR groups, there was no significant change (p>0.05). The

Table 1. Glucose levels before and after 28 days of probiotic tape treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Before injection STZ</th>
<th>Three days after STZ injection / before treatment</th>
<th>Changes (%)</th>
<th>After 28 days of treatment</th>
<th>Changes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFHR</td>
<td>68.82±5.17</td>
<td>73.61±2.61</td>
<td>+6.69</td>
<td>76.55±1.72AcD</td>
<td>+3.99</td>
</tr>
<tr>
<td>SFDR</td>
<td>70.82±4.83</td>
<td>262.50±5.58f</td>
<td>+270</td>
<td>268.75±4.44df</td>
<td>+0.02</td>
</tr>
<tr>
<td>PTDR</td>
<td>72.39±3.55</td>
<td>257.47±3.83b</td>
<td>+255.67</td>
<td>155.78±2.29gb</td>
<td>-39.49</td>
</tr>
<tr>
<td>PPTDR</td>
<td>70.17±2.59</td>
<td>260.32±1.54b</td>
<td>+270.98</td>
<td>109.88±3.05bA</td>
<td>-57.79</td>
</tr>
</tbody>
</table>

Values are presented as mean±SD. Values with different lowercase superscript within the same column are significantly different between treatment groups (One Way Anova, p<0.05). Values with different uppercase superscripts within the same row are significantly different in terms of before and after treatment (Paired Sample T-Test, p<0.05).


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Based on the results obtained, it can be seen that probiotic parboiled rice tape gave a greater decrease in glucose and an increase in insulin compared to the group given probiotic rice tape. This may be related to the RS content of each type of rice in this study, namely parboiled Ciherang rice contains RS (5%) higher than non-parboiled Ciherang rice (1%). The results of this study are in accordance with previous studies by Sun et al. (2018) who showed that giving RS2 of corn starch at 200 g/kg per day had an effect of increasing insulin by 40.7% compared to RS2 with a dose of 150 g/kg per day which was 24% and lowering plasma glucose by 41.22% (RS2 200 g/kg) and 26.29% (RS2 150 g/kg). Firdaus et al. (2018) reported that giving cassava flour (containing RS3) which is made with the addition of lactic acid bacteria, can lower plasma glucose and can increase the secretion of glucagon-like-protein-1 (GLP-1). GLP-1 can improve glucose homeostasis by stimulating pancreatic beta cells to secrete insulin, reducing glucagon secretion in pancreatic beta cells and inhibiting appetite (Timper et al., 2016; Wang et al., 2016). According to Sun et al. (2018), RS2 treatment in diabetic rats can modulate enzyme gluconeogenesis and glycogen synthesis in the liver. The decreased expression of Phosphoenolpyruvate carboxykinase (PEPCK) can reduce glucose-6-phosphatase (G6Phase) which plays a role in gluconeogenesis in the liver so that it triggers a decrease in blood glucose. RS2 is able to increase the activation of insulin receptors in the pancreas to secrete insulin so that plasma insulin levels increase.

A similar study was conducted by Niibo et al. (2019) and they reported that giving $6 \times 10^7$ Lactobacillus gasseri per rat per day for 4 weeks can reduce serum glucose levels and increase insulin sensitivity. Insulin levels in the probiotic tape diet group were higher than in the control group. The increase in blood insulin in rats was shown in the probiotic tape diet group containing 1.7×$10^9$ CFU of L. plantarum Dad-13 per 1.8 g of probiotic rice tape, and 3.4×$10^9$ CFU per 1.8 g of probiotic parboiled rice tape.

Hyperglycemia is an indicator of diabetes mellitus. Probiotics can increase glucose homeostasis through the activation of glucose transport type-4 (GLUT-4) which functions as a facilitator of glucose transfer and is able to increase glucose uptake and sensitivity including through the activation of adenosine monophosphate-activated protein kinase (Xue et al., 2019; Khursheed et al., 2020). Insulin is a hormone produced by the pancreas to balance glucose levels in the blood. Insulin is produced by beta cells and works through insulin receptors which have 2 subunits. The alpha subunit outside the cell binds insulin to move across the cell membrane and activates tyrosine kinase from the intracellular beta subunit. Tyrosine kinase will activate protein kinase B which plays a role in encouraging glycogen synthesis and increasing glucose storage into glycogen (McCracken et al., 2018).

Probiotics are beneficial in that they affect the balance of the gut microbes, whereas prebiotics supply energy, and nutrients for the growth of probiotic bacteria. The combination of the two is called synbiotics which can provide superior health effects (Markowiak and Śliżewska, 2018). Based on a study conducted by Tabrizi, et al. (2018) giving symbiotic feeding (probiotic dose $10^7$-$10^9$) can reduce plasma glucose by 10-29% and plasma insulin by 8-26%. Symbiotic can be used as antidiabetic food through their ability for supporting systemic low-grade inflammation to modulate the immune system (de Moreno de Leblanc and Perdigón, 2010).

Based on the description above and its association with this study, it can be seen that the group given probiotic parboiled rice tape had the effect of lowering glucose and increasing insulin levels. This is possible because the material used was parboiled rice which had a high RS (5%) and the supplementation with L. plantarum Dad-13 made it serve as a symbiotic food.
3.2 Lipid profile

The results of lipid analysis including cholesterol, triglycerides, HDL, and LDL are presented in Table 3 and Table 4. From the tables, it is established that prior to STZ injection, the cholesterol, triglyceride, HDL, and LDL levels were all within the normal range and after the STZ injection, the rats experienced an increase in cholesterol levels (from 180.54-184.83 mg/dL), triglycerides (from 128.27-130.39 mg/dL), and LDL (from 76.01-78.43 mg/dL), and decreased in HDL (from 24.94-27.21 mg/dL). This shows that the rats injected with STZ had metabolic syndrome, which as stated by He and Shi (2017) is characterized by an increase in cholesterol, triglyceride, and LDL levels and a decrease in HDL. A similar study was conducted by Wahjuningsih et al. (2018) who found that there was an increase in cholesterol levels (from 181.61-185.81 mg/dL), triglycerides (from 126.50-129.34 mg/dL), and LDL (from 72.47-76.32 mg/dL) and a decrease in HDL (from 24.39-25.46 mg/dL) after STZ injection.

In Table 3, it can be seen that after treatment for 28 days, there was a decrease in cholesterol levels in the PPTDR group by 37.65%, followed by the PTDR group by 21.93%, while in the SFDR or control group there was an increase of 2.98%. An increase of 2.91% was also found in the SFHR group. Triglyceride levels decreased by 34.50% in the PPTDR group, followed respectively by the PTDR group of 20.33%, SFHR group of 5.39%, and SFDR group of 3.72%. The HDL levels before being treated in the diabetic rats' group were all low (24.94-27.21 mg/dL). After 28 days of treatment in the PPTDR group there was a significant increase (171.21%), much greater than the PTDR group (117.95%), in contrast to the control group there was a decrease of 12.86% in SFDR and 4.95% in SFHR. LDL levels showed a significant difference between the control group (standard feed) and the tape diet group, where the PTDR and PPTDR groups experienced a decrease of 42.50% and 59.26% respectively, whereas the SFHR and SFDR groups experienced an increase of 11.26% and 3.54%, respectively. Based on the results obtained, it was established that the provision of probiotic rice tape had a greater effect on lipid profile improvement compared to standard feed and the improvement of lipid profile on probiotic parboiled rice tape is stronger than that of probiotic rice tape.

Regarding the known RS levels of the research material, the PPTDR group that had a higher RS had a greater effect on lowering cholesterol, triglycerides, LDL, and increasing HDL levels. This is similar to the research of Aggarwal et al. (2017) who found that giving formulated biscuit (FB) enriched with RS from whole grains (RS2) as much as 29.41% per day per rat can reduce total cholesterol level by 7.87%, triglycerides by 17.2%, LDL by 25% and increased HDL level by 13.21% in diabetic rats. In this study, the existence of 5% RS was able to reduce cholesterol levels with a higher percentage of 37%. Therefore, differences in the level of RS will result in differences in the reduction of cholesterol. According to Wahjuningsih et al. (2018), RS3 was able to reduce cholesterol levels by 47.7%, triglycerides by 31.14%, and LDL by 32.39%, which was similar to this study.

The consumption of RS and probiotics can affect lipid metabolism in the blood. RS intake can increase the activation of Insig-1 and Insig-2 which can inhibit the activity of sterol regulatory element-binding protein-1 (SREBP-1) in the liver. SREBP-1 plays a role in lipogenesis which is characterized by decreasing fatty acids synthase (FAS) activity so that fatty acid synthesis decreases (Wang et al., 2017). RS also plays a role in reducing peroxisome proliferator-activated-receptor-y (PPARY) which is able to change the expression of a gene to convert cholesterol to bile acids, trigger adipocyte differentiation and proliferation, thereby increasing lipogenesis, and adipogenesis (Balakrishnan et al., 2018).

In addition, RS can act as a fermentation substrate by the action of L. acidophilus and Bifidobacterium in animals (Arshad et al., 2018). In this study, L. plantarum Dad-13 was used as a probiotic source and the results were similar to previous studies conducted by Da Costa et al. (2019) which stated that giving 10^6 L. plantarum per rat for 14 days was able to significantly reduce total cholesterol levels in metabolic syndrome rats (from 63.75 to 42.75 mg/dL). The same result was also reported by Memarrast et al. (2017), giving Lactobacillus as much as 10^{10} CFU/mL for 8 weeks can reduce triglyceride levels by 32%, cholesterol by 20%, LDL by 20% and increase HDL levels by 30% in diabetic rats. The combination of probiotics and prebiotics (synbiotics) has a beneficial effect in improving the lipid profile. Similarly to this study, Hadi et al. (2020) reported that giving synbiotic yoghurt to diabetic rats at 5 g per day per head for 6 weeks can reduce cholesterol levels by 28.1%, triglycerides by 28.9%, and LDL by 26.8%.

Probiotics play a role in improving lipid profiles in diabetics. Probiotics are also able to reduce cholesterol absorption in the intestine by increasing cholesterol excretion through faeces (Mahboobi et al., 2018). LDL serves to mediate the uptake of cholesterol by plasma from the liver, while HDL functions to mediate cholesterol from plasma to the liver (Kim et al., 2016).
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<th>Changes (%)</th>
</tr>
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<tbody>
<tr>
<td>SFHR</td>
<td>92.49±2.29</td>
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<td>+22.07</td>
<td>97.16±2.50&lt;sup&gt;bD&lt;/sup&gt;</td>
<td>+2.91</td>
<td>69.16±3.12</td>
<td>71.49±3.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+3.37</td>
<td>75.34±2.87&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+5.39</td>
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<tr>
<td>SFDR</td>
<td>90.38±4.91</td>
<td>183.11±3.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+102.6</td>
<td>188.56±1.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+2.98</td>
<td>77.35±2.93</td>
<td>129.21±2.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+67.04</td>
<td>134.02±3.17&lt;sup&gt;D&lt;/sup&gt;</td>
<td>+3.72</td>
</tr>
<tr>
<td>PTDR</td>
<td>90.49±4.12</td>
<td>180.54±4.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+99.51</td>
<td>140.96±2.68&lt;sup&gt;B&lt;/sup&gt;</td>
<td>-21.93</td>
<td>69.56±6.45</td>
<td>130.39±2.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+87.45</td>
<td>103.88±3.64&lt;sup&gt;Ec&lt;/sup&gt;</td>
<td>-20.33</td>
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<tr>
<td>PPTDR</td>
<td>90.84±4.18</td>
<td>184.83±3.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+127.6</td>
<td>115.25±3.28&lt;sup&gt;bA&lt;/sup&gt;</td>
<td>-37.65</td>
<td>64.65±4.01</td>
<td>128.27±1.83&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+98.41</td>
<td>84.02±1.77&lt;sup&gt;Ec&lt;/sup&gt;</td>
<td>-34.50</td>
</tr>
</tbody>
</table>

Table 3. Cholesterol and triglyceride levels before and after 28 days of probiotic tape treatment

Values are presented as mean±SD. Values with different lowercase superscript within the same column are significantly different between treatment groups (One Way Anova, p<0.05). Values with different uppercase superscripts within the same row are significantly different in terms of before and after treatment (Paired Sample T-Test, p<0.05).


<table>
<thead>
<tr>
<th>Groups</th>
<th>Before injection STZ</th>
<th>Three days after STZ injection / before treatment</th>
<th>Changes (%)</th>
<th>After 28 days of treatment</th>
<th>Changes (%)</th>
<th>Before injection STZ</th>
<th>Three days after STZ injection / before treatment</th>
<th>Changes (%)</th>
<th>After 28 days of treatment</th>
<th>Changes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFHR</td>
<td>82.75±3.95</td>
<td>81.29±4.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-1.76</td>
<td>77.27±4.51&lt;sup&gt;C&lt;/sup&gt;</td>
<td>-4.95</td>
<td>25.37±1.25</td>
<td>26.99±1.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+6.38</td>
<td>30.03±2.43&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+11.26</td>
</tr>
<tr>
<td>SFDR</td>
<td>83.25±1.45</td>
<td>27.21±1.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-67.75</td>
<td>23.71±1.56&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>-12.86</td>
<td>25.25±2.65</td>
<td>76.01±1.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+201.1</td>
<td>78.70±2.22&lt;sup&gt;D&lt;/sup&gt;</td>
<td>+3.54</td>
</tr>
<tr>
<td>PTDR</td>
<td>80.73±4.83</td>
<td>25.85±3.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-69.86</td>
<td>56.34±2.16&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>+117.95</td>
<td>23.12±2.58</td>
<td>78.43±1.13&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+239.23</td>
<td>45.10±2.77&lt;sup&gt;BD&lt;/sup&gt;</td>
<td>-42.50</td>
</tr>
<tr>
<td>PPTDR</td>
<td>82.41±2.62</td>
<td>24.94±1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-69.74</td>
<td>67.64±1.94&lt;sup&gt;aC&lt;/sup&gt;</td>
<td>+171.21</td>
<td>23.68±1.67</td>
<td>77.28±1.49&lt;sup&gt;C&lt;/sup&gt;</td>
<td>+220.35</td>
<td>31.48±1.71&lt;sup&gt;Ac&lt;/sup&gt;</td>
<td>-59.26</td>
</tr>
</tbody>
</table>

Table 4. HDL and LDL levels before and after 28 days of probiotic tape treatment

Values are presented as mean±SD. Values with different lowercase superscript within the same column are significantly different between treatment groups (One Way Anova, p<0.05). Values with different uppercase superscripts within the same row are significantly different in terms of before and after treatment (Paired Sample T-Test, p<0.05).

With high HDL, it will be able to reduce LDL, triglycerides, and plasma cholesterol.

3.3 Microbial profile and SCFA digesta

The results of the LAB, pH and SCFA analyses of rats are presented in Table 5. It can be seen from the table that there was no significant difference (p>0.05) between the number of LAB in the control group (SFHR and SFDR) and the treatment group (PTDR and PPTDR). This means that giving probiotic tape does not affect the number of microbes in the intestines. Even though it was not statistically significant, parboiled rice which had 5% RS and 1% RS was able to produce relatively more LAB compared to those given standard feed alone. The number of LAB in the PPTDR group reached 8.2×10^7 CFU/g, PTDR reached 7.72×10^7 CFU/g, SFHR reached 7.34×10^7 CFU/g and SFDR reached 7.2×10^7 CFU/g. These results are similar to those reported by Lei et al. (2020) that giving RS lotus seeds were able to modulate the number of cecum faecal bacteria in diabetic rats, where the faeces were found to be 5.6 - 7.1×10^7 CFU/g. Likewise, it was shown in the pH of rats’ faeces, that there was no significant difference (p>0.05) between the probiotic tape diet group (PTDR and PPTDR) and the control group (SFHR and SFDR), which had a range of 6.41-6.95. These results are similar to those reported by Saw et al. (2018) and Zhu et al. (2018) that the faecal pH in healthy rats was 6.51-7.3.

The SCFA levels of propionic, acetic, and butyric acid in the treatment group and the control group also did not show a significant difference (p>0.05). In this study, it was shown that the order of the SCFA content of rats digesta from the highest level was acetic acid, propionic, and butyrate, both in the probiotic tape diet group and the standard feed. Acetic acid levels ranged from 6.31 to 7.97 µmol/g (73.04-76.25%), propionate levels from 1.66 to 1.93 µmol/g (18.2-19.6%), and butyrate 0.49-0.68 µmol/g (4.15-7.46%). Meanwhile, Mandaliya and Seshadri (2019) reported that the concentrations of SCFA found in the colon consisted of approximately 60% acetic acid, 25% propionic, and 15% butyrate. SCFAs can rapidly move from the digestive tract into the bloodstream. Butyrate is used as the main energy source in the intestines, brain, and liver. Propionate is useful for gluconeogenesis in the liver. Acetate is used by peripheral tissues as fuel. According to Firdaus et al. (2018), butyric acid is also able to stimulate the hormone glucagon-like peptide-I (GLP-I) which plays a role in glucose homeostasis and stimulates insulin secretion. In this study, butyric acid production for PPTDR was 0.68%, while it was 0.49% for SFDR. In contrast to research, Zeng et al. (2017) reported that the administration of RS3 dose at 10% showed significant differences (p<0.05) with the control group. The RS3 produced 8.46 µmol/g acetic acid, 2.34 µmol/g propionic acid, and 2.35 µmol/g butyric acid, while the control group produced 7.29 µmol/g acetic acid, 0.82 µmol/g propionic acid, and 1.13 µmol/g butyric acid. The difference in results is because in this study only 1-5% RS (rice as raw material for tape) was used. It is possible that it was not enough to make a significant increase compared to standard feed.

Zheng et al. (2020) reported that the intake of RS as much as 15.9% in 100 g of modified starch can help the proliferation and growth of bacteria, improve lipid profiles, and improve microbiota disruption. Cabello-olmo et al. (2019) indicated that the provision of fermented foods with LAB supplementation to diabetic rats can induce modification of the digestive tract microbiota, improve glucose metabolism, and protect the body from deteriorating conditions due to diabetes mellitus. Probiotic treatment in diabetic rats has a hypoglycemic effect and repairs mucosal damage to the small intestinal wall (Xue et al., 2019). Treatment with a combination of probiotics and prebiotics (synbiotics) in diabetic rats, namely synbiotic yoghurt at 10 mg per day per rat can increase the abundance of bacteria types such as Lactobacillus, Akkermansia, and Bifidobacterium (Ban et al., 2020).

The existence of RS can encourage the growth of beneficial bacteria such as Lactobacillus and Bifidobacterium, so that it enriches SCFA production, improves nutrition in the intestinal cells, and influences the physiological functions of the body (Zeng et al., 2017). Probiotics are able to suppress growth and reduce

<table>
<thead>
<tr>
<th>Group</th>
<th>LAB (log 7 CFU/g)</th>
<th>pH</th>
<th>SCFA Acetic (µmol/g)</th>
<th>Propionic (µmol/g)</th>
<th>Butyric (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFHR</td>
<td>7.3±0.47</td>
<td>6.95±0.58</td>
<td>6.72±0.70</td>
<td>1.72±0.16</td>
<td>0.55±0.03</td>
</tr>
<tr>
<td>SFDR</td>
<td>7.2±0.22</td>
<td>6.41±0.15</td>
<td>6.31±0.91</td>
<td>1.66±0.13</td>
<td>0.49±0.02</td>
</tr>
<tr>
<td>PTDR</td>
<td>7.72±0.24</td>
<td>6.76±0.62</td>
<td>7.37±1.20</td>
<td>1.93±0.18</td>
<td>0.61±0.06</td>
</tr>
<tr>
<td>PPTDR</td>
<td>8.2±0.25</td>
<td>6.85±0.26</td>
<td>7.97±1.30</td>
<td>1.93±0.17</td>
<td>0.68±0.07</td>
</tr>
</tbody>
</table>

No significant difference observed.

the number of pathogenic bacteria such as Firmicutes and Actinobacteria (Bagarolli et al., 2017). Probiotics work to inhibit lipid peroxidation and increase endogenous antioxidants that prevent diabetes severity (Zhang et al., 2016). Probiotics can directly secrete insulinotropic polypeptide metabolites which increase glucose storage in tissues and glycogen in the liver (Ostadrahimi et al., 2015).

4. Conclusion
Dietary intake of probiotic tape significantly affected glucose, insulin, and lipid levels in diabetic rats. After treatment, giving the probiotic tape diet using either rice (PTDR) or parboiled rice (PPTDR) in diabetic rats can reduce glucose, cholesterol, triglyceride, and LDL levels, also increase insulin and HDL levels. However, the number of lactic acid bacteria, levels of SCFA (acetic acid, propionate, and butyrate), and pH in rats’ faeces did not show any significant changes. Probiotic parboiled rice tape treatment was better in improving diabetic rats metabolic syndrome than probiotic rice tape treatment.

Conflict of interest
The authors declare no conflict of interest.

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