

RESEARCH ARTICLE

***Lacticaseibacillus rhamnosus* Probiotics Improve Fasting Blood Glucose, HOMA-IR, and Reduce Body Weight in Diabetic Rat Model**

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Abstract

BACKGROUND: Indonesian indigenous probiotics have been found to improve disruptions of tight junctions in the intestinal epithelium and reduce total cholesterol levels. Improvement in the tight junction could decrease the LPS level and further reduce the blood glucose and insulin resistance. The effects of indigenous Indonesian *Lacticaseibacillus rhamnosus* (Lr) probiotics on glucose metabolism and inflammatory marker levels in diabetic rats was studied to find if these probiotics are suitable as potential supplementation treatment in diabetes.

METHODS: Sixteen female Wistar rats were induced with diabetes using streptozotocin and fed a high-fat, high-sucrose diet. The rats were separated into four groups: LrFBB81, LrFSMM22, LrSKG34, and a control group. Each intervention group received daily dosages of 1 mL probiotic-suspension containing 10^9 CFU/mL cells given orally for 14 days, whereas the control group received saline. Fasting blood glucose (FBG), insulin, homeostatic model assessment for insulin resistance (HOMA-IR), lipopolysaccharide (LPS), and body weight were evaluated.

RESULTS: FBG was significantly reduced in LrFSMM22 group ($\Delta=120.75$ mg/dL, $p=0.035$), while significant reduction was not observed in LrFBB81, LrSKG34, and control groups. No statistically significant differences were found in HOMA-IR before and after intervention in all groups, but Δ HOMA-IR in LrFSMM22 group was reduced more than the control group (-3.90 vs. 2.02 , $p=0.028$). All groups showed no significant differences in LPS level, meanwhile statistically significant reduction in body weight was observed in all probiotic groups, LrFBB81 ($\Delta=-15.7$ gram, $p=0.040$), LrSKG34 ($\Delta=-20.43$ gram, $p=0.006$), and LrFSMM22 groups ($\Delta=-18.33$ gram, $p=0.037$).

CONCLUSION: The administration of *L. rhamnosus* could improve FBG, HOMA-IR, and reduce body weight without suppressing the LPS.

KEYWORDS: diabetes, probiotic, *Lacticaseibacillus rhamnosus*, fasting blood glucose, HOMA-IR, lipopolysaccharide, insulin resistance

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Introduction

Diabetes is a chronic metabolic syndrome characterized by hyperglycemia due to defects in insulin secretion, action, or

both.(1) Around 90% of all diabetes cases globally are type 2 diabetes mellitus (T2DM). Over time, it leads to chronic complications and is considered a significant burden owing to its prevalence and associated costs. The prevalence of diabetes is increasing annually. In 2019, diabetes affected

463 million individuals worldwide. According to the International Diabetes Federation (IDF) Diabetes Atlas, this number is estimated to have reached 578 million by 2023 and is projected to reach 700 million by 2045, representing 10% of the adult population.(2)

The pathophysiology of T2DM outlining in multiple target sites, including the pancreas, liver, muscle, adipose tissue, brain, gut microbiota, and immune dysregulation, that can affect insulin signaling and action.(3) Among these targeted sites in diabetes mellitus are the intestine and microbiome.(4) Consumption of a high-fat diet (HFD) can lead to the decline of Gram-negative bacteria, resulting in increased lipopolysaccharide (LPS) production in the gut. This process activates inflammatory cytokines, phosphorylations of serine residues in insulin receptor substrate-1, and decreases insulin sensitivity.(5) Imbalances in the proportions of *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* contribute to the development of metabolic syndrome.(6) Diabetic patients have been observed to have lower levels of *Bifidobacterium* and *Faecalibacterium prausnitzii*, both of which are Gram-positive bacteria that contribute to dysbiosis and reduced anti-inflammatory substances.(7) Overgrowth of Gram-negative bacteria and elevated LPS levels can lead to increased gut barrier permeability, also known as a leaky gut. Under these circumstances, metabolic endotoxemia and induced inflammatory pathways can affect insulin signaling.(8) LPS may induce insulin resistance and affect adipose tissue inflammation, potentially contributing to T2DM.(7)

Probiotics play a role in enhancing the diversity of the gut microbiota, improving dysbiosis, and preventing LPS-induced inflammation.(9) Indonesian indigenous probiotics, such as *Weissella confusa* F213 and *Lacticaseibacillus rhamnosus* FBB81 (LrFBB81), have been found to have positive effects on rectifying disruptions in tight junctions in the intestinal epithelium (10), and another probiotic strain, *L. rhamnosus* SKG34 (LrSKG34), reducing total cholesterol levels in the bloodstream (11). As these effects are specific to particular strains of probiotics, they may also affect glucose metabolism. Given the inconsistent results from earlier studies, with a limited number of trials using Indonesian indigenous probiotics for diabetes treatment, therefore this study was conducted to assess the effectiveness of LrFBB81, LrSKG34, and *L. rhamnosus* FSMM22 (LrFSMM22) in diabetic rats. To the best of our knowledge, this is the first study to utilize Indonesian indigenous probiotic strains to treat T2DM. The primary goal of this study was to determine the effects of probiotics on fasting blood glucose (FBG) levels and insulin resistance. The secondary objective was

to evaluate the effects of probiotics on LPS, insulin, and body weight.

Methods

Preparation of Bacterial Cell Suspensions

The research involved the use of three probiotic strains: LrFBB81, LrSKG34, and LrFSMM22. The LrFBB81 was isolated from healthy infant feces, meanwhile LrSKG34 and LrFSMM22 were isolated from Sumbawa's mare milk. All probiotic strains were stored and monitored in deep freezers (-80°C) at Bioscience and Biotechnology Laboratory, Universitas Udayana, Denpasar, Indonesia. Probiotics were cultured on de Man Rogosa Sharpe broth (Oxoid) medium at 37°C for 18 hours under anaerobic conditions using CO₂-generating gas Thermo Scientific Anaerogen™ 3.5L sachets (Thermo Scientific, Waltham, MA, USA). The cultures were then centrifuged at 4000 g (4°C) for 10 minutes to obtain cell mass, which was then dissolved in phosphate buffered saline with pH 7.2 (Sigma-Aldrich, St. Louis, MO, USA) at a final concentration of cell population about 10⁹ CFU/mL.

Animals and Experimental Design

Sixteen female Wistar rats (*Rattus norvegicus*) weighing 150–250 grams were sourced from the Pharmacology Department at Universitas Udayana, Denpasar, Indonesia. The animals were housed in an experimental room with a temperature of 22±2°C, humidity of 50±10%, and a 12 h light/dark cycle for illumination. The animal procedures were approved by the Ethics Committee of the Faculty of Medicine at the University (No. 1976/UN14.2.2.VII.14/LT/2023).

After a week of acclimatization, rats were provided with a daily high fat and sucrose diet (modified AIN-93M diet, comprising 25% sucrose, 40% fat, and 20% protein) with unrestricted access to water. Diabetes was induced to the rats by streptozotocin (STZ) (Sigma-Aldrich) in a dose of 35 mg/kg BW, injected intraperitoneally. Sodium citrate buffer (50 mM, 0.1 mol/L, pH4.5) was used to dissolve the STZ. Diabetes was confirmed by monitoring FBG levels, with a target level above 200 mg/dL 72 h post-injection. The rats were fasted for 10 h from night to morning in accordance with the circadian pattern before blood glucose measurements. The diabetic rats were then randomly assigned to one of four groups: 1) LrFBB81, 2) LrFSMM22, 3) LrSKG34, and 4) placebo as control group.

The rats received a daily intraoral dose of 1 mL of a probiotic cell suspension containing 10⁹ CFU/mL for 14

days, whereas the control group was administered 1 mL of buffered normal saline. Daily evaluations of all rats were conducted, including the monitoring of body weight and physiological appearance. Blood samples were obtained on the 3rd day after STZ injection and on the 15th day after euthanasia. All groups were assessed for FBG, homeostatic model assessment for insulin resistance (HOMA-IR), insulin, and LPS levels. The rats were fasted overnight and anesthetized using 0.1 mg/100 gram BW ketamine/xylazine before euthanized at the end of the trial (Figure 1).

Biochemical Parameter Analysis

The blood glucose test required extracting a blood sample from the tail vein of the rats and utilizing a glucometer OneTouch Ultra Plus Flex™ meter, (LifeScan, Malvern, PA, USA) following a 10 h period of fasting. To examine insulin and LPS levels, blood samples were collected from the venous sinus of the retro-orbital while the rats were under anesthesia on the day when diagnosis of diabetes was made and at the end of the trial. One and half mL of blood was

obtained and centrifuged, to collect serum. Serum insulin and LPS levels were analyzed using Sandwich Enzyme Linked Immunosorbent Assay (ELISA) method. Insulin level was determined using the Rat INS (Insulin) ELISA Kit (Cat. No. E-EL-R3034; ElabScience, Houston, TX, USA) with detection range of 6.25–400 pg/mL and sensitivity of 3.75 pg/mL. The HOMA-IR was calculated using the formula: $[HOMA-IR = \text{glucose (mg/dL)} \times \text{insulin (mU/L)} / 405]$, which the insulin unit was converted from pg/mL to mU/L. LPS level was determined using Rat Lipopolysaccharides (LPS) ELISA Kit (Cat. No. MBS268498; MyBioSource, San Diego, CA, USA), with detection range of 1000 ng/mL–15.6 ng/mL and sensitivity up to 5 ng/mL. Each ELISA sample was tested in triplicate.

Statistical Analysis

Data were subjected to univariate and bivariate analyses. Initially, the numeric data were examined to assess normality and are presented as mean and standard deviation (SD). The test of normality was conducted by the Shapiro-Wilk test.

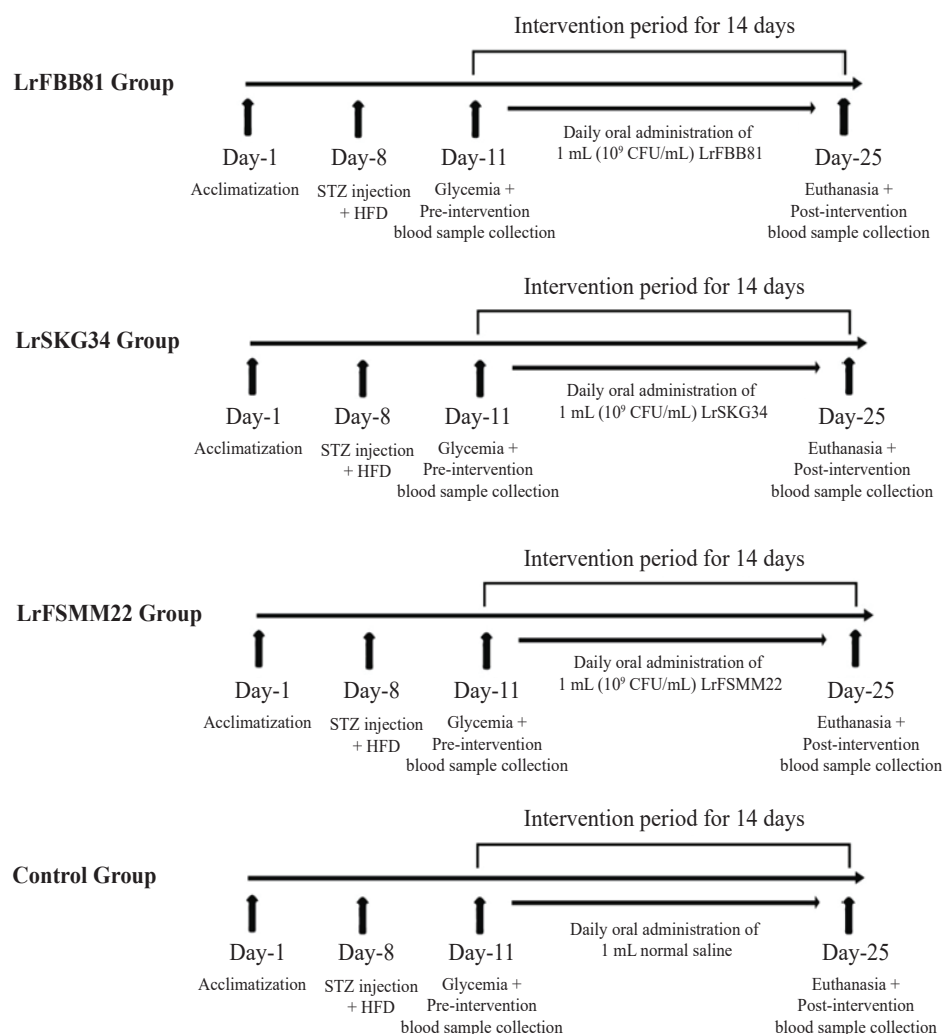


Figure 1. Flowchart of the experimental design.

Bivariate analysis comparing the pre- and post-intervention values within each group was performed employing a paired sample t-test. Bivariate analyses were conducted between the Δ value of intervention and control groups using a one-way analysis of variance (ANOVA). The post hoc Fisher least significant difference (LSD) test followed in cases of the assumption of equal variance, and Dunnett's T3 post hoc test was performed with the assumption of unequal variance. Statistical significance was determined as a p -value < 0.05 using SPSS® Statistics software Version 29 (IBM Corporation, Armonk, NY, USA) and Prism Version 9 (GraphPad Software, San Diego, CA, USA).

Results

After rats were administered with probiotics for two weeks, it was observed that the probiotics were safe and did not cause diarrhea, mortality, or any toxic effects on the behavior of the treated rats. The data for all parameters in all groups between the initial and final stages were presented in Figure 2 and Table 1-5. While the comparison between groups was provided in Table 6.

L. rhamnosus Administration Improved FBG, Insulin Resistance, and Insulin Level

The results before and after the experiment showed a notable contrast on FBG levels in LrFSMM22 group ($\Delta = -120.75$ mg/dL, $p = 0.035$), with FBG levels being significantly lower

after taking probiotic (Figure 2A, Table 1). However, no significant differences were observed in the Δ FBG of all probiotic groups if compared with control group. Weak evidence ($p = 0.093$) was seen in LrFSMM22 group after Dunnett's T3 post hoc test (Table 6).

A significant difference in Δ HOMA-IR was also noted in the LrFSMM22 group compared to that in the control groups ($\Delta = -3.90 \pm 4.64$, $p = 0.028$), although the use of probiotic did not result in a significant decrease in HOMA-IR after the experiment ($p = 0.096$). No significant reduction in HOMA-IR was observed after the experiment in the other groups (Figure 2B, Table 2).

There were no significant differences in the insulin levels between the probiotic and placebo groups before and after the experiment (Figure 2C, Table 3). LrFBB81 exhibited decreases in both HOMA-IR ($\Delta = -1.25 \pm 3.14$) and insulin levels ($\Delta = -106.89 \pm 129.56$ pg/mL) but not in FBG ($\Delta = 23.75 \pm 296.66$ mg/dL). In contrast, LrSKG34 only displayed an improvement in insulin levels ($\Delta = -26.67 \pm 68.53$ pg/mL). The changes in both probiotics groups were not statistically significant.

L. rhamnosus Administration Did Not Affect LPS Level, But Reduced Body Weight

After probiotics administration, only LrFBB81 group showed reduction in LPS level ($\Delta = -31.27 \pm 85.81$ ng/mL). The control group also showed a decrease in LPS levels ($\Delta = -24.43 \pm 30.14$ ng/mL) at the end of the experiment, but both the LrFBB81 and control groups difference were not

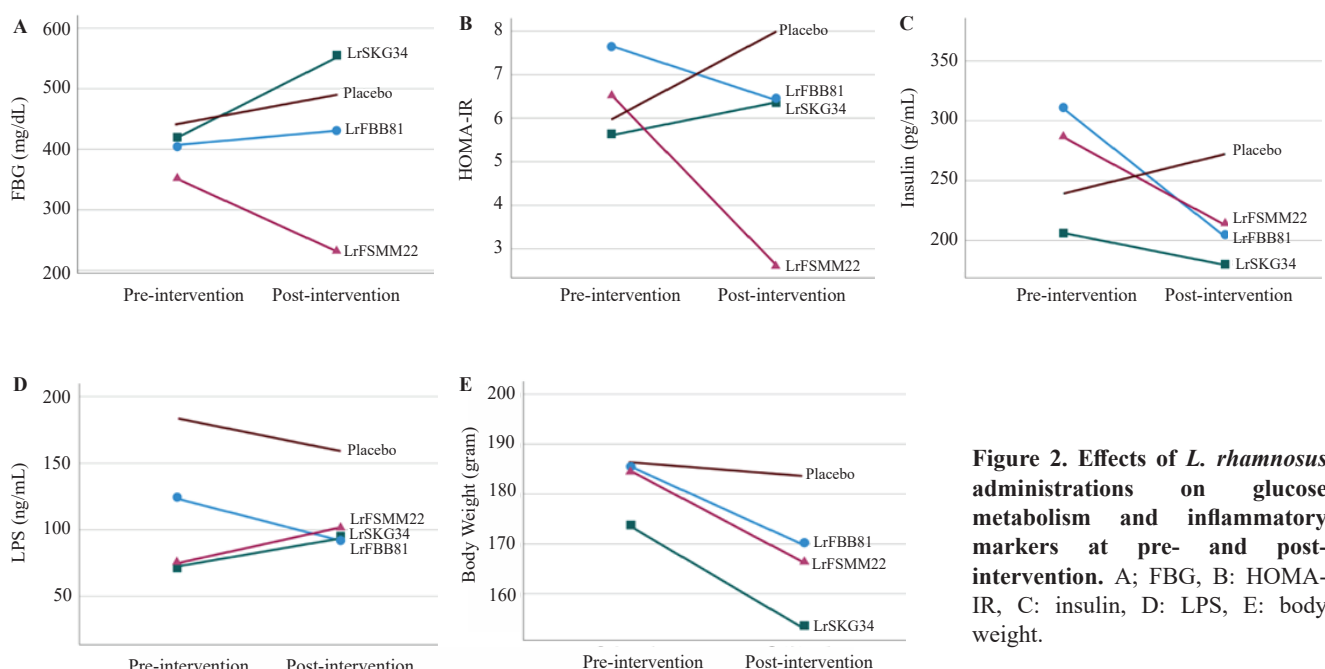


Figure 2. Effects of *L. rhamnosus* administrations on glucose metabolism and inflammatory markers at pre- and post-intervention. A; FBG, B: HOMA-IR, C: insulin, D: LPS, E: body weight.

Table 1. Effect of probiotics on FBG in intervention and placebo groups.

	LrFBB81	LrSKG34	LrFSMM22	Placebo
FBG Pre-intervention (mg/dL)	407.00±92.89	418.00±173.42	352.25±178.81	441.25±182.65
FBG Post-intervention (mg/dL)	430.75±204.99	551.75±55.71	231.50±119.95	490.00±132.22
Δ FBG (mg/dL)	23.75±296.66	133.75±170.38	-120.75±87.45	48.75±51.27
<i>p</i> -value	0.441	0.107	0.035*	0.077

p-value refers to the *p*-value between the pre- and post-intervention, analyzed with paired sample t-test.

**p*<0.05 is statistically significant.

statistically significant (*p*=0.259 and *p*=0.102, respectively) (Figure 2D, Table 4). No significant difference was observed from post hoc analysis in LPS (Table 6).

Changes in body weight before and after the experiment showed significant differences in the LrFBB81 group (Δ = -15.70±11.99 gram, *p*=0.040), LrSKG34 group (Δ =-20.43±7.24 gram, *p*=0.006), and LrFSMM22 group (Δ =-18.33±13.66 gram, *p*=0.037) (Figure 2E, Table 5). When analyzing Δ body weight differences, both LrSKG34 and LrFSMM22 group showed a significant difference compared to the placebo (*p*=0.029 and *p*=0.049, respectively) (Table 6).

Discussion

This study observed the effects of the Indonesian indigenous probiotic strains LrFBB81, LrSKG34, and LrFSMM22. The LrFBB81 is usually used to validate the *in vitro* translocation test system (10) and has functional properties as an antioxidant both *in vitro* and *in vivo* (12). In addition to their antioxidant properties, LrFBB81 improve tight junction disturbances in the intestinal epithelium induced by H₂O₂ and reduce epithelial permeability, thus playing an important role in the maintenance of mucosal integrity.(13) LrFSMM22 demonstrates good results in the binding of bacterial cells to laminin, a glycoprotein derived from mice that serves as an extracellular matrix.(14) This interaction functions as a model to investigate the role of cell surface proteins in laminin binding (15), exhibited high adhesion to porcine colonic mucin and extracellular matrix protein,

which indicate good adhesion to the intestinal mucosal surface. Furthermore, LrFSMM22 showed significantly higher adhesion to laminin than the GG strain of *L. rhamnosus* (LGG); the health-beneficial effects of these probiotic strains depend in part on the length of time they remain in the gastrointestinal tract and may be affected by their adhesion to the intestinal mucosa.(16) LrSKG34 can deconjugate glycodeoxycholic acid (17), and it was reported that the consumption of bio-yoghurt produced by LrSKG34 lowers the concentrations of serum total cholesterol, low-density lipoprotein (LDL), and triglycerides in hypercholesterolemia subjects.(11,18)

In our study, we found that only the probiotics LrFSMM22 significantly reduced FBG levels in rats compared to those in the control group. The results of present study are consistent with those of several previous studies. The db/db mice, an animal model of T2DM, showed that the administration of LGG resulted in improved glucose tolerance compared with control. The results of this study suggest that the positive effects of LGG on diabetes in db/db mice are associated with reduced endoplasmic reticulum (ER) stress and inhibition of macrophage activation, ultimately resulting in improved insulin sensitivity.(19) Another indigenous Indonesian probiotic, *Pediococcus acidilactici* DNH16, showed reduction in post-prandial glucose level in T2DM rat and considered safe for kidney and liver.(20) The effect of *L. rhamnosus* Hao9 (Hao9) on T2DM was investigated along with the underlying mechanisms in diabetic rats induced by an HFD and STZ. Diabetic rats administered with Hao9 showed lower insulin and FBG levels and the beneficial effects were

Table 2. Effect of probiotics on HOMA-IR in intervention and placebo groups.

	LrFBB81	LrSKG34	LrFSMM22	Placebo
HOMA-IR Pre-intervention	7.66±2.11	5.60±3.89	6.53±5.04	5.97±2.01
HOMA-IR Post-intervention	6.41±1.87	6.36±2.43	2.62±1.58	7.98±1.41
Δ HOMA-IR	-1.25±3.14	0.76±1.60	-3.90±4.64	2.02±3.31
<i>p</i> -value	0.243	0.208	0.096	0.155

p-value refers to the *p*-value between the pre- and post-intervention, analyzed with paired sample t-test.

Table 3. Effect of probiotics on insulin in intervention and placebo groups.

	LrFBB81	LrSKG34	LrFSMM22	Placebo
Insulin Pre-intervention (pg/mL)	310.01±76.58	206.20±103.20	286.21±78.81	239.23±75.85
Insulin Post-intervention (pg/mL)	203.12±61.67	179.53±62.03	212.95±129.65	272.13±62.09
Δ Insulin (pg/mL)	-106.89±129.56	-26.67±68.53	-73.31±134.96	32.9±50.35
<i>p</i> -value	0.099	0.493	0.178	0.141

p-value refers to the *p*-value between the pre- and post-intervention, analyzed with paired sample t-test.

achieved by enhancing the antioxidant capacity of the liver and significantly reducing the expression of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase in the liver of diabetic rats. Additionally, Hao9 decreased the serum levels of pro-inflammatory cytokines.(21)

In this study, LrFBB81 and LrSKG34 showed no improvement in FBG and HOMA-IR levels. While, the impact of LGG and *Lactobacillus delbrueckii* subsp. *bulgaricus* LB3 (*L. bulgaricus*) were evaluated in FBG levels of KK-Ay mice. Both FBG and postprandial glucose levels were lower in LGG group, compared with *L. bulgaricus* and control group.(22) Anti-obesity effects and mechanisms of action of four human-derived lactic acid bacteria strains (*L. rhamnosus* MG4502, *Lactobacillus gasseri* MG4524, *Limosilactobacillus reuteri* MG5149, and *Weissella cibaria* MG5285) in obese mice fed an HFD were evaluated. The study revealed that the *L. reuteri* MG5149 and *W. cibaria* MG5285 groups exhibited significantly reduced glucose levels compared to those in the HFD group.(23) Therefore, various studies show the beneficial effects of probiotics could vary depending on the strain.

A systematic review revealed that 27 probiotic interventions (*Lactobacillus* spp., *Bifidobacterium* spp., *Clostridium*, and *Akkermansia*) enhanced insulin resistance in experimental animals.(24) Probiotics may not consistently enhance insulin resistance in humans, only five from seven clinical trials demonstrated improvements in terms of insulin resistance parameters.(24) A recent clinical trial involving patients diagnosed with T2DM revealed that those who received a symbiotic containing *L. rhamnosus*, *Bacillus coagulans*, *Lactobacillus acidophilus*,

and fructooligosaccharide for a 12-week period experienced a decrease in FBG, insulin levels, HOMA-IR, homeostasis model assessment of β-cell function (HOMA-B), and high sensitivity C-reactive protein (hs-CRP) compared with those of the group that received a placebo.(25) The improvements in insulin resistance don't always occur simultaneously with improvement in insulin levels. While, HOMA-IR level was improved after administration of *Bifidobacterium animalis* 01 in diabetic Sprague–Dawley rats, but no significant difference was observed from the insulin level (26), similar results were observed in present study.

A study have demonstrated that probiotic supplementation can modify the gut microbiota, leading to a decrease in low-grade intestinal inflammation and enhancement of intestinal barrier integrity.(27) Administration of probiotic *L. acidophilus* FNCC 0051 at dose of 1.5×10^8 – 1.5×10^9 CFU/ mL/day for 21 days in STZ-induced diabetic rats showed statistically significant decrease of insulinitis scores, explaining the possible action of *L. acidophilus* FNCC 0051 in suppressing inflammatory responses on pancreas.(28) Administering heat-killed *W. cibaria* JW15 reduced nitric oxide and prostaglandin E production by suppressing inducible nitric oxide synthase and cyclooxygenase-2. Furthermore, it also inhibited the expression of pro-inflammatory cytokines.(29) The inflammatory pathway from gut dysbiosis is not the only pathway that could influence glucose metabolism. Gut microbiota also produce short chain fatty acids (SCFAs), which have a crucial part in glucose homeostasis.(30) SCFAs stimulate the gut hormone peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) by activating enteroendocrine L

Table 4. Effect of probiotics on LPS in intervention and placebo groups.

	LrFBB81	LrSKG34	LrFSMM22	Placebo
LPS Pre-intervention (ng/mL)	123.09±100.73	72.60±53.98	75.05±38.95	183.49±284.25
LPS Post-intervention (ng/mL)	91.82±59.10	93.51±64.93	101.80±70.75	159.06±258.52
Δ LPS (ng/mL)	-31.27±85.81	20.91±65.88	26.75±104.19	-24.43±30.14
<i>p</i> -value	0.259	0.285	0.322	0.102

p-value refers to the *p*-value between the pre- and post-intervention, analyzed with paired sample t-test.

Table 5. Effect of probiotics on body weight in intervention and placebo groups.

	LrFBB81	LrSKG34	LrFSMM22	Placebo
Body Weight Pre-intervention (gram)	185.65±33.40	173.70±6.5	184.80±23.67	186.37±31.72
Body Weight Post-intervention (gram)	169.95±24.29	153.27±6.28	166.47±34.39	183.62±29.60
Δ Body Weight (gram)	-15.70±11.99	-20.43±7.24	-18.33±13.66	-2.75±4.71
<i>p</i> -value	0.040*	0.006*	0.037*	0.164

p-value refers to the *p*-value between the pre- and post-intervention, analyzed with paired sample t-test.

**p*<0.05 is statistically significant.

cells.(31) GLP-1 stimulates insulin secretion from β-cells and suppresses glucagon from α-cells in the pancreas, which help manage blood glucose levels in diabetes.(32)

It was noted that inflammation contributes to the insulin resistance observed in diabetes. Present study focused on examining LPS to assess inflammatory response, which LrFBB81 and placebo groups showed reductions in LPS levels without statistically significant difference. A study conducted in Zuckerrats demonstrated that *Lactocaseibacillus paracasei* CNCM I-4034, *Bifidobacterium breve* CNCM I-4035, and *L. rhamnosus* CNCM I-4036 have the potential to decrease hepatic steatosis by reducing serum LPS levels and exerting anti-inflammatory effects.(33) LGG strain not only increased the diversity of beneficial bacteria in the small intestine, but also restored the gut permeability in the duodenum by increasing duodenal tight junction protein. There was a significant reduction in portal LPS in the liver of C57BL/J6 mice that were fed a high fructose diet supplemented with LGG.(34) Chronic diseases are linked to systemic inflammation, which is considered the primary pathogenic mechanism of metabolic conditions. Microbes in the host's gut release signaling byproducts, such as LPS from their cell walls (35), which can exert local effects. Once these LPS molecules cross the intestinal barrier and

enter the bloodstream, they can contribute to increased concentrations of LPS, which are associated with chronic diseases and metabolic conditions.

The findings of this current study revealed a statistically significant reduction in body weight across all groups. While other study revealed that introducing a modified *Lactobacillus* strain expressing Amuc-1100 on its surface improved obesity in adult mice fed an HFD.(36) Mice that received LGG were protected from developing adiposity and/or insulin resistance caused by an HFD when LGG was administered after an HFD, but not when given simultaneously. These findings indicated that in the presence of an HFD, supplementation with LGG reverses insulin resistance but does not prevent its onset.(19) *L. gasseri* SBT2055 and *Lactobacillus amylovorus* reduced body weight in healthy, overweight humans.(37) The same result also reported in an animal model, which showed reduction in body weight and fat collection in mice fed with HFD and probiotic *Lactiplantibacillus plantarum*.(38)

This study offers probiotic insights into lactic acid bacteria strains that have an impact on diabetes management as a supplementary treatment. Further comprehensive research in various scenarios and environments, along with extended follow-ups, particularly in molecular mechanisms

Table 6. Post hoc analysis from comparison of Δ in intervention and placebo group.

	LrFBB81	LrSKG34	LrFSMM22	Placebo
Δ FBG	23.75±296.66	133.75±170.38	-120.75±87.45	48.75±51.27
<i>p</i> -value	1.000	0.889	0.093	
Δ HOMA-IR	-1.25±3.14	0.76±1.60	-3.90±4.64	2.02±3.31
<i>p</i> -value	0.194	0.605	0.028*	
Δ Insulin	-106.89±129.56	-26.67±68.53	-73.31±134.96	32.9±50.35
<i>p</i> -value	0.078	0.428	0.169	
Δ LPS	-31.27±85.81	20.91±65.88	26.75±104.19	-24.43±30.14
<i>p</i> -value	0.902	0.419	0.363	
Δ Body Weight	-15.70±11.99	-20.43±7.24	-18.33±13.66	-2.75±4.71
<i>p</i> -value	0.094	0.029*	0.049*	

p-value compared to placebo, analyzed with Dunnett's T3 post hoc test. **p*<0.05 is statistically significant.

and human studies, has the potential to thoroughly assess the beneficial effects of probiotic strains. This study had some limitations. We believe that the improvement in FBG and HOMA-IR may be attributed to the healing of the leaky gut caused by a decrease in LPS levels. However, the present study revealed that LrFSMM22 improved FBG and HOMA-IR but not LPS levels. Inadequate probiotic dosages or intervention duration may be the reason why LPS didn't improve in this study. Meanwhile, the improvement in glucose metabolism even in the absence of change in LPS may be related to other pathway, such as SCFA, GLP-1 and PYY pathway, and further studies are required for clarification. Additionally, our study did not report gut microbiota diversity of a STZ-induced diabetic rat model. Gut dysbiosis is one of the mechanisms of diabetes mellitus, and assessing the composition and diversity of the gut microbiota could help elucidate the pathways or mechanisms by which indigenous probiotics function. Further investigations are needed to understand the molecular mechanisms of probiotic actions and the composition and diversity of the gut microbiota.

Conclusion

The results of this study showed that different probiotic strains have varying effects on glucose metabolism and inflammatory responses. LrFSMM22 notably decreased FBG and HOMA-IR in diabetic rat model, demonstrating significant differences compared to those in the placebo. However, there were no statistically significant differences in FBG and HOMA-IR values between the LrFBB81 and LrSKG34 groups. Body weight reduction was found across all groups, meanwhile the probiotics administration couldn't suppress the increases of LPS level.

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Authors Contribution

AVS developed the study, handled and treated the animal model, analyze the results, and wrote the final paper draft.

IGRW oversaw the experimental setup, data collecting, wrote portions of the procedures and findings, and carried out the statistical analysis. AANJK helped with the research design and did a literature analysis on the significance of probiotics in diabetes treatment. NNDF created probiotic formulations, examined the biochemical data and helped to write the commentary, with a special emphasis on the metabolic implications of the results. KS contributed clinical thoughts on the findings' applicability to diabetic patients, helped with data interpretation, and checked the text for correctness. INS created probiotic formulations, oversaw the experimental setup, data collecting, and analyzed the findings. All authors agreed to the final revised version of the manuscript.

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