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Effect of Buffalo Curd Milk-Edamame Pudding Snack Consumption on Fasting Blood Glucose Levels and Lipid Profile in Diabetes Mellitus Patients

Pengaruh Pemberian Snack Puding Dadih Susu Kerbau dan Edamame terhadap Kadar Glukosa Darah Puasa dan Profil Lipid pada Pasien Diabetes Mellitus

Liri Sisca Wulandari[1], Inggita Kusumastuty[1]*, Anggun Rindang Cempaka[1], Fajar Ari Nugroho[1]

[1]Nutrition Departement, Faculty of Health Sciences, Universitas Brawijaya, Malang, Indonesia

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****Correspondent:***
Inggita Kusumastuty
inggita@ub.ac.id

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ABSTRACT

Background: Buffalo milk curd, a native probiotic source from Indonesia, is dominated by live indigenous Lactic Acid Bacteria known for their ability to lower blood glucose levels. Edamame contains amino acid arginine, chromium, antioxidants, and fibre, aiding in blood glucose control and lipid profile reduction.
Objectives: This study aimed to assess the effects of buffalo milk curd and edamame-based pudding snacks on fasting blood glucose levels and lipid profiles in Type 2 diabetes mellitus (T2DM) patients.
Methods: This study used a True Experiment Design with Pretest-Posttest Control Group Design. The study involved 32 patients divided into treatment and control groups. During the study, the treatment group received 250 g of snacks in the morning and evening for one week. Blood glucose levels and lipid profiles were measured using the enzymatic colourimetry method.
Results: The results showed decreased fasting blood glucose levels before and after treatment in both control (-17.06 ± 40.17) and treatment (-48.38 ± 40.27 mg/dl) groups (p=0.036). Total cholesterol levels also decreased in control (-15.87 ± 23) and treatment (-41.4 ± 19 mg/dl) groups (p=0.001). There were decreased LDL levels in control (-6.81 ± 29.09) and treatment (-27.3 ± 25.09 mg/dl) groups (p=0.04). However, HDL and TG levels showed no differences at the end of the study.
Conclusions: Buffalo curd milk-edamame pudding snacks can reduce fasting blood glucose levels and lipid profiles, particularly total cholesterol and LDL levels, in Type 2 DM patients.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a non-communicable disease with an increasing global prevalence. The projected number of people with type 2 diabetes mellitus may reach 537 million by 2021[1]. Managing T2DM is both complicated and financially demanding. This disease is associated with severe complications that impact health, productivity, and overall quality of life. It has been indicated that over 50% of people with diabetes die from cardiovascular disease, experience blindness due to retinal damage, and have a 25-times greater risk of lower extremity amputation, compared to non-diabetics[2]. Previous studies reported the need for improved dietary management and self-care among T2DM patients. Current patient interventions include dietary plans, food selection, physical exercise, and the development of low glycaemic index products[3]. One strategy for improving dietary management involves incorporating traditional food that has the potential to reduce complications in T2DM patients.

Indonesia, particularly in West Sumatra, has a unique probiotic product, buffalo milk curd. This curd is a result of natural fermentation, where buffalo milk undergoes fermentation within a bamboo tube covered with banana leaves at room temperature for 24-48 hours. This process yields approximately 10^8 colonies of indigenous live lactic acid bacteria (LAB) per gram[4]. Recent findings from a study on mice showed that LAB present in buffalo milk curd contributes to immune system maintenance, as seen by increased anti-inflammatory cytokines[5]. Edamame, the premature form of soybeans encased in their pods, is classified as Glycine max or green soybeans (vegetable soybeans) and is widely known as healthy food[6]. Edamame contains

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antioxidants, isoflavones, beta-carotene, fibre, and nine essential amino acids required by the body and has a good effect on impaired glucose tolerance, hyperlipidaemia, and decreased insulin sensitivity conditions[7]. Despite these attributes, the effectiveness of the combination of buffalo milk curd and edamame in a product has never been directly tested, particularly for their effectiveness in improving blood glucose and lipid profiles in type 2 DM patients. While seeing the huge benefit of these two ingredients and in line with promoting buffalo milk curd as a valuable local food, these reasons encourage further exploration.

Considering the benefits of buffalo milk curd and edamame for diabetes mellitus, and acknowledging the current limited research on buffalo milk curd, this study aimed to investigate the effect of buffalo milk curd and edamame consumption on improving blood glucose levels and lipid profiles in patients with type 2 DM.

METHODS

This study was single-blind true experiment with a pretest-posttest control group design. Following Frederer's calculation, a total of 32 participants were divided into the treatment group and the control group through purposive random sampling. The inclusion criteria for this study were type 2 diabetes mellitus patients, able to communicate well orally and in writing, and had no history of cow's milk allergy. The preparation of the mixed buffalo milk curd-edamame pudding snack involved grinding 25 g of curd and 25 g of boiled edamame until smooth. The puree resulted underwent filtration and was mixed with 2 g of agar-agar, ¼ tsp of salt, 2 g of corn sugar, and food flavouring. The mixture was stirred and added 250 ml of water while heated at 80˚C for ± 15 minutes. The treatment group received 250 g of mixed buffalo milk curd-edamame pudding snacks in the morning and evening for one week. Conversely, the control group obtained a pudding snack made from diabetic commercial milk with the same portion and duration as the treatment. The determination of the intervention portion was calculated as part of the recommendations for meeting energy and nutrients for all participants. Based on the Harris-Benedict formula, the total energy provided had been adjusted by considering gender, age, weight, height, and physical activity; thus, the given intervention corresponded to 15-20% of each subject's total daily energy. All procedures have been approved by the research ethics committee of Faculty of Medicine, Universitas Brawijaya through the Ethic Approval number 367/EC/KEPK-S1-GZ/12/2021. All participants were requested to provide their informed consent by signing the inform consent letter prior to their inclusion in the research.

During the study, participants recorded their food intake using a food record form. Nutrisurvey 2007 software (EBIspro, Willstätt, Germany) was utilized to process the data and determine the participants' total energy and nutrient intake[8]. Participants documented their snack consumption through photos or videos to ensure compliance. All groups underwent blood glucose and lipid profile measurements before and after the intervention, preceded by 8-hour fasting. Blood samples were drawn from the elbow crease vein and placed into EDTA tubes. Blood glucose levels and lipid profiles were then measured from the collected samples using the enzymatic colourimetry method. Pretest analysis of cholesterol, TG, and HDL, as well as pretest and posttest of blood glucose, were evaluated through independent T-test. Mann-Whitney tests were conducted for analysing cholesterol, TG, HDL post-test, and LDL pre- and posttest. Statistical significance was determined at $p < 0.05$. Data analysis was done using SPSS software (version 16.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Participant Characteristics

During the pre-intervention period, comprehensive education was provided to all participants regarding the right type, amount, and schedule of eating patterns according to the principles of the diabetes mellitus diet. In addition, participants received information on completing the food record form and using the Exchanged Food Ingredients List (EFIL). This information was intended to encourage varied and balanced eating patterns according to the dietary recommendations given.

The participants involved in this study were dominated by women. Most participants have been living with DM for less than three years (59.4%). Half of the participants were non-working or housewives (53.1%). Notably, 75% of the total participants reported a history of nutritional consultation. Lack of physical activity was found in approximately 40.6% of participants, and the nutritional status of Obesity I was detected in 37.5% of participants (Table 1).

Table 1. Participants' Characteristics

Characteristic		N	%
Sex	Male	7	21.9
	Female	25	78.1
Age	Late adult (35-44 years)	1	3.1
	Early elderly (45-54 years)	12	37.5
	Late seniors (55-64 years)	16	50.0
	Seniors (> 65 years)	3	9.4
Time suffering from DM	< 3 years	19	59.4
	3-5 years	2	6.3
	>5 years	11	34.4
Occupation	Not working/ Housewife	17	53.1
	Farmer/ Laborer	1	3.1
	Trader/ Entrepreneur	7	21.9

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Characteristic		N	%
	PNS / TNI / Polri	3	9.4
	Other	4	12.5
History of nutrition consultation	Never	8	25.0
	Once	24	75.0
Physical activity	Leisure strolling	16	50
	Gymnastics	4	12.5
	Fitness	3	6.3
	No sporting activities	10	31.3
Physical activity duration	Sufficient (≥ 150 minutes/week)	9	28.1
	Insufficient (< 150 minutes/week)	13	40.6
	No sporting activities	10	31.3
Nutrition status	Normal	7	21.9
	Overweight	11	34.4
	Obesity I	12	37.5
	Obesity II	2	6.3

Most of the respondents in this study were in the 55-64 years old range (50%). According to data from the Indonesian Baseline Health Research (*Riskesdas*) (2018), advancing age is associated with increasing blood glucose, consequently heightening the susceptibility to diabetes. This condition does not only occur in Indonesia but also throughout the world, encompassing both developing and developed countries[9,10]. The association between advanced age and T2DM has been reported to be related to genetic background, decreased insulin secretion, and changes in several environmental factors associated with central obesity and insulin resistance. Lack of physical activity coupled with changes in eating further contributes to the occurrence of diabetes mellitus. Several recent studies have also demonstrated the role of other factors, such as arginine vasopressin (AVP) or its terminal fragment, called Copeptin, which play a role in the mechanism of diabetes in elderly individuals by influencing insulin sensitivity, hepatic glycogenolysis regulation, and glucagon secretion[11,12].

Food Intake

At the end of the study, energy and nutrient intake in both the treatment and control groups did not follow the established principles of balanced nutrition for diabetes mellitus. Specifically, energy, carbohydrate, and fat intake in both groups surpassed the recommended levels, each exceeding 120% of the requirement. Meanwhile, protein intake exhibited a moderate deficit, within the range of 70-79% of the needs in both groups. Fibre intake in both the treatment group and the control group was classified as a severe intake deficit, less than 70% of the requirement (Table 2). The results of the independent T-test between groups on energy, carbohydrate, protein, fat, and fibre intake showed no significant difference (p>0.05) (Table 3).

Table 2. Distribution of Participants' Energy and Nutrient Intake during the Study

	Treatment			Control		
	Intake	Needs	Fulfilment	Intake	Needs	Fulfilment
Energy (kcal)	1831.34	1467.62	124.80%	1788.67	1467.62	121.90%
Protein (g)	55.78	73.38	76.10%	52.63	73.38	71.70%
Fat (g)	59.46	40.76	145.80%	61.16	40.76	150.10%
Carbohydrate (g)	267.45	201.79	132.50%	256.20	201.79	126.90%
Fibre (g)	10.40	25.00	41.80%	8.80	25.00	35.10%

Table 3. Results of Difference Test Analysis in Average Fulfilment of Energy and Nutrient Intake

	Treatment	Control	p-value[a]
	Mean ± SD	Mean ± SD	
Energy (kcal)	1831.34 ± 142.33	1788.67± 191.20	0.479
Protein (g)	55.78 ± 9.44	52.63 ± 13.98	0.462
Fat (g)	59.46 ± 11.77	61.16 ± 16.99	0.743
Carbohydrate (g)	267.45 ± 36.96	256.20 ± 40.77	0.420
Fibre (g)	10.46 ± 2.90	8.78 ± 2.29	0.079

[a]Independent t-test

At the beginning of the study, all respondents received education concerning dietary recommendations. Throughout the research activity, respondents were required to record their food intake in a food record form. The results of the food record show that while the intervention and control snack puddings were fully consumed by all participants, there existed challenges in adhering to the recommended dietary guidelines, particularly in terms of portion sizes and food selections. Thus, both the control and treatment groups had higher intakes of energy, fat, and carbohydrates than needed, while protein and fibre intakes were lower (Table 2).

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Dietary compliance refers to an individual's adherence to dietary recommendations, with energy intake serving as one of the important indicators. Several previous studies have reported that patients with chronic ailments, on average, achieve only a 50% adherence rate to long-term dietary regimens in developed countries and tend to be lower in developing countries. Low compliance has also been found in T2DM patients in this study[13,14].

Blood Glucose Level and Lipid Profile

The measurement results showed fasting blood glucose levels and lipid profiles; the average post-intervention score was lower than pre-intervention in all groups (Table 4). The data analysis between groups showed that post-intervention cholesterol and LDL levels were significantly different between the intervention and treatment groups (Table 5). Furthermore, the reduction in fasting blood glucose levels, total cholesterol, and LDL were also significantly different between the two groups (Table 6).

Table 4. Average Participants' Fasting Blood Glucose Levels and Lipid Profile

Parameter	Average ± SD			
	Control		Treatment	
	Pre	Post	Pre	Post
Fasting blood glucose (mg/dl)	219.3 ± 61.4	197.0 ± 59.7	246.4 ± 63.5	205.8 ± 57.5
Total cholesterol (mg/dl)	279.6 ± 34.9	263.8 ± 32.6	277.9 ± 29.2	236.5 ± 23.7
Triglycerides (mg/dl)	215.6 ± 89.0	172.6 ± 87.3	247.1 ± 83.3	182.9 ± 40.5
LDL (mg/dl)	192.2 ± 39.4	185.4 ± 30.3	188.6 ± 32.1	161.2 ± 24.8
HDL (mg/dl)	46.1 ± 7.8	43.6 ± 9.3	41.5 ± 8.8	38.7 ± 15.0

Table 5. Results of Intergroup Difference Test Analysis on Participants' Fasting Blood Glucose Levels and Lipid Profile

Parameter		Statistical Test Value of Differences Between Groups (p-value)
Fasting blood glucose	Pre	0,840[b]
	Post	0,963[b]
Total cholesterol	Pre	0,883[b]
	Post	0,018[a]*
Triglycerides	Pre	0,309[b]
	Post	0,365[a]
LDL	Pre	0,880[a]
	Post	0,040[a]*
HDL	Pre	0,133[b]
	Post	0,345[a]

[a]Mann Whitney; [b]Independent t-test; *Significantly different (p<0,05)

Table 6. Means and Results of Difference Test Analysis in Changes of Participants' Fasting Blood Glucose Levels and Lipid Profiles Before and After the Treatment

Parameter	Average Delta ± SD		Statistical Test Results (p-value)
	Control	Treatment	
Fasting blood glucose	-17.1 ± 40.2	-48.4 ± 40.3	0,036[b]*
Total cholesterol	-15.9 ± 23.2	-41.4 ± 19,7	0,001[a]*
Triglycerides	-42.9 ± 104.4	-64.2 ± 91.6	0,545[b]
LDL	-6.8 ± 29.1	-27.3 ± 25.1	0,041[b]*
HDL	-2.4 ± 9.0	-2.8 ± 14.7	0,931[b]

[a]Mann Whitney; [b]Independent t-test; *Significantly different (p<0,05)

The administration of mixed buffalo-edamame milk curd led to a significant decrease in blood glucose levels in the treatment group compared to the control group at the end of this study (Table 6). This may be attributed to the potency of buffalo milk curd in terms of its bioactive substances, such as prebiotics, probiotics, amino acids, indigenous lactic acid bacteria (LAB), and antioxidants. The activity of LAB facilitates the breakdown of complex compounds into simpler forms, enhancing digestion and absorption within the body[4]. This mechanism triggers a hypoglycaemic effect through the modulation of the glucosidase enzyme, which subsequently enhances lactose absorption in the intestines[15,16].

Several previous studies have also utilized probiotics in glycaemic control. Research involving a six-week regimen of probiotic fermented milk has proven beneficial in glycaemic control[17]. Similarly, a study employing probiotic preparations containing *Lactobacillus casei* Shirota for four weeks has shown maintenance of glycaemic control and fasting insulin levels[18]. Clinical research on humans receiving two types of probiotics, *Lactobacillus reuteri* ADR-1 and ADR-3, have exhibited significant enhancements in glycaemic control and blood pressure among individuals with diabetes mellitus[19]. In patients with metabolic syndrome, intake of yoghurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb1 for two

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months resulted in reduced blood glucose levels and significant changes in insulin resistance (HOMA-IR)[20].

Adipose tissue is a storage site for lipids, which also play important metabolic[21] and hormonal roles for energy homeostasis and glycaemic regulation[22]. Excessive accumulation of lipids within adipose tissue can induce hypertrophy, cellular stress, and local inflammation. Prolonged lipid excess can cause adverse health conditions, including obesity, insulin resistance, hyperglycaemia, and type 2 diabetes[23]. Probiotics influence adipose tissue, reducing inflammation, reducing fat deposition, repairing damage to adipose tissue, improving insulin signalling routes, and increasing glucose absorption. These lead to reduced insulin resistance, improved insulin sensitivity, reduced hyperglycaemia, improved tissue glucose absorption, and improved glucose metabolism and homeostasis[24].

Meanwhile, edamame (soybean), containing a range of physiologically active ingredients like soy phytosterols, soyasaponins, soy protein, and lecithin, lowers blood glucose and serum cholesterol[25]. The structural similarity between edamame (soy) isoflavones and endogenous 17-β-oestradiol allows them to bind to oestrogen receptors, thereby activating genes that were beneficial in glucose and lipid metabolism[26,27].

Soybean genistein content has also been reported to reduce blood glucose by up to 18% in women and 43% in men[28], accomplished through mechanisms of increasing cAMP accumulation, activating PKA and ERK1/2, and increasing INS-1 cell proliferation[29]. In lipid metabolism, soy isoflavones also significantly regulate metabolism independently of the oestrogen receptor, reduction in TG, an increase of lipolysis, induce metabolism of HDL, and an improvement of fatty acid metabolism[30,31].

CONCLUSIONS

The results of this study indicate that the consumption of mixed buffalo milk curd-edamame can significantly reduce blood glucose levels, cholesterol, and LDL levels in type 2 DM (T2DM) patients. However, this study also found that adherence to dietary recommendations remains a challenge for T2DM patients. Based on these results, the combination of mixed buffalo milk curd-edamame along with comprehensive adherence to recommendations holds the potential to serve as an effective strategy for blood glucose control in patients. The results of this study also encourage further research on the use of Indonesian native functional food products as a practical part of implementing nutrition management in DM patients in the future.

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Conflict of Interest and Funding Disclosure

All authors have no conflict of interest in this article.

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