The Effect of Fortified Dadih (Fermented Buffalo Milk) with Vitamin D$_3$ on Caecum Short Chain Fatty Acids (SCFA) Concentration and HOMA-IR of T2DM-Rats

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**Abstract**

The prevalence of T2DM continues to increase along the years. Probiotics and vitamin D have antidiabetic effects and a synergism between them is evident. Fermented milk such as dadih is a great source of probiotics, specifically lactic acid bacteria (LAB). Probiotics are involved in the formation of short-chain fatty acids (SCFA) which can increase insulin production and improve Homeostasis Model Assessment-Insulin Resistance (HOMA-IR). The objective of this study was to investigate the effect of vitamin D$_3$-fortified dadih on caecum SCFA concentration and HOMA-IR of T2DM-induced Wistar rats. A total of thirty rats were randomly split into five groups: four diabetic groups (C2, T1, T2, and T3) and one healthy control group (C1). Intervention groups were either given vitamin D$_3$ (T1), unfortified dadih (T2), or vitamin D$_3$-enriched dadih (T3). Concentration of SCFA, glucose, and insulin were measured by gas chromatography, GOD-POD, and ELISA, respectively. T3 group showed significantly lower fasting blood glucose and higher insulin than T1 or T2 at post-intervention. The HOMA-IR index at the end of intervention indicated that T3 was significantly different from T1. Total caecum SCFA and butyrate concentrations were significantly higher in T3 than T1 or T2. The HOMA-IR had an inverse correlation with total caecum SCFA ($r=-0.600$, $p=0.001$) and butyrate concentration ($r=-0.692$, $p=0.000$). The decreased insulin resistance might be partially attributed to total caecum SCFA and butyrate concentrations. In conclusion, vitamin D$_3$-fortified dadih had better efficacy in improving glycemic status, insulin, and SCFA concentration, leading to improved insulin resistance in T2DM rats.
**Introduction**

Type 2 Diabetes Mellitus (T2DM) occurs when the Pancreatic gland is unable to produce and use insulin effectively, thus disrupting the delivery of glucose to body cells and making blood sugar levels increased.\(^1\) Worldwide prevalence of DM in 2017 was 8.8% and was expected to increase to 9.9% by 2023.\(^2\) The pathogenesis of T2DM might involve intestinal microbiota due to its potential role in insulin signaling and systemic inflammation amelioration.\(^3,4\) An imbalance in the number and diversity of microorganisms in the digestive tract are commonly found in people with obesity and T2DM.\(^5\) Modulation of intestinal microbiota with probiotics is one approach to achieve balanced intestinal microbiota in order to improve host health. Probiotics can protect humans from infection and disease as well as produce short-chain fatty acids (SCFA) and vitamin.\(^6\) SCFA subsequently activates intestinal hormones, in particular peptide YY and glucagon-like-peptide-1, to stimulate insulin signaling and proliferation of pancreatic cells.\(^3,5\) Probiotics are present in traditionally fermented milk such as dadih.

Dadih is a buffalo milk fermentation product from West Sumatera, Indonesia. Dadih contains lactic acid bacteria (LAB) such as *Lactobacillus plantarum*, *Lactococcus lactis* subsp cremoris, and *Lactococcus lactis* subsp lactis.\(^7\) Furthermore, some LAB in dadih are also found in yogurt and curd such as *Lactobacillus plantarum* and *Lactococcus lactis*.\(^8–11\)

Vitamin D and its receptors (VDR) regulate the diversity and functions of intestinal microbiota, other than their role in energy metabolism balance, immunity, and inflammatory response.\(^12–14\) Several studies showed that vitamin D deficiency was associated with the pathogenesis of T2DM. Vitamin D\(_3\) in its active form (1α, 25-dihydroxyvitamin D\(_3\)) affects pancreatic β cells and insulin secretion, and through other mechanisms can affect insulin sensitivity. Probiotics can also increase VDR activity as well as improve 25 (OH) D circulation in the body. One study stated that *Lactobacillus plantarum* increased the expression and activity of VDR.\(^13,15,16\) Administration of fermented goat milk fortified with vitamin D\(_3\) to T2DM rats was reported to reduce glucose and leukocyte levels, which are both markers of low-grade inflammation.\(^17\) Fortification of vitamin D\(_3\) in dadih is expected to increase vitamin D\(_3\) intake and improve the balance of intestinal microbiota so as to improve glycemic status through SCFA therefore making it a possible additional therapy for diabetic patients.

The study aimed to determine the effect of fortified dadih (fermented buffalo milk) with vitamin D\(_3\) on caecum Short Chain Fatty Acids (SCFA) concentration and HOMA-IR of T2DM-Rats

**Materials and Methods**

**Dadih Preparation**

![Fig. 1: Dadih and Vitamin D3-fortified dadih](image-url)
Experimental Procedure
A total of thirty, 8-12 weeks old Wistar rats weighing 150-200 g were allocated to five groups, specifically healthy controls (C1), diabetic controls (C2), and three intervention groups (T1, T2, and T3). Rats were kept in individual cages and fed with standard diet (15 g/day) during the whole experiment, as well as provided ad libitum access to water. Laboratory animal care was performed according to the Guidelines of Laboratory Animal Management from the Central Laboratory of Food and Nutrition Studies, Gadjah Mada University, Yogyakarta.

High fat diet (HFD) was introduced for 14 days and followed by the injection of streptozotocin (STZ) to induce type 2 diabetes in rats. Afterwards, the intervention groups were given a dose of 36IU/200gbodyweight/day of vitamin D₃ (T1), unfortified dadih of 4g/200g body weight/day (T2), and vitamin D₃-fortified dadih of 4g/200gbodyweight/day (T3), respectively, for 28 days. Blood sample and caecum digesta were taken for analysis, with the former collected pre- and post-intervention while the latter only at post-intervention. The entire procedure involved in our study was precedingly ethically approved through certificate no. 140/EC/H/KEPK/FK-UNDIP/X1/2019 released by the Health Research Ethics Committee of Faculty of Medicine Diponegoro University Indonesia.

Serum Insulin Determination
Serum insulin determination was carried out through ELISA-Kit according to manufacturer instructions (Fine Test, Wuhan, China). Briefly, 100µl of standard and serum of each sample was transferred into a well plate, later the plate was sealed and incubated at 37°C for 90 minutes. Afterwards, the plate was rinsed 3 times using wash buffer where the buffer remained in the wells for at least 2 minutes every time. Every well was then filled with 100µl of SABC working solution, before getting sealed and incubated for half an hour at 37°C. Post-incubation rinse was done in a similar manner but for 5 times. TMB substrate at 90µl and 50µl of stop solution were added into each well, and the plate was sealed and incubated at 37°C for 20 minutes in the dark. The colour would immediately turn yellow. The absorbance of samples in well plates was read by ELISA Reader at 450 nm.¹⁸

Plasma Glucose Measurement
Plasma glucose levels were measured with GOD-PAP where glucose oxidase (GOD) enzymatically oxidize glucose into gluconic acid. Quinoneimine dye was the chosen colorimetric indicator. Briefly, 10 µl standart and serum of each sample were add into tube. Furthermore, 1000 µl reagen kit of GOD PAD was add into tube, which mixed with vortex and incubated at 20-25°C for 20 minutes. The sample absorbance was futher read by spectrofotometer at 500 nm.¹⁹

HOMA-IR Measurement
The Homeostatic model assessment of insulin resistance (HOMA-IR) as calculated with the following formula
HOMA IR= Fasting insulin × (Fasting plasma glucose) / 405

where fasting insulin was expressed in IU/mL and fasting plasma glucose in mg/dL.\textsuperscript{20}

**SCFA Analysis**

SCFA levels were determined with gas chromatography (SHIMATZU). Approximately 0.05 g of caecal content was acidified with equal amounts of sulphuric acid, and then extraction of SCFA was done by adding 0.6 ml of diethyl ether and putting the sample in a centrifuge for 30 seconds at 14000 rpm. The capillary column (Nukol) of the gas chromatography was equipped with a flame ionization detector and injected with 1µl of organic phase, with the initial temperature set at 80°C was maintained for 0.5 minutes, the raised to 180°C with ramping 8°C/minute to be constant for 1 minute. Furthermore, the temperature was increased to the final at 200°C (ramping 20°C/minute) and then sustained for 5 minutes. Nitrogen was the carrier gas and calibration curves were set for acetic acid, butyrate, and propionic acid in order to quantify the concentration of those fatty acids in the sample.\textsuperscript{21}

**Statistical Analysis**

Normally distributed data were expressed as mean ± SD and analysed with One-Way ANOVA followed by Bonferroni post hoc test. Kruskal Wallis test followed by Mann-Whitney-U-test was used as the alternative. The relationship between variables were tested using Spearman's correlation test. All statistical analyses were done with a statistical software (SPSS 16) with significant differences noted at p<0.05 (95% confidence interval).

**Results**

**Rats' Weight Changes**

Figure 1 presents the weight changes of rats in all groups during the experiment. No significant difference was observed amongst groups at the inclusion (p > 0.05). The body weight of the rats changed during T2DM induction by HFD and STZ-NA. There was an increase of body weight (rat-BW) during HFD period in C2, T1, T2 and T3 (the groups had p = 0.000, ANOVA; Fig. 4). This significant difference was confirmed by the analysis of the changes (Δ) of rat-BW between C2, T1, T2 and T3 compared with C1 (p<0.05; Fig.4). Although HFD induced an increase of BW in T2DM groups, STZ-NA injection resulted in a significantly lower BW of T2DM groups (C2, T1, T2, T3) than C1 (p< 0.05). After the intervention, all intervention groups (T1, T2, T3) showed significantly higher BW than C2 and no differences in comparison to C1. ΔBW in C1 was not significantly different than any intervention groups (p>0.05; Fig.4). Vitamin D3, dadih, and vitamin D3-fortified dadih treatments significantly caused weight gain in T1, T2 and T3.

**Fasting Blood Glucose, Serum Insulin, and HOMA-IR**

T2DM induction resulted in elevated serum fasting blood glucose (FBG) (>250 mg/dL) in C2, T1, T2 and T3. No difference was found among those groups (p>0.05; Fig 5.A). After the treatment, the FBG levels of T1, T2 and T3 groups were significantly lower than C2, almost at the same level of those in C1 (p< 0.05; Fig 5.B). Post-intervention FBG levels of T3 were considerably lower in comparison to T1 (p =0.000; Fig 5.B). In addition, Δ FBG level of T3 was notably lower than other intervention groups (p =0.004 and p = 0.037; Fig 5.C).

All diabetic groups showed noticeably lower insulin levels than the healthy C1 group at pre-intervention period. In contrast, measurement at post-intervention revealed that all intervention groups had notably higher insulin levels than C2. T3 exhibited considerably higher insulin levels compared to both T1 and T2. All intervention groups showed significantly lower insulin levels than healthy C1 group at post-intervention. The findings of the Δ insulin level analysis were in line with the insulin level at post-intervention.

Before the intervention, HOMA-IR of all diabetic rat groups were found to benotably higher than the healthy controls. However, no difference was observed among C2, T1, T2, and T3 groups. At the end of the intervention, HOMA-IR of all intervention groups were significantly lower than C2. T1 group showed significantly higher post-intervention HOMA-IR in comparison to the other intervention groups, while no difference was found between the latter two intervention groups. Most of Δ HOMA-IR analysis supported the findings of the HOMA-IR at post-intervention.
The Effect of Fortified Dadih on Caecum SCFA

The post-intervention caecum total SCFA, acetate, propionate and butyrate concentrations were significantly different among groups (p=0.002, p=0.001, p=0.0001 and p=0.0001, respectively; Table 1). T3 group, in particular, had significantly higher concentrations in comparison to C2 and the other intervention groups. Additionally, no difference was observed between T3 and the healthy C1 group. Those of the T1 and T2 groups were also not considerably different than C2. T3 group showed noticeably higher levels of caecum acetate and propionate in contrast to C2, but no difference was observed with C1. On the other hand, those of T1 and T2 groups were difficult to interpret due to the lack of difference found in caecum acetate and propionate levels when compared to the C2 or C1 groups. Interestingly, all analyses of butyrate levels were in favour of the total SCFA levels.

![Graph](image)

**Fig. 4:** Vitamin D3-fortified dadih improved the weight loss of T2DM rats. The values are shown as mean±SD. *p < 0.05,**p < 0.01,***p < 0.001 in comparison to DM group

![Graph](image)

**Fig. 5:** A. Pre-treatment FBG levels, B. Post treatment, and C. Delta FBG. The values were shown as mean ± SD. *p < 0.05,**p < 0.01,***p < 0.001 in comparison to C2 group. ### p < 0.001 in comparison to C1 group
Table 1: Insulin Level, HOMA-IR, Total SCFA, Acetate, Propionate, and Butyrate

<table>
<thead>
<tr>
<th>Groups</th>
<th>C1</th>
<th>C2</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>p1</th>
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<tbody>
<tr>
<td>Marker</td>
<td>Insulin Level (pg/ml)</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>Pre</td>
<td>16.92 ± 0.16&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>12.36 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.26 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.32 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.34 ± 0.16 a</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>16.80 ± 0.18&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>12.18 ± 0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.68 ± 0.32&lt;sup&gt;b,d&lt;/sup&gt;</td>
<td>15.68 ± 0.14&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>16.07 ± 0.20&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>∆</td>
<td>-0.11</td>
<td>-0.19</td>
<td>2.32</td>
<td>3.45</td>
<td>3.77</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pre</td>
<td>2.95±0.06&lt;sup&gt;b,c,d&lt;/sup&gt;</td>
<td>8.79±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.60±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.70±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.82±0.17&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>Post</td>
<td>3.02±0.07&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>8.75±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.69±0.07&lt;sup&gt;b,d&lt;/sup&gt;</td>
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<td>3.18±0.08&lt;sup&gt;b,c&lt;/sup&gt;</td>
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<tr>
<td></td>
<td>∆</td>
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<td>-4.95</td>
<td>-5.43</td>
<td>-5.62</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.001</td>
<td>0.019</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
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<tr>
<td>Total SCFA (mmol/kg)</td>
<td>137.44</td>
<td>84.71</td>
<td>103.52</td>
<td>106.36</td>
<td>139.42</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>(97.7-171.06)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(58.20-97.08)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(42.85-122.87)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(82.43-142.33)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(137.36-150.61)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Asetate (mmol/kg)</td>
<td>60.60 ± 11.20</td>
<td>41.63 ± 9.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.72 ± 16.85</td>
<td>54.14 ± 11.13</td>
<td>70.28 ± 2.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionate (mmol/kg)</td>
<td>48.74 ± 84.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.15 ± 5.44&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30.36 ± 10.92</td>
<td>37.03 ± 10.28</td>
<td>49.66 ± 11.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Post</td>
<td>(22.74-37.31)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(11.49-16.77)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(7.31-19.54)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>(11.20-23.39)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(20.71-22.65)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Notes: Five groups of rats (n=6 for each group) consist of C1: healthy control and C2: T2DM control; T1: Treatment group 1; T2: Treatment group 2; T3: Treatment group 3: changes between pre-post value; differences among groups were analyzed using ANOVA (Insulin level, HOMA-IR, Acetate and Propionate) Kruskall wallis (SCFA total, Butyrate); p: Paired t-test/ Wilcoxon test were used to evaluate pre-post treatment values; alphabetical superscripts showed a significance level of a: p<0.05 as opposed to C1; b: p<0.05 as opposed to C2; c: p<0.05 as opposed to T2; d p<0.05 as opposed to T2; e p<0.05 as opposed to T3. Normally distributed data are written as mean±SD and median (Min-Max) if otherwise; p1: value among all of groups were analyzed using ANOVA for the data which were normally distributed and Kruskall wallis for non-normally distributed data.

Post-intervention correlation test on all variables revealed a significantly inverse correlation (Table 2). Total SCFA appeared to be strongly correlated to HOMA-IR index (r=-0.600; p=0.001; Table 2). Likewise, a similar relationship was also observed between butyrate and HOMA-IR index (r=-0.692; p=0.000; Table 2).

**Discussion**

The interventions used in this present study were expected to influence the BW of T2DM rats, because their protective effects might target the mechanisms related to BW. The weight loss in diabetic rats induced with HFD-STZ experience was in fact countered by the interventions as giving vitamin D3-
fortified *dadih* apparently caused an increase of BW in T2DM-rats (Figure 4). The same effect was also observed in groups given either *dadih* or vitamin D3. The weight gain occurred was not different among treatment groups and they all managed to return the BW of T2DM-rats to normal. HOMA-IR analysis showed that all interventions reduced insulin resistance (Table 1). HOMA-IR index at the end of all interventions were significantly lower than those at pre-interventions and post-intervention diabetic controls. Furthermore, giving vitamin D3-fortified *dadih* simply *dadih* exhibited better effects in decreasing insulin resistance of T2DM-rats than merely vitamin D3. Post-intervention HOMA-IR of all intervention groups were close to that of healthy control group, and there was no notable difference between those given *dadih* only and healthy controls. Measurements of other IR indexes and biomarkers are required to confirm these findings. Because of the relationship between HOMA-IR and cardiovascular diseases, a further study is needed to confirm whether vitamin D3-fortified *dadih* has better protective effects in preventing cardiovascular illnesses in T2DM individuals in comparison to its single intervention (*dadih* only or vitamin D3 only).

Vitamin D3-fortified *dadih* attenuated HOMA-IR of T2DM-rats, as seen by the pre and post indices in T2DM-rats given vitamin D3-fortified *dadih* (Table 1). Moreover, post-intervention values were lower than both diabetic controls and vitamin D3 intervention group. Since *dadih* is a probiotic-rich food, the findings in this study were in line with probiotic studies. Probiotics increase SCFA production by activating GLP-1 andYY peptides in the intestine, leading to improved insulin resistance and insulin production. Probiotic bacteria improve insulin resistance through its anti-inflammatory effects, specifically by reducing TNF-α release and NF-κB binding activity. Another study also reported beneficial effects of providing *Lactobacillus casei* 108 CFU strains for T2DM patients towards fasting blood glucose and HOMA-IR levels. The consumption of *Lactobacillus acidophilus NCFM* at 10^10^ CFU/g for four weeks was proven to increase insulin sensitivity compared to the control group. Three types of intervention were used in this study: vitamin D3, *dadih*, and vitamin D3-fortified *dadih* and no difference was found among the three in their effect towards BW of T2DM rats. All interventions proved to positively affect BW, possibly due to restored pancreatic β cells that consequently means improved insulin production. Increased insulin secretion inhibits the lipolysis and fatty acid oxidation thus resulting in weight gain.30,31

<table>
<thead>
<tr>
<th>SCFA</th>
<th>r</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Total SCFA</td>
<td>-0.600</td>
<td>0.001*</td>
</tr>
<tr>
<td>Acetate</td>
<td>-0.477</td>
<td>0.008</td>
</tr>
<tr>
<td>Propionat</td>
<td>-0.518</td>
<td>0.003</td>
</tr>
<tr>
<td>Butyrate</td>
<td>-0.692</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

- 0.00-0.25 = very weakly correlated
- 0.25-0.50 = weakly correlated
- 0.50-0.75 = moderately correlated
- 0.75-0.99 = strongly correlated

HFD and STZ were used to induce T2DM in rats. HFD increases weight and size of adipocytes. It also increases oxidative stress which causes various conditions that result in T2DM, including compromised glucose tolerance, insulin resistance, pancreatic β-cell damage, and dyslipidemia. Reactive oxygen species produced during oxidative stress causes insulin resistance in peripheral tissues due to decreased expression of GLUT4 transporters in cellular membranes. HFD-induced weight gain is associated with intestinal colonization of gram-negative bacteria, causing lipopolysaccharide (LPS) levels to rise. High levels of LPS triggers the secretion of proinflammatory cytokines, further aggravating insulin resistance. Weight loss indiabetic rats induced with HFD-STZ was attributed to lipolysis and proteolysis processes as a result of insulin resistance, leading to decreased muscle mass and adipose tissue volume. Significant weight loss was also found in rats induced by STZ at 45 mg/kg, as reported by Asiltas *et al.* Three types of intervention were used in this study: vitamin D3, *dadih*, and vitamin D3-fortified *dadih* and no difference was found among the three in their effect towards BW of T2DM rats. All interventions proved to positively affect BW, possibly due to restored pancreatic β cells that consequently means improved insulin production. Increased insulin secretion inhibits the lipolysis and fatty acid oxidation thus resulting in weight gain.30,31

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repair insulin resistance. Vitamin D affects insulin resistance through parathyroid hormone (PTH). Vitamin D deficiency causes an increase in PTH concentration, consequently reducing the amount of glucose transporters in cell membranes, particularly GLUT1 and GLUT4, thus decreasing glucose uptake in cells which will eventually lead to insulin resistance. Despite that, giving vitamin D3-fortified dadih to T2DM rats did not prove to attenuate HOMA-IR in this study. This may have been caused by poor synergy between lactic acid bacteria and vitamin D3.

In the present study, vitamin D3-fortified yoghurt managed to prove its efficacy in improving HOMA-IR compared to plain yoghurt in T2DM post-menopause women. These contrasting results might be due the difference in subjects. The same study also reported that the group consuming vitamin D3-enriched yoghurt showed significantly higher 25 (OH)D serum than the plain yoghurt group. Further research on serum 25 (OH)D levels might be able to complete the vitamin D3-fortified dadih study.

Vitamin D3-fortified dadih improved to have more prominent effects than the single interventions (either dadih or vitamin D3) as shown by the significantly higher insulin levels (Table 1) and improved glycemic status of T2DM-rats (Figure 5). Vitamin D in its form as 1,25 (OH) D2 has an important role in insulin secretion because it activates insulin gene transcription in humans. It also activates VDR in the pancreas gland and increases the expression of 1α-hydroxylase. Calcitriol or active vitamin D metabolites is known to stimulate insulin gene transcription in humans as well. HOMA-IR in this present study was only partially in line with the findings of insulin and FBG. HOMA-IR is calculated based on both FBG and insulin levels, therefore the measurement of biomarkers in insulin signalling, including GLUT1 and GLUT4, needs to be performed to re-confirm the results in this study.

Furthermore, giving vitamin D3-fortified dadih appeared to be more effective in elevating concentrations of caecum total SCFA and butyrate in comparison to its single intervention. These findings are supported by a previous study where consumption of yoghurt fortified with 2000 IU of vitamin D for 12 weeks significantly increased fasting serum insulin in menopausal T2DM patients. Increased insulin after the intervention was mediated by raised SCFA concentrations in the caecum. SCFA is an important energy source for de novo lipogenesis and ligands for FFAR2 and FFAR3 in intestinal enteroendocrine cells. SCFA reduces insulin resistance and promotes proliferation and development of pancreatic cells by activating the receptors. SCFA produced by intestinal microbiota and other bacteria during food matrix fermentation has many beneficial effects. Production of SCFA in the large intestine inhibits the growth of pathogenic bacteria through lowering local pH. SCFA has several functions, namely regulating the metabolism of lipid and glucose, lessening risks of inflammatory diseases, and promoting antioxidant functions.

In addition, SCFA positively influences host metabolism. SCFA increases insulin production, lowers the production of pancreatic glucagon, and reduces liver gluconeogenesis through increased AMPK activity. AMPK is an enzyme that functions in energy metabolism. Activation of AMPK stimulates glucose transporter (GLUT4) on cell membrane so that it will raise glucose uptake by muscles and adipocytes. Butyrate plays an essential part in the development of diabetes and obesity. It can stimulate pancreatic beta cells through incretin. Numerous studies have reported the beneficial impacts of butyrate for diabetic or obese subjects. A notable increase of plasma insulin was observed in mice orally fed with sodium butyrate. Another animal study reported improved insulin sensitivity and decreased adiposity in C57BL/6 mice after given butyrate supplementation, successfully preventing insulin resistance despite being previously treated with high-fat diet. A similar study with high-fat diet-fed mice found an association of increased butyric production by microbiota, lowered blood glucose, suppressed weight gain, and ameliorated insulin resistance with the administration of VSL probiotic #. The inverse moderate correlation between SCFA concentration and HOMA-IR was found in this present study (Table 2), implying that somehow SCFA contributed in lowering HOMA-IR in T2DM subjects, also supported by the studies mentioned earlier. Although HOMA-IR index of vitamin D3-fortified dadih group was not significantly different than the dadih only group, it was still lower than the latter. Additionally, the average total SCFA, acetate, propionate and butyrate concentrations of vitamin D3-fortified dadih group was higher compared to other groups. It is concluded that vitamin D3-fortified
**dadih** proved to significantly reduce the FGB and HOMA-IR index and increase insulin and SCFA.

**Conclusion**
This study concludes that vitamin D3 fortified **dadih** can improve glycemic status, increase insulin levels and improve insulin resistance in T2DM-induced Wistar rats through increasing concentrations of total SCFA and Butyrate. The strong inverse correlation between HOMA-IR and Total SCFA and butyrate indicated that increases in total SCFA and butyrate are associated with improved insulin sensitivity.

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**Conflict of Interest**
The authors declare no conflict of interest.

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