

Predinical Study of Goat Milk Kefir as an Antihyperglycemic Food

by Siti Susanti

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³ Preclinical study of goat milk kefir as an antihyperglycemic food

Siti Susanti¹, Nurwantoro Nurwantoro¹, Jessen Juan Elto¹, Trilaksana Nugroho², Ade Erma Suryani³, Heni Rizqiati^{1*}

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¹Food Technology, Department of Agriculture, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia ²Faculty of Medicine, Diponegoro University, Semarang, Indonesia ³Research Center for Food Technology and Processing-National Research and Innovation Agency, Yogyakarta, Indonesia

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***Corresponding Author:** Heni Rizqiati, Food Technology, Department of Agriculture, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia.

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ABSTRACT

Background: Goat milk kefir is a functional food with numerous health benefits, including blood sugar control, cholesterol reduction, and improved digestive health. This study aims to determine the effect of kefir consumption on blood glucose levels in hyperglycaemic mice (*Mus musculus*) without affecting another blood chemistry. This study compared goat milk kefir products to other processed milk products considered to be capable of controlling and reducing blood sugar levels.

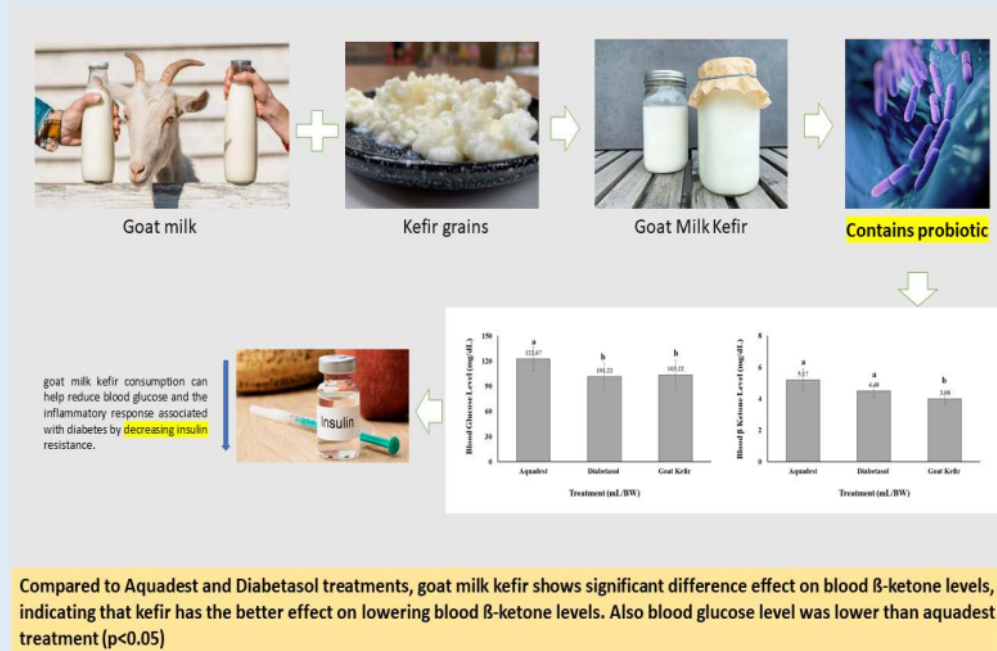
Objective: This study aimed to compare goat milk kefir products to other processed food products considered to be capable of controlling and reducing blood sugar levels.

Methods: Twenty-one mice, as experimental animals, were induced for 21 days with the high-fat and high fructose diet to increase blood sugar levels. After induction, mice were divided into 3 groups according to treatment, namely oral administration of additional distilled water (placebo), diabetasol[®] (positive control), and goat's milk kefir with each group consisting of 9 individuals.

Results: Treatment showed significant effect on the blood glucose and β -ketone level. Diabetasol[®] and Goat kefir supplemented mice had a lower blood glucose level than control ($p < 0.05$) and goat kefir supplemented mice had lower blood β -ketone level than control and Diabetasol[®] ($p < 0.05$). Meanwhile, hematocrit and hemoglobin levels did not show significant differences between treatments.

Conclusion: The results suggested that goat milk kefir has preclinical potential as an antihyperglycemic functional food without affecting other blood chemistry profiles.

Keywords: Antihyperglycemic, blood sugar, goat kefir



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INTRODUCTION

Goat milk kefir is a fermented food made from goat milk fermented by lactic acid bacteria (LAB), acetic acid bacteria, and yeast [1]. Goat milk kefir is a functional food because it contains probiotic bacteria that have benefits such as reducing the growth of pathogenic bacteria and enhancing the intestinal microflora system [2]. Goat milk kefir contains bioactive peptides as antioxidants. According to Jafar [3], the active peptides in dairy milk fermented with LAB have antioxidant properties. Due to interacting with free radicals, antioxidants can help control diabetes mellitus [4].

Hyperglycaemia occurs when an individual's blood glucose levels rise abnormally. When a person's blood

sugar level exceeds 160 mg/dL, they have hyperglycaemia [5]. Hyperglycaemia is caused by excessive sugar consumption, obesity, heredity, and insulin resistance [6]. Hyperglycaemic patients who acquire insulin resistance are also referred to as having diabetes mellitus.

According to Basic Health Research data in 2018, the prevalence of hyperglycaemia in Indonesia is 2% of the total population over the age of 15 years, as confirmed by a diagnosis of the doctor. According to the Central Java Health Office's profile for 2019, the estimated number of people living with hyperglycaemia in Central Java is 652,882. There is currently no medication that can definitively lower and cure

hyperglycaemia [7]. Patients with hyperglycaemia can be treated with oral hypoglycaemic medications (OHMs) such as glibenclamide and metformin, which may cause adverse effects such as increased body weight, nausea, muscle pain, and shortness of breath [8]. Insulin injection therapy is possible for people who have hyperglycaemia as a result of insulin resistance [9]. Insulin injection therapy takes up to 6 injections per day, therefore it is rather expensive. The long-term treatment of insulin injection therapy causes several negative effects, including an increased risk of lipodystrophy, allergies, and insulin overdose. As a result, it is important to seek non-toxic alternatives to medications in the form of functional foods.

According to research conducted by Rahayu *et al.* [10], goat milk kefir consumption can help reduce blood glucose and the inflammatory response associated with diabetes by decreasing insulin resistance. However, no study has been conducted to determine the effect of kefir consumption on blood chemistry profiles, other than blood glucose levels. Additionally, no research has been conducted to compare goat milk kefir products to other processed food products considered to be capable of controlling and reducing blood sugar levels. Therefore, this study aims to determine the effect of kefir consumption on blood chemistry profiles other than blood glucose levels in mice (*Mus musculus*). This study compared goat milk kefir products to other processed food products considered to be capable of controlling and reducing blood sugar levels.

MATERIALS AND METHODS

Materials: The materials used in this study included goat milk, kefir grains, distilled water, mice (*Mus musculus*), standard feed (comfeed AD-2), fructose solution 66%, margarine, diabetasol*, alcohol 70%, and iodine

antiseptic. On the strips test, the materials used included blood glucose, hematocrit, hemoglobin, and β -ketone strips. Blood was taken by surgically cutting 0.5 cm from the base of the tail.

METHODS

The procedure of goat kefir milk making: The method for making goat milk kefir was conducted based on Rosiana *et al.* [11] with some modifications. All equipment was sterilized. Fresh goat milk was homogenized for 5 minutes. Afterwards, the goat milk was transferred to a sterile, closed container. Kefir grain at a concentration of 5% (w/v) was added to the milk and slowly mixed, covered, and sealed with plastic wrap. Following that, the mixture was incubated at room temperature for 24 hours. The kefir product was filtered to separate the kefir grains, resulting in liquid goat milk kefir. The coarse sieve used has a hole diameter of 2 mm. The obtained product was then placed in a 20 mL bottle and stored in the refrigerator at 4°C.

The preparation of experimental animals: The procedure for preparing experimental animals was conducted based on Tudang *et al.* [12] with modifications. Twenty-one mice (*Mus Musculus*) strain BALB/c with an age of 8-12 weeks and a bodyweight of 20-30 grams were used as experimental animals. Mice were kept in cages with a floor size ≥ 97 cm² per mouse. The mice cages were put in an area with natural light, 12 hours of light, 12 hours of darkness, and a temperature range of approximately 18-26 °C with adequate air circulation. The mice cages were provided with a rice husk or sawdust substrate and were cleaned every 5 days.

Antihyperglycemic preclinical test of goat milk kefir:

Preclinical tests were carried out under Ethical Clearance No. 139/EC/H/FK-UNDIP/XII/2021. Mice were adapted for 7 days on a regular diet of 6 g/mouse/day and ad

libitum water. After that, twenty-one mice were induction with a high fat/high fructose diet for 21 days to elevate blood sugar levels. Fructose solution was given at 2 ml/mouse/day through a feeding tube, and liquid margarine was mixed with standard feed at 0.238 g/mouse/day. After 21 days, mice with an average fasting blood glucose level of 168 mg/dL were obtained, which indicate that the mice were suffering from hyperglycaemia.

Mice were separated into three groups according to treatment, using 0.52 mL/mouse/day of distilled water (placebo), diabetasol® (positive control), and goat's milk kefir. Nine mice were used in each treatment group. The treatment began with a 2-hour fast, followed by additional food according to the treatment group. The induction process was carried out every day for 21 days. Mice then fasted for 8 hours before blood collection. Three-in-one test strips (blood glucose, hematocrit, and hemoglobin) and β -ketone strips were used. When the test strip is inserted into a specified spot on the instrument, an order to drip blood appears, showing that the instrument is ready to use. Blood was dripped onto the strip, and the blood sugar profile value was displayed on the screen after a few moments. The tails of mice were cleaned, iodine antiseptic was applied to avoid

infection, and the wound was squeezed until it dried, and the bleeding stopped.

Data analysis: All data was analyzed by using analysis of variance method to evaluate the treatment effect. Furthermore, Duncan multiple range tests (significance level: 5%) were conducted to analyze the significant effect [13].

RESULTS

Supplementation of goat kefir and Diabetasol® on mice showed the significant effect on the blood glucose and β -ketone level (Figure 1 and 2). Figure 1 shows that goat kefir supplemented mice had lower blood glucose level than control ($p < 0.05$), which are 103.22 and 122.67 mg/dL, respectively. However, there was no significant difference on goat kefir and Diabetasol® supplemented mice. Meanwhile, blood β -ketone level of goat kefir supplemented mice was lower ($p < 0.05$) than aquadest and Diabetasol® supplementation (Figure 2). It showed that blood β -ketone level of goat kefir supplemented mice was 3.98 mg/dL, while the aquadest and Diabetasol® treatments were 5.17 and 4.48 mg/dL, respectively. There was no significant difference in both aquadest and Diabetasol® treatment group (Figure 2). On the other hand, there were no effects of those supplementation on the mice hemoglobin and hematocrit level (Figure 3 and 4).

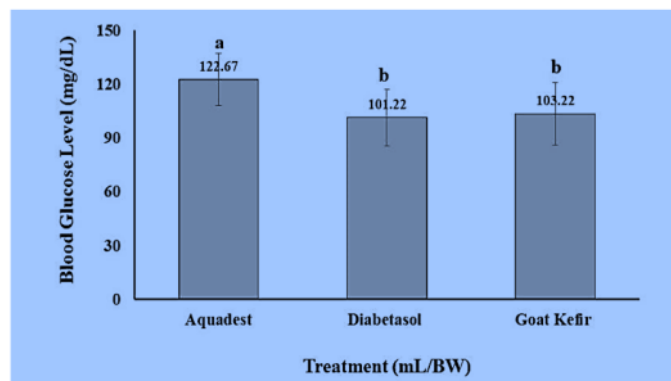


Figure 1. The blood glucose level of mice supplemented with 0.52 mL/BW of aquadest (control), Diabetasol® (positive control), and goat kefir.

*The data are presented as means \pm SD (n=6). The letters above each bar graph represent the significant differences between the group.

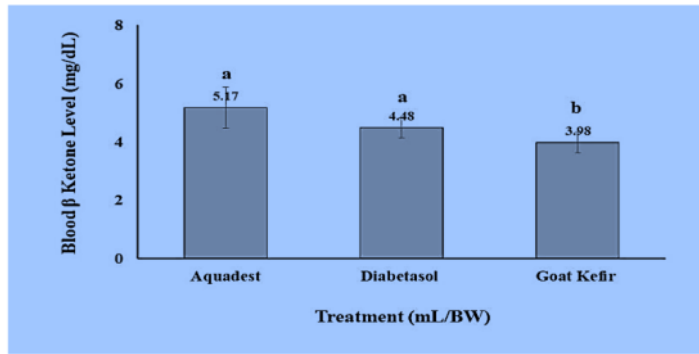


Figure 2. The blood β -ketone level of mice supplemented with 0.52 mL/BW of aquadest (control), Diabetasol* (positive control), and goat kefir.

*The data are presented as means \pm SD (n=6). The letters above each bar graph represent the significant differences between the group.

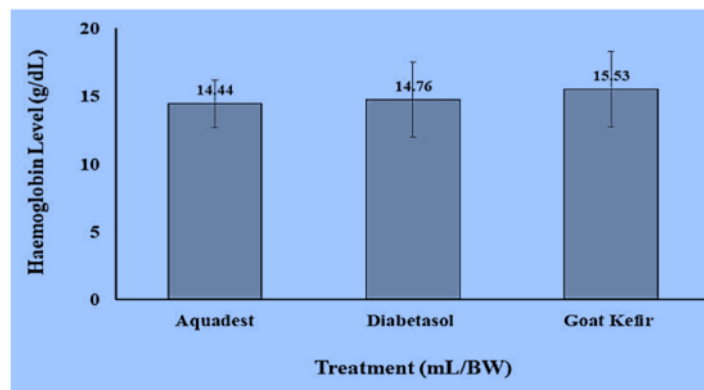


Figure 3. The hemoglobin level of mice supplemented with 0.52 mL/BW of aquadest (control), Diabetasol* (positive control), and goat kefir.

*The data are presented as means \pm SD (n=6).

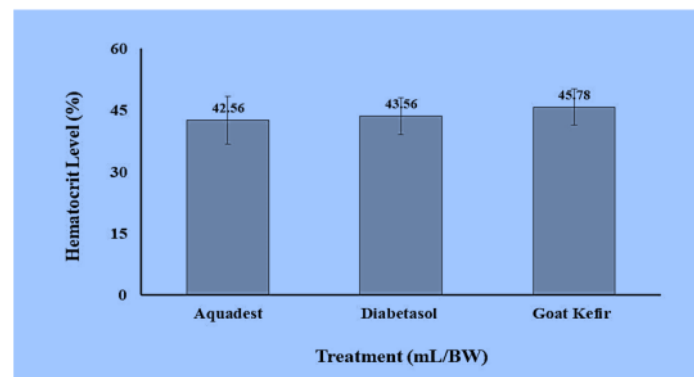


Figure 4. The hematocrit level of mice supplemented with 0.52 mL/BW of aquadest (control), Diabetasol* (positive control), and goat kefir.

*The data are presented as means \pm SD (n=6).

DISCUSSION

The level of glucose in the blood is referred to as the blood glucose level [14]. Normal mice have fasting blood glucose levels between 73 - 96.6 mg/dL [15]. The consumption of diabetasol[®] in persons with type 2 diabetes mellitus for only 2 days shows that it can help control the blood glucose levels by up to 2.34%. According to Dalimartha and Adrian [16], diabetasol[®] can resist elevated blood glucose levels due to the presence of vita digest, a combination of beneficial carbohydrate fibers. The consumption of fermented milk for 14 days has also been shown to help control the blood glucose levels by up to 16% in people with type 2 diabetes mellitus, compared to those who do not consume fermented milk [17]. According to Ostadrahimi *et al.* [18], probiotics in kefir affect the pathogen bacteria in the digestive system and produce insulinotropic polypeptides and glucagon-like peptides, which can boost muscle glucose induction. The intake of diabetasol[®] and goat milk kefir had no significant difference effect on blood glucose levels in this research, indicating that both had the same effect on blood glucose levels.

β -ketone is an acid that is created as a result of fat metabolism as an alternative energy source, when glucose cannot be used by the body. Blood β -ketone levels in healthy mice range between 1.5 - 3 mg/dL [19]. According to Mardiana *et al.* [20], when the pancreas gland can't produce insulin, the body will use fat as an energy source. The consumption of diabetasol[®] didn't significantly affect the amount of β -ketone in blood, as the main ingredients in it are maltodextrin, soy-based oil, casein, whey protein, fructo-oligosaccharide, and aspartame [21]. The consumption of goat kefir for 10 weeks can reduce blood fat level up to 18% [22] and having the properties to lower the amount of fat in blood and help boost muscle glucose induction will help reduce the amount of β -ketone in blood [23]. The intake of diabetasol[®] and goat milk kefir shows a significant

difference on blood β -ketone levels in this research, indicating that goat milk kefir has the better effect on lowering blood β -ketone levels. Hence, goat milk kefir could be classified as a functional food which has been clinically proven to produce lower blood sugar and β -ketone levels, which makes it a great product for regular consumption for antihyperglycemic individuals. A functional food is one that claims to have a desired functionality by combining two or more existing ingredients.

Both hematocrit and hemoglobin levels in the blood are linked. Hemoglobin is a protein found in red blood cells, whereas hematocrit refers to the proportion of red blood cells in total blood cells [24]. Healthy mice have blood hematocrit and hemoglobin levels of 33.1-49.9% and 13-16 g/dL respectively [25, 26]. One of the consequences that can arise in patients with type 2 diabetes mellitus is kidney failure, which occurs when the kidneys stop generating the hormone erythropoietin and lower the amount of red blood cells [27]. Treatment was hoped to help keep the amount of blood hematocrit and hemoglobin levels on the healthy area. The consumption of diabetasol[®] helps maintain the level of blood hematocrit and hemoglobin because of the B12 vitamin in it. Diabetasol[®] has slight amount of B12 vitamin which are needed to produce red blood glucose. Consumption of goat kefir shows help maintain the level of blood hematocrit and hemoglobin because of the higher iron content of kefir. According to Hardiansyah [28], goat milk kefir is a fermented milk product that has a considerable amount of minerals (456 mg calcium and 4.96 g iron per 200 mL) that are needed to produce red blood cell. The intake of diabetasol[®] and goat milk kefir had no significant difference effect on hematocrit and hemoglobin levels in this research, indicating that they both had the same effect on hematocrit and hemoglobin levels.

CONCLUSION

The results of our study concluded that goat milk kefir has preclinical potential as an antihyperglycemic functional food without affecting other blood chemistry profiles. Diabetasol® and Goat kefir supplemented mice had lower blood glucose level than control and goat kefir supplemented mice had lower blood β -ketone level than control and diabetasol®.

Ethical Clearance: No. 139/EC/H/FK-UNDIP/XII/2021.

List of abbreviations: BW, body weight; BALB/c, Bagg's albino, an albino, laboratory-bred strain of the house mouse from which a number of common sub-strains are derived; LAB, lactic acid bacteria; OHMs, oral hypoglycaemic medications.

Author contributions: S.S., H.R., N.N., J.J.E., T.N., A.E.S. designed the research. J.J.E. conducted the research and performed statistical analysis. S.S., H.R., N.N., T.N., A.E.S. provided guidance and supervision. S.S., H.R., N.N. supported the research. J.J.E wrote the manuscript. S.S. had primary responsibility for the final manuscript. All authors read and approved the final version of the manuscript.

Competing interests: the authors have no conflicts of interest to declare.

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