Effect of Traditional Fermented Buffalo Milk (Dadih) On Body Weight, Adipose Tissue Mass and Adiposity Inflammation in High Fat-Induced Obese Rats

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Abstract

Background: Changes in the gut microbiota composition have been proposed to play a major role in obesity development and chronic low grade inflammation in human. Manipulating the gut microbiota composition through diet exerts potential role in obesity treatment and prevention. The aim of this study was to study the anti-obesity and anti-inflammatory effect of dadih, a traditional fermented buffalo milk from Indonesia, in rats fed high fat diet.

Method: Obesity was induced in 20 male Sprague Dawley rats by giving high fat diet for 6 weeks. Five rats were added as a control group by giving standard chow diet. Rats were randomly divided into dadih 2 g/ml, 4 g/ml and 8 g/ml and negative control. Rats were sacrificed on day 98 and white adipose tissue (WAT) was extracted for malondialdehyde (MDA) analysis.

Result: Reduced body weight and adiposity were observed in all dadih groups compared with the negative control group. MDA was significantly lower in rats received dadih 4 g/ml and 8 g/ml. Supplementation of dadih 4 g/ml and 8 g/ml significantly increased fecal lactic acid bacteria count in rats.

Conclusion: Dadih was effective for reducing adiposity, weight gain and adiposity inflammation in high fat induced obese rats.

Keywords: Fermented Milk; Obesity; Lactic Acid Bacteria

Introduction

Obesity is an emerging health problem worldwide. There is an increasing prevalence of obesity and overweight in both developed and developing countries [1,2]. It is estimated that around 1.5 billion of people worldwide are overweight and 500 million of them are obese which can be translated as a twofold increase over three decades [3,4]. Obesity and overweight also attributed to 3.4 million of death in 2010 [5]. Although weight loss in obesity is the best way to reduce the burden of obesity and overweight-related disease [6-11], lifestyle/dietary modification and pharmacological intervention for decreasing weight are challenging because of the modest effect of treatment and weight regain in the long term treatment [12-14].

Studies have demonstrated that gut microbiota plays an important role in obesity and other metabolic disease development, such as diabetes mellitus and cardiovascular disease [15-17]. In addition, gut microbiota composition is a strong predictor of obesity compared with the genome-associated obesity [18,19]. This effect was linked with an effective ability of the gut to harvest energy from food [15,20-23]. The disturbance in the microbiota composition also correlated with increasing levels of lipopolysaccharide in the blood, supporting the chronic inflammatory theory in obesity [24-26] and underlines the central role of the gut microbiota in obesity development.

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Several ways have been developed to modulate the alteration of the gut microbiota, i.e., probiotics, prebiotics, antibiotics, synbiotic, and fecal transplant. Probiotic, a living micro-organism when administered in the adequate amount confers the health benefit of the host [27], has been gained attention for obesity treatment due to its ability in alleviating chronic intestinal disorder such as inflammatory bowel disease, antibiotic-associated diarrhea, infectious diarrhea, traveler’s diarrhea, irritable bowel disease, and necrotizing enterocolitis [28-35]. It is also has hypocholesteromic activity [36-39] and weight reducing activity [40-43].

Dadih, a traditional fermented buffalo milk from west Sumatra, Indonesia, has been used as food condiment by people in that region which is believed to confer health benefit due to the presence of lactic acid bacteria in dadih [44,45]. Several lactic acid bacteria have been characterized in dadih, i.e., *Leuconostoc mesenteroides*, *Streptococcus faecalis*, *S. lactis* supsp. *lactis*, *S. cremoris*, *L. casei* subsp. *casei* and *Lactobacillus casei* subsp. *rhamnosus*. However, in our knowledge, study regarding dadih for obesity prevention isn’t conducted yet. Thus, the aim of this study is to evaluate the anti-obesity effect of dadih in rats fed a high fat diet.

Method

Preparation of Dadih

Dadih was prepared according to local method. Briefly, 200 ml of buffalo milk was pasteurized at 70°C for 30 minutes with mild stir. Buffalo milk, then left at room temperature until 37°C and incubated at the closed-sterile bamboo with banana leaves for 2 days. After two days, bamboo was cut and dadih is stored at 4°C prior to future use.

Chemical Analysis of Dadih

Protein content in dadih was analyzed using Kjehdahl method. Fat was analyzed using soxhlet method. Gravimetric method was used to analyze the moisture content in dadih. All analysis was performed by a standard protocol on the AOAC official method (1990). The pH of dadih was analyzed using Orion 2 Star pH meter (Thermo Scientific).

Animals

Twenty five (25) male Sprague dawley rats aged 2 months were obtained from faculty of pharmacy University Gadjah Mada (UGM), Yogyakarta, Indonesia. They were healthy before and during the experiment. Rats were caged individually in plastic cages with 12 hours of light cycle. Food and water were given ad libithum in adaptation period for 1 week. After the adaptation period, twenty (20) rats were fed a high fat diet for 6 weeksto induce obesity and other was fed a standard diet as controls. The composition of the diet was presented in Table 1.

<table>
<thead>
<tr>
<th>Composition</th>
<th>Standard Diet</th>
<th>High Fat Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize starch</td>
<td>530</td>
<td>430</td>
</tr>
<tr>
<td>Egg white</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Sucrose</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>50</td>
<td>150</td>
</tr>
<tr>
<td>Vitamin mix*</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Mineral mix*</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Carboxy methyl cellulose (CMC)</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

*Based on American Institute of Nutrition (AIN)-93 vitamin and mineral mix composition [46].

Table 1: Composition of experimental diet (as fed g/kg).

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Experimental Design
After high fat diet, rats were divided into 5 groups with 5 rats in each group. Group 1 was the obese rats + dadih 2 gram/ml, group 2 was the obese rats + dadih 4 gram/ml, group 3 was the obese rats + dadih 8 gram/ml, group 4 was the obese rats, which serves as negative control and group 5 was normal rats fed a normal diet which serves as a control group. Dadih was prepared by homogenized 100 gram of dadih with 100 ml distilled water prior to administration to give 1 gram/ml dadih concentration. Then, dadih was given at concentration mentioned above. Body weight was weighted once in a week. Dadih was given for 8 weeks and at the end of the experiment, all rats were sacrificed for white adipose tissue malondialdehyde analysis. Feces were collected after high fat diet and eight weeks of experiment for fecal lactic acid bacteria counting.

White Adipose Tissue Malondialdehyde (MDA) Analysis
Malondialdehyde in white adipose tissue was analyzed using Ohkawa, et al. [47] method with modification. In brief, white adipose tissue was extracted, weighed, and homogenized with cold PBS (10% w/v). The mixture then centrifuged at 3000 RPM for 15 minutes to get a supernatant. Supernatant then mixed with 0.2 ml SDS 8.1% (w/v), 1.5 ml acetate buffer (20% v/v, pH 3.5), and 1.5 ml 0.8% TBA (w/v). The solution then incubated at 37°C in a water bath for 60 minutes. A 15:1 ratio (v/v) mixture of n-butanol and pyridine was added into the supernatant, mixed by vortexes and centrifuged at 4000 RPM for 15 minutes. The absorbance was measured from supernatant at 532 nm. Standard curve linear was performed using TMP (1,1,3,3-tetramethoxypropane) at different concentration to calculate the concentration in the sample.

Total Lactic Acid Bacteria Count
Lactic acid bacteria in feces and dadih were measured according to Foo, et al. [48]. Briefly, 1 gram of feces or dadih was homogenized with 9 ml of sterile peptone water and incubated for 1 hour prior to 10-fold dilution (v/v) for total lactic acid bacteria count. Total lactic acid bacteria count was measured by measuring the colonies after spreading the solution in each fold dilution into MRS agar and incubated 30°C for two days.

Statistical Analysis
Data were presented as mean ± standard deviation and analyzed using Graphad PRISM 6. One way ANOVA followed by Tukey HSD was used for data analysis. Alpha was set at 0.05.

Result
Proximate Analysis of Dadih
Based on the proximate analysis, dadih has 8.25 ± 0.09% of protein, 31.145 ± 0.12% of fat, 4.54 ± 0.30% of carbohydrate and 56.07 ± 0.09% of water. Dadih also has high acidity with the pH level of 4.68 ± 0.01. The decreases of the pH in dadih were due to the activity of the lactic acid bacteria. The lactic acid bacteria in dadih counted to be 11 ± 1.13x10^8 CFU/gram.

Body Weight and Adipose Weight
After high fat diet for 6 weeks, all rats have significantly increased body weight (p < 0.0001). There is a difference in body weight of rats (p < 0.0001) after a high fat diet, with dadih 8 g/ml groups has the highest weight (321.67 ± 4.60 gram) compared with other groups. After 4 weeks of treatment, there is a significant difference in final body weight of rats between groups (p < 0.0001). Interestingly, only rats in negative control have gained weight compared with others (Figure 1). All dadih groups shown a lower weight or decreased body weight compared with during the high fat period.

In addition, there is a difference in white adipose tissue weight (p < 0.0001). The control group has the lowest weight (1.76 ± 0.75 g) while the negative control has the highest weight of white adipose tissue (8.2 ± 1.30 g) compared with other treatment groups. There is no difference in white adipose tissue weight among dadih treatment group with weight of 6.88 ± 1.25 g, 6.2 ± 1.09 g, and 6.0 ± 1.23 g for dadih 2 g/ml, dadih 4 g/ml and dadih 8 g/ml respectively (Figure 2a).

Malondialdehyde in White Adipose Tissue

After 4 weeks of treatment, there is a difference between groups in adipose tissue inflammation, measured by the MDA level in the white adipose tissue (p = < 0.0001) (Figure 2b). Dadih 2 g/ml has the highest adiposity MDA level (292.25 ± 49.43 mmol/gram) but the level isn't statistically different compared with the adiposity MDA level in negative control (277.60 ± 21.52 mmol/gram). Rats that received dadih 8 g/ml, on average, have the lowest adiposity MDA level (197 ± 2.55 mmol/gram) and statistically not different with dadih 4 g/ml group (210 ± 12.75 mmol/gram) or control group (237.50 ± 16.98 mmol/gram).

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Fecal Lactic Acid Bacteria

High fat diet for 6 weeks significantly (p = 0.0001) reduced lactic acid bacteria count in rats as indicated by no significant difference found in all high fat diet groups (Table 2). However, after 4 weeks of treatment, there is a significant difference in lactic acid bacteria count between the groups of treatment (p < 0.0001). Dadih 8 gram/ml group has the highest lactic acid bacteria, whereas the lowest lactic acid bacteria count was found in the negative control group (Figure 3).

Table 2: Total fecal lactic acid bacterial count (x10^4 CFU/g)*.

<table>
<thead>
<tr>
<th></th>
<th>Dadih 2 g/ml</th>
<th>Dadih 4 g/ml</th>
<th>Dadih 8 g/ml</th>
<th>Negative control</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>After High fat</td>
<td>33.67 ± 9.34</td>
<td>40.25 ± 17.33</td>
<td>64.25 ± 15.72</td>
<td>64.50 ± 15.77</td>
<td>103.5 ± 31.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>After Treatment</td>
<td>144.5 ± 37.85</td>
<td>17350 ± 3165.83</td>
<td>36350 ± 8525.99</td>
<td>46.20 ± 16.50</td>
<td>520.75 ± 67.66</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± standard deviation.
abcDifferent notation indicated p < 0.05.

Discussion

In this study, we evaluated the beneficial effect of dadih, traditional fermented buffalo milk from Indonesia, for obesity prevention. We focused on bodyweight, adipose and white adipose tissue inflammation in this study.

We showed that dadih has an ability to reduce and prevent weight gain in rats fed high fat diet. We proposed that this effect was due to the ability of dadih in increasing lactic acid bacteria in the gut to the amount that confers health benefits in host [49]. Several types of lactic acid bacteria have successfully identified in dadih such as Leuconostoc mesenteroides, S. lactis supsp. lactis, S. cremoris L. casei subsp. casei, Lactobacillus casei subsp. rhamnosus, Lactococcus sp., Leuconostoc sp., Enterococcus faecium IS-27526 and Lactobacillus plantarum IS-20506 which act as probiotic for modulating the gut microbiota composition [45,50]. Modulation of gut microbiota through lactic acid bacteria administration was linked to the prevention of obesity [51].
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Several studies conducted in animal and human have reported the anti-obesity effect of lactic acid bacteria [51-54]. Lactobacillus gasseri, for instance, has been studied to have an anti-obesity effect by affecting the expression of fatty acid synthesis and oxidation in animal trials [56-58]. However, studies reported that Lactobacillus species might also contribute to the development of obesity in human [59-61]. A further comparative genomic study revealed that the discrepancies between weight-gain and weight protection-associated Lactobacillus is associated with the presence of several enzymes that involvesin carbohydrate metabolism, acetate production and defense against oxidative stress [62]. Although we didn’t sequence the lactic acid bacteria species in our study, we hypothesized that the indigenous lactic acid bacteria in dadih is comprised with the weight protection lactic acid bacteria instead of the weight gained lactic acid bacteria.

In addition, bacterial endotoxemia plays an important role in the development of intestinal inflammation and chronic low-grade inflammation in obesity [24-26, 63]. A study conducted by Li., et al [64] observed a close association between intestinal inflammations with adiposity inflammation. Modulating the gut microbiota with probiotic that can compete with the pathogenic bacteria-producing LPS in the gastrointestinal wall is the important factor in preventing the inflammation in obesity. Studies reported that indigenous lactic acid bacteria in dadih is able to resist gastrointestinal harsh environment and able to adhere into the gastrointestinal tract [44,45]. The ability to adhere and to compete with the pathogenic bacteria in the gastrointestinal wall are the proposed mechanism of anti-inflammation of dadih in preventing the development of chronic low-grade inflammation from bacterial endotoxemia in obesity, which in line with another study conducted by Miyoshi., et al. (2013) [65].

Conclusion
In conclusion, our study shown that dadih has an anti-obesity and anti-inflammatory effect in rats fed high fat diet. Further study is needed to elucidate the anti-obesity and anti-inflammatory mechanism of dadih for therapeutic diet therapy in obesity prevention.

Acknowledgement
R.J.K. and E.H. designed the research and R.J.K wrote the manuscript; R.J.K. conducted the research and analyzed the data; F.A., G.P., R.S., and F.N., conducted the research. Each author contributed to the development of this article.

Conflict of Interest
The author declares no conflict of interest in this study.

Bibliography
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