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Original research paper

FERMENTED BUFFALO MILK (DADIH) POWDER WITH REDUCED WATER ACTIVITY AS PARABIOTICS FOR PROTECTION AGAINST OXIDATIVE STRESS AND LIVER INJURY

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Abstract: *Dadih* powder is dried fermented buffalo milk. This study evaluated the effectiveness of dadih powder enriched with modified cassava flour (MOCAF) on metabolic syndrome parameters in Sprague-Dawley rats. Formulations containing 10, 30, and 50% MOCAF were assessed for physicochemical properties and lactic acid bacteria (LAB) viability. The optimal formulation was determined using the deGarmo method, based on the proximate composition and total LAB, and identified the 10% MOCAF formulation as having the highest effectiveness score. This formulation was used in the intervention study. The rats were divided into four groups: normal diet (N), high-fat-high-sucrose diet (KN), and two treatment groups receiving dadih powder supplementation at 0.335 mg/g (D1) and 0.670 mg/g (D2) of body weight for eight weeks. The rats were then sacrificed, and blood and liver samples were collected for biochemical and histological analyses. Traditionally, fresh *dadih* is highly perishable, with a shelf life of approximately three days. Drying reduced the water activity from 0.70 to 0.66, thereby enhancing stability and prolonging its shelf life. The drying process effectively maintained the viability of LAB at level up to 10^2 cfu/g, thereby allowing the product to be classified as parabiotic. *Dadih* powder supplementation significantly reduced malondialdehyde levels, indicating antioxidative activity, although the reductions in glucose and lipid profiles were not significant. Histopathological analysis revealed severe hepatic steatosis in the KN group, whereas the D2 group exhibited near-normal liver structure. These findings indicate that *dadih* powder may serve as a parabiotic food to protect against oxidative stress and liver damage associated with metabolic syndrome.

Key words: *antioxidative activity, high-fat-high-sucrose diet, metabolic syndrome, modified cassava flour, water activity*

INTRODUCTION

Innovating dairy products in various forms and formulations enhances the accessibility and acceptance of fermented food. Fermented dairy products are known as functional foods due to the presence of probiotic lactic acid bacteria (LAB), which contribute not only to organoleptic properties, but also to health benefits exceeding their fundamental nutritional value

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(Tamang, Shin, Jung & Chae, 2016; Wan-Mohtar, Ilham, Jamaludin, David & Mohd Zaini, 2022). Probiotics provide health benefits by inhibiting the colonization of pathogenic bacteria and fermenting prebiotics to support the gut microbiota (Koirala et al., 2024). *Lactobacillus rhamnosus* exhibits probiotic properties, including acid and bile tolerance (survival at pH 2.5–4.5 and bile salt concentrations of 0.3–0.5%), adhesion and aggregation capabilities, and antimicrobial activity (Behbahani, Barzegar & Taki, 2025). These traits ensure high viability and functional benefits as probiotics. Nevertheless, probiotic cells, whether live, dead, or lysed, produce bioactive metabolites known as parabiotics and postbiotics. These compounds enhance gut microbiota function, reduce liver damage, and improve insulin sensitivity (Liu, Wang & Wu, 2022).

Dadih, a traditional functional dairy product made from buffalo milk, contains various strains of LAB with proven *in vitro* and *in vivo* probiotic properties (Kazancıgil, Demirci, Öztürk-Negiş & Akın, 2019; Ary, Dadrasnia, Ameen, Alwakeel & Ismail, 2021). LAB from *dadih* have been reported *in vitro* and animal studies to exhibit antimicrobial, antioxidant, immunomodulatory, hypocholesterolemic, and antimutagenic activities, as well as the ability to produce γ-aminobutyric acid (GABA) and folate, which may support gastrointestinal health and immune function (Arnold, Rajagukguk & Gramza-Michałowska, 2021). However, diverse *dadih* products are not yet widely popular, where is typically consumed as a side dish with rice, mixed with sliced red onion and chili, or blended into beverages with puffed rice and palm sugar. One major limitation of *dadih* is its short shelf life, which lasts only approximately three days at room temperature (Herlina & Setiarto, 2024). Processing *dadih* into *dadih* powder could extend its shelf life and make it a versatile raw material for various processed food products. However, research on the development of *dadih* powder is limited.

Metabolic syndrome has become a global health issue, characterized by a cluster of risk factors, including abdominal obesity, dyslipidemia, hypertension, and insulin resistance. It significantly increases the risk of cardiovascular disease, type 2 diabetes, and nonalcoholic fatty liver disease (Cheng, Yen, Huang, Chen & Hsu, 2022). Based on global estimates, more than 1 billion people worldwide are affected by metabolic syndrome (Saklayen, 2018). The prevalence of metabolic syndrome is 30.4% among the adult population in Iran (Farmanfarma et al., 2019), 33% in Brazil (Valadares et al., 2022), and 41% in Indonesia (Santoso, Bramantoro, Kardos, Szakacs & Nagy, 2022). Fermented foods have gained attention for their potential to improve metabolic health by producing bioactive compounds, such as short-chain fatty acids (SCFAs), peptides, and vitamins (Mathur, Beresford & Cotter, 2020). These compounds help modulate the gut microbiota, enhance intestinal integrity, reduce inflammation, and support glucose and lipid metabolism. While current evidence supports the beneficial effects of fermented foods on metabolic health (Pramana et al., 2025), the effectiveness of new product innovations, such as *dadih* powder, has not been confirmed as a functional food in the prevention of metabolic syndrome.

Modified cassava flour (MOCAF) is a type of cassava flour produced through lactic acid fermentation, resulting in improved functional properties compared to conventional cassava flour (Nainggolan, Yudianto & Sayekti, 2019). MOCAF is gluten-free, rich in resistant starch and dietary fiber, and has a lower glycemic index, making it suitable for the development of functional foods (Khasanah, Indrianingsih, Triwitono & Murdiati, 2024). The presence of resistant starch in MOCAF has been associated with prebiotic effects, improved lipid metabolism, and enhanced insulin sensitivity (Firdaus, Sulistyaningsih & Subagio, 2018). Incorporating MOCAF into dairy-based fermented products could improve physicochemical characteristics, extend shelf life, and potentially support the survival of LAB. Moreover, the use of MOCAF adds economic and nutritional value to locally available cassava, which is abundant in Indonesia. Therefore, MOCAF was selected in this study as a complementary ingredient for *dadih* powder to optimize its functional and technological properties. This study aimed to optimize the formulation of *dadih* powder and examine the effectiveness of *dadih* powder in metabolic syndrome.

MATERIALS AND METHODS

This research was conducted in two stages: optimization of the formula and analysis of the effectiveness of *dadih* powder in metabolic

syndrome. The optimization stage focused on developing *dadih* powder from fermented buffalo milk to obtain an optimal composition, the details of which are described in the following section. The selected *dadih* powder formulation was then applied in the intervention study. Its effectiveness against metabolic syndrome was evaluated in an animal model induced by a high-fat and high-sucrose diet.

Materials

This study employed *dadih* collected from buffalo milk producers in Kamang, Agam Regency, West Sumatra, Indonesia. MOCAF was used as an additional ingredient in the processing of *dadih* powder. Analytical-grade reagents were used to determine the physicochemical and microbiological characteristics of the samples. Animal feed was obtained from Indo Feed (Indonesia Formula Feed).

Preparation of *dadih* powder

The *dadih* powder samples were formulated by combining fresh *dadih* with MOCAF at three different concentrations: 10% (F1 formulation), 30% (F2 formulation), and 50% (F3 formulation). MOCAF was added at varying concentrations and served as a parameter to determine the optimal formulation for producing the *dadih* powder. The *dadih*-MOCAF was homogenized and dried using a drum dryer at a drum surface temperature of 115°C and modified time of 10 s (Aalaei, Rayner, Tareke & Sjöholm, 2016).

Physical characterization

Physical properties were performed on yield, bulk density, solubility, water absorption, and color. The yield was defined as the ratio of the product's weight or yield to the initial weight. The bulk density was determined by measuring 2 g of *dadih* powder placed in a 100 mL graduated cylinder. The weight of the cylinder containing the sample was then recorded. Bulk density was calculated as the ratio of the sample weight to its volume of 100 mL (Yüksel, 2021).

The water solubility was determined using a gravimetric method adapted from (Tafu & Jideani, 2022). *Whatman* No. 42 filter paper was dried in an oven at 105 °C for 3 h and weighed (W_1). One gram sample (W_0) was mixed with 20 mL of distilled water in a 25 mL Erlenmeyer flask and homogenized using a vortex mixer at 1000 rpm for 10 min. The mixture was then filtered through filter paper using a vacuum pump and weighed (W_2). Water solubility was calculated using Equation [1], and the water absorption capacity was determined using Equation [2].

$$\text{Solubility} = \frac{100-(W_2-W_1)}{\left\{100-\left(\%\frac{\text{moisture content}}{100}\right)\right\}\times W_0} \times 100 \quad (1)$$

$$\text{Water absorption} = \frac{(W_2-W_1)}{W_0} \times 100 \quad (2)$$

The color attributes of the *dadih* powder were measured using a chromameter based on the Hunter Lab color. The instrument was calibrated using standard white and black tile samples. The sample was placed in a sample holder, and the color readings were recorded directly from the device. The measured parameters included lightness (L*), red-green axis (a*), and yellow-blue axis (b*). The L* value represents brightness, ranging from 0 (black) to 100 (white). The a* value ranged from +a (0 to +100), indicating red, to −a (0 to −80), indicating green. Similarly, the b* value ranged from +b (0 to +70) for yellow to −b (0 to −70) for blue (Tkacz, Modzelewska-Kapitula, Wiek & Nogalski, 2020; Machado et al., 2023).

Water activity and proximate composition

The water activity (a_w) of the *dadih* powder was measured using an a_w-meter with a sensitivity of 0.001. The sample was placed in the designated sample chamber of the instrument and allowed to equilibrate for 15 min. The a_w value was measured and recorded after sample stabilization (Janczuk, Brodziak, Krol & Czernecki, 2023). The proximate composition consisted of moisture, ash, protein, lipid, and carbohydrate content, which were quantitatively analyzed using standardized methods (AOAC, 2019). Moisture content was measured by drying samples at 105°C in a hot air oven until a constant weight was achieved (standard AOAC method 952.08). The crude protein content was determined using the Kjeldahl method (standard AOAC method 992.23), with the total nitrogen content converted to protein using a 6.25 conversion factor. The lipid content was assessed through acid hydrolysis (standard AOAC method 948.15) using Soxhlet extraction at 60 °C until weight stabilization. The ash content was determined by gravimetric analysis (standard AOAC method 930.30) through sample incineration at 550°C to a constant weight.

Total carbohydrates were calculated by difference.

Count of total LAB colonies

The enumeration of LAB colonies was performed according to the ISO 15214:1998 procedure. One gram of *dadih* was diluted in Buffered Peptone Water (BPW) medium and homogenized using a vortex. The solution was serially diluted, and each dilution was pour-plated on de Man, Ragosa, and Sharpe (MRS) agar. The plates were incubated at 30 °C for 72 h. The colonies were enumerated, and the CFU per gram was calculated. Total colony counts were performed on Petri dishes containing between 15 and 300 colonies (Kang et al., 2020).

Determination of the selected formula

The optimal treatment was determined using a weighted effectiveness method based on the deGarmo method. The selected formula was eva-luated using parameters that represent dairy product quality, including proximate composition (moisture, protein, fat, carbohydrate, and ash content) as well as total LAB. These parameters were chosen because proximate composition reflects the nutritional value and stability of the product, while LAB viability indicates its functional potential as a probiotic, containing fermented dairy products. Each parameter was assigned a weight, and the treatment with the highest cumulative score was designated as the most favorable.

Effectiveness of *dadih* powder test in metabolic syndrome

Animal model

This study used healthy adult male *Sprague Dawley* (SD) rats (8 weeks old, 213-263 g), which were obtained from PT. Biomedical Technology Indonesia (BMTI). The health status of the animals was confirmed by clear eyes, clean white fur without shedding, and normal activity levels. The rats were individually caged with wood chip bedding, which was changed twice weekly. Each cage contained ad libitum access to food and water supply.

The animals housing conditions were maintained at temperature of 22 ± 3 °C, relative humidity of 60-70%, adequate ventilation, and a 12-hour light/dark cycle. Prior to the experiment, all animals underwent a one-week acclimatization period during which they were fed a standard diet (Kapar & Ciftci, 2020). All experimental procedures were approved by the IPB University Animal Ethics Committee (No. 263-2024 IPB) and conducted at the Experimental Animal Facility of the Tropical Biopharmaca Research Center, IPB University, Bogor, Indonesia.

Experimental animal group

Sample size determination was conducted using a one-way ANOVA group comparison approach based on the formula 10/k+1, where k presents the number of experimental groups. Based on this calculation a minimum of four animals per group was required. A total of 16 rats were used in this study. Following acclimatization period, the rats were randomly allocated into four experimental groups (n=4 per group). One group received a standard diet (normal control), while the remaining 12 rats were fed a high-fat high-sucrose (HFHSD) diet and future divided into three parallel groups, namely the HFHSD control group and two *dadih* powder treatment group. The experimental groups were defined as follows:

- Group N (n=4): Standard feed + plain water for 8 weeks
- Group KN (n=4): High-fat feed + 100 g/L sucrose water for 8 weeks
- Group D1 (n=4): High-fat feed + 100 g/L sucrose water + 0.335 mg/g body weight *dadih* powder for 8 weeks
- Group D2 (n=4): High-fat feed + 100 g/L sucrose water + 0.670 mg/g body weight *dadih* powder (double dose) for 8 weeks

Diet-induced animal model

Two types of experimental diets were administered for 8 weeks: standard and high-fat feed. The standard feed was formulated according to the American Institute of Nutrition (AIN-93G) (Reeves, 1997), while the high-fat high-sucrose diet was adapted and modified from previous studies (Buszewski et al., 2017; Silva et al., 2017; Kobi et al., 2023). In this study, the diet contained 200 g/kg of beef visceral fat and 200 g/kg milk fat as fat sources, with drinking water being supplemented with 100 g/L sucrose water. This modification was made to replace lard used studies with beef and milk fat, which better reflect local dietary pattern and comply with cultural and ethical considerations. The nutritional composition of both diets, including carbohydrates, protein, fat, and energy content, are detailed in Table 1. *Dadih* powder was administered once daily via oral gavage in the morning.

Table 1.
Nutritional composition of experimental diets

Component	Unit	Standard feed	High-fat feed
Moisture	%	9.76	6.04
Ash	%	7.56	4.70
Protein	%	24.38	15.51
Fat	%	4.01	25.34
Crude fiber	%	4.73	5.15
Carbohydrate	%	54.31	51.07
Energy	kcal	351	494

Prior to administration, the powder was diluted 1:10 in distilled water. The dosage was determined based on: (1) typical daily *dadih* consumption (1 tube ≈ 60 g fresh *dadih*), (2) powder yield characteristics, and (3) human-to-animal dose conversion using body surface area alometric scaling. The simplified interspecies factor 0.018 to the total daily human dose to obtain the rat daily dose, thereby accounting for interspecies metabolic-rate differrences and avoiding a direct 1:1 body-weight conversion (Laurence & Bacharach, 1964).

Pre-sacrifice assessments

Body weights were measured weekly using a digital scale. Food intake was recorded daily throughout the 8-week study period by weighing the residual feed every morning. Water consumption was monitored every two days by measuring the remaining volume of water.

Serum analysis

Blood samples were collected at week 0 (baseline, tail vein) and week 8 (terminal, via cardiac puncture). For both baseline and terminal measurements, rats were fasted for 14-16 hours prior to blood collection. Before sampling at baseline, the animals were placed in a warming chamber for 15 min and restrained in a cylindrical holder. The tail was disinfected with 70% alcohol, and 1-2 mL of blood was collected using a 1 cc syringe. At week 8, terminal blood samples were obtained via cardiac puncture after euthanasia to provide sufficient sample volume for multiple biochemical analyses. To minimize variation between sampling sites, all samples were processed immediately under the same conditions and handled consistently within groups.

In addition, 100 µL of blood was collected from the lateral tail vein for glucose levels were measured using EasyTouch® blood glucose test strips and meter (Bioptik Technology, Inc., Taiwan) according to the instructions (Shimizu et al., 2019). All samples were centrifuged at 1,200 rpm for 15 min at 4 °C to separate serum. The obtained serum samples were aliquoted and stored at -80 °C until further analysis. Serum biochemical parameters included total cholesterol, LDL cholesterol, triglycerides, and malondialdehyde (MDA) levels (Taranto et al., 2021; Tsutsumi et al., 2021; He et al., 2022). MDA concentration, as an oxidative stress marker, was determined spectrophotometrically at a wavelength of 532 nm.

Hepatic histopathology

Liver tissues were immediately excised after euthanasia and rinsed with 0.9% NaCl solution for 30 min to remove residual blood. The tissues were then fixed in 10% neutral buffered formalin (NBF) for at least 24 h. The liver samples were trimmed into approximately 10×10×3 mm sections and placed in tissue cassettes for histological processing. Dehydration was carried out through a graded series of alcohol (70%, 80%, and 90% ethanol, 1-2 hours each). The tissues were subsequent clearing was performed using xylene I, II, and III solutions. The processed tissues were embedded in liquid paraffin to form paraffin blocks.

The paraffin blocks containing liver tissue were sectioned at a thickness of 5 µm using a microtome, and the sections were floated on warm water (38-40 °C) to remove wrinkles before drying.

The dried sections were stained with hematoxylin for 10 min, rinsed with running water, and counterstained with eosin. Following staining, the sections ware rehydration through a graded ethanol series and cleared in xylene. Finally, permanent mounting was achieved using the Entellan mounting medium on glass slides. Histological slides were examined under a light microscope, and representative photomicrographs were captured for docu-

mentation. Observations focused on histopathological features including hepatocyte degeneration, inflammatory cell infiltration, necrosis, and Kupffer cell activation. All images were captured with an embedded scale bar calibrated to 75 μm.

Statistical analysis

The optimization of *dadih* powder formulation (10, 30, and 50%) was conducted in duplicate. Data were analyzed using of one-way of variance (ANOVA) followed by an LSD post-hoc test ($p \leq 0.05$) to compare differences among the three formulations.

The animal study employed a completely randomized design (CRD) with four groups (n=4 rats/group). The treatment effects on the measured variables were analyzed using one-way ANOVA in IBM SPSS Statistics (version 17), and data are presented as mean±standard deviation. When ANOVA revealed significant differrences ($p<0.05$), LSD post-hoc test was conducted to identify specific treatment differences.

RESULTS AND DISCUSSION

Optimal formulation of *dadih* powder

Dadih powder was produced using a drum dryer, with the addition of MOCAF as a filler to optimize the product quality and distribution. Drum drying is effective for semi-solid materials like *dadih* because it allows for even heating through direct contact with the heated drum surface. Heat is transferred from the drum wall to the slurry by the steam condensation (Almena, Goode, Bekalis, Fryer & Lopez-Quiroga, 2019). The drying process was carried out at a drum surface temperature of 115 ± 2 °C for 10 s, allowing for rapid and efficient moisture evaporation without damaging the functional compounds. The temperature used in this study is lower than Hossain and Jayadeep (2024) studies, which employed a temperature of 120 °C, thereby preserving most of the nutrients after drying. The product was then dried in a drum dryer to attain a moisture content of less than 7%. The physical, chemical, and total LAB characteristics of the *dadih* powder are listed in Table 2.

The addition of 10, 30, and 50% MOCAF resulted in significantly different physical characteristics of the *dadih* powder, including yield, bulk density, absorption, and color, but showed not significantly in solubility. The F3 formulation (50% MOCAF) had a significantly higher yield compared to F1 (10% MOCAF), indicating that a higher MOCAF concentration results in greater *dadih* powder production.

The F1 formulation (10% MOCAF) exhibited distinct physical characteristics compared with the other formulations. It had the highest bulk density, with a more compact particle structure and low porosity. Pore formulation refers to the development of void spaces within the powder matrix, which is influenced by starch content from MOCAF. A lower MOCAF concentration reduced pore formation, resulting in denser particles. While low porosity increases bulk density and product stability, it may limit rehydration capacity, whereas higher porosity generally facilitates solubility and dispersibility. The absorption index of F1 was significantly lower than those of F2 and F3, although there were no significant differences in solubility among F1, F2, and F3. Color analysis based on L*, a*, and b* values indicated a brownish color in the *dadih* powder. Color changes in the *dadih* powder with MOCAF were monitored and detected visually in Figure 1A. The region of the CIE diagram in Figure 1B was obtained the relationship between concentration of MOCAF and color variations in *dadih* powder. F1 formulation color shifts towards yellow region. Meanwhile, F2 and F3 formulation exhibit a shift towards white region of the diagram. There were significant differences in lightness (L*) observed between the 10 and 30% MOCAF formulation, as well as between the 10 and 50% MOCAF formulation. Meanwhile, no significant difference was detected between the 30 and 50% MOCAF formulations.

MOCAF is potentially a filler agent to *dadih* powder processing. The results of this study revealed that the yield of *dadih* powder ranged from 23.23 to 55.23%. The yield of powdered dadih in this study exceeded the yield of kefir powder reported in the range of 13−22% using skim milk as a filler (Rizqiati, Nurwantoro, Susanti, Apriliani & Prayoga, 2024). Furthermore, this study demonstrated that each 20% increment of Mocaf resulted in an approximately 16% increase in the yield of *dadih* powder, which is in agreement with the findings (Setyadjit & Sukasih, 2015), who reported a similar increase in yield using 20% cassava starch. These findings suggest that MOCAF can be utilized as an effective filler

and anti-caking in the production of *dadih* powder, which is consistent with the role of fillers in increasing the total solid content and volume of powder (Mulyadi, Febrianto, Kumalaningsih & Aswari, 2012), and value of a_w above 0.70 increased caking and cohesiveness of drying products.

The addition of MOCAF at a higher concentration (50%) results in significantly a lower a_w (0.66) than powdered *dadih* with 10% MOCAF (a_w = 0.70). This is influenced by the resistant starch content in MOCAF, which helps bind free water. *Dadih* powder with 50% MOCAF contains a higher amount of resistant starch, thus producing a lower a_w. During the drying process with a drum dryer using heat, the resistant starch forms a gel that binds water more strongly. This gel slows down the water evaporation process, resulting in higher moisture content in *dadih* powder with 50% MOCAF. On the other hand, *dadih* powder with 10% MOCAF showed a_w of 0.7 (within intermediate zone), with a relatively lower resistant starch content, meaning it has a reduced ability to bind free water. When heated, the structure of the resistant starch in the 10% MOCAF addition changes and releases the bound water. This allows the water to evaporate more easily, resulting in lower moisture content in the product with 10% MOCAF.

The lower moisture content in *dadih* powder with 10% MOCAF makes the product denser, as seen in the higher bulk density value, resulting in lower flowability. Lower flowability indicates that the product is more difficult to absorb water from the environment, which is reflected in the lower water absorption index. This makes *dadih* powder with 10% MOCAF more stable and gives it a longer shelf life. Maintaining the product at a $a_w < 0.8$ can preserve shelf life and the stability of flowability, ensuring that the flow factor remains high and the powder flows easily without clumping (Juarez-Enriquez et al., 2019).

The a_w of the *dadih* powder ranged from 0.66 to 0.70, classifying it as an intermediate-moisture product. A decreasing trend in a_w was observed with increasing MOCAF content, with the 10% MOCAF formulation (F1) exhibiting the highest a_w (0.70±0.02) and total LAB count. Although F1 showed numerically higher LAB counts, the differences among formulations were not statistically significant.

Table 2
Physicochemical and total LAB characteristics of *dadih* powder

Parameters	Unit	Formulation (Concentration of MOCAF)		
		F1 (10%)	F2 (30%)	F3 (50%)
Physical parameters				
Yield	%	23.23±4.05 ^{a}	39.03±2.38 ^{ab}	55.23±2.77 ^{b}
Bulk density	g/cm^3	0.55±0.03 ^{b}	0.38±0.01 ^{a}	0.39±0.01 ^{a}
Solubility index	%	78.79±0.38 ^{a}	78.87±0.79 ^{a}	78.28±1.12 ^{a}
Absorption index		68.26±1.91 ^{a}	76.08±0.15 ^{b}	78.04±1.02 ^{b}
Color				
L*		61.70±3.49 ^{a}	72.89±1.20 ^{b}	75.86±0.72 ^{b}
a*		12.81±1.73 ^{b}	8.28±0.33 ^{a}	6.37±0.80 ^{a}
b*		18.67±0.68 ^{c}	15.91±0.56 ^{b}	13.57±0.02 ^{a}
Whiteness	°hue	55.64±4.57 ^{a}	62.47±1.73 ^{b}	64.91±2.79 ^{b}
Microbiological parameters				
Total LAB	log10 cfu/g	2.09±0.08 ^{a}	1.89±0.09 ^{a}	1.87±0.16 ^{a}
Chemical parameters				
Water activity		0.70±0.02 ^{b}	0.70±0.01 ^{b}	0.66±0.01 ^{a}
Moisture content	% wb	5.64±0.29 ^{a}	4.67±0.44 ^{a}	7.77±0.89 ^{b}
Ash content	% db	3.41±0.03 ^{c}	2.31±0.01 ^{b}	1.73±0.03 ^{a}
Protein content	% db	19.81±0.32 ^{c}	14.82±0.97 ^{b}	8.28±0.19 ^{a}
Fat content	% db	29.00±0.13 ^{c}	15.04±0.17 ^{b}	6.04±0.29 ^{a}
Carbohydrate content	% db	22.04±7.96 ^{a}	54.11±5.88 ^{b}	77.67±1.88 ^{c}

F: formulation; L: lightness; a*: red, green; b*: yellow, blue; all display results are expressed as mean±SEM, different small letters in the superscript in rows indicate statistically significant differences at the level α=0.05.*

A

B

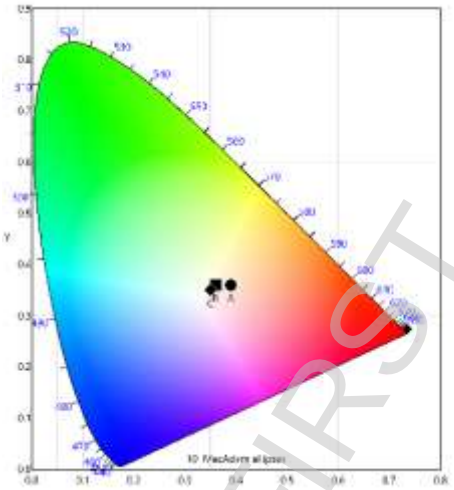

Figure 1. Photograph of powdered *dadih* with addition of mocaf (A): F1 formulation (10% MOCAF), F2 formulation (30% MOCAF), F3 formulation (50% MOCAF). CIE diagram (B): F1 formulation (●), F2 formulation (■), F3 formulation (♦)

Quantitative analysis showed a considerable reduction in total LAB in *dadih* powder, ranging from 74% to 76% compared to fresh *dadih*, which initially contained 8 log cfu/g of LAB.

The a_w value of *dadih* powder in this study ranged between 0.66 and 0.70, which is considered moderate. This condition allows the growth of LAB, where bound water is still available, and the solution phase begins to form. This supports the Maillard reaction between reducing sugar of MOCAF and *dadih* protein, resulting in the formation of brown color (Sitanggang, Firdausi & Budijanto, 2021). This color change can be observed through the analysis of L*, a*, and b* values in the color measurement of the *dadih* powder. The higher the addition of MOCAF, the lower the water activity of the *dadih* powder tends to decrease. Lower water activity causes osmotic stress, which inhibits the growth of LAB, leading to a lower LAB count (Serrano, Grujović, Marković, Barreto-Crespo & Semedo-Lemsaddek, 2015). Optimal LAB growth was recorded at a_w of 0.70. Nevertheless, excessive increases in a_w can compromise product quality by promoting the growth of contaminating microorganisms, including molds and pathogenic bacteria. The risk of contamination increases, particularly at a_w values $\geq$ 0.85, where pathogenic and spoilage bacteria begin to proliferate (Labuza & Altunakar, 2020). The formulation with 10% MOCAF produced the highest LAB compared to other formulas, although no significant differences were found between the MOCAF addition treatments. These findings are consistent with those of previous studies, such as those by (Jimenez, Flores-Andrade, Pascual-Pineda & Beristain, 2015; Sornsenee, Chimplee, Saengsuwan & Romyasamit, 2022), which demonstrated that a_w range of 0.346-0.536 can maintain the viability of *Lactobacillus paracasei* cells while inhibiting mold growth.

Dadih powder with a higher total LAB content indicates enhanced nutritional profile. The protein and lipid content of *dadih* powder provides substrates that maintain LAB viability during processing. Proximate composition and total LAB were used as parameters to deter-

mine the optimal formulation using an effectiveness index approach, which identified the 10% MOCAF formulation as the most favorable (Table 3). The results showed that a 20% increment in MOCAF addition significantly reduced the protein content by 5% to 6%. Furthermore, higher MOCAF concentrations resulted in a significant decrease in lipid content, whereas F1 exhibited a lower carbohydrate level than the 50% MOCAF formulation (F3). Based on the effectiveness index approach, which considers nutritional value and total LAB count, the formulation F1 with 10% MOCAF yielded the highest results and was selected for the effectiveness of the *dadih* powder.

Table 3
Effectiveness index of *dadih* powder formulation

Formula (percentage of MOCAF)	Effectiveness index
F1 (10%)	0.806
F2 (30%)	0.375
F3 (50%)	0.200

The drying of *dadih* powder with the addition of 10% MOCAF can lower the water activity (a_w 0.70) compared to fresh *dadih* (a_w 0.9). Low water activity can inhibit oxidative reactions in the production of free radicals (Vu, He, McClements & Decker, 2020). Oxidative compounds such as hydroperoxides and aldehydes are formed when products are exposed to oxygen, light, or heat (Clarke, McCarthy, O'Sullivan, Kerry & Kilcawley, 2021). The optimization of a_w can maintain the stability of the parabiotic properties of *dadih* powder, thereby providing health benefits to the body. Parabiotics can have a positive effect on metabolism, even when bacterial cells are in a lysed condition.

Feed intake, water consumption, energy intake, and body weight

As shown in Fig. 2, rats fed a high-fat diet with 10% sucrose water (KN group) exhibited a significant 38.15% reduction in daily feed intake compared with the standard diet group (N). In contrast, water consumption in the KN group increased significantly ($p<0.05$). Administration of *dadih* powder at doses of 0.335 mg/g (D1) and 0.67 mg/g (D2) did not result in statistically significant differences in feed or water intake compared with the KN group. Despite the lower feed intake, the KN group exhibited a significantly higher energy intake than group N ($p<0.05$). Although groups D1 and D2 showed no significant differences the from KN group, their energy intake tended to move toward normal levels (not significantly different from the N group).

Water intake in the D1 group tended to be higher than that in the KN group, although the difference was not statistically significant. This may be due to the stimulation of type III taste cells by the fermentation acid of *dadih* powder through the otopterin 1 (Ahmad & Dalziel, 2020). Consumption of *dadih* powder at higher doses (D2) potentially contains more BAL lipase (Yao et al., 2021) and SCFAs (Fusco et al., 2023), which enhance insulin sensitivity (Subramaniyan & Hanim, 2025) and reduce thirst by improving sodium reabsorption (Nizar, Shepard, Vo & Bhalla, 2018; Singh, Sharma, Kumari & Tiwari, 2019). Therefore, the water intake of the D2 group was significantly lower than that of the D1 group.

The high-fat, high-sucrose diet led to increased energy intake despite reduced food intake, which was attributable to the high caloric density of fatty foods and sucrose water. Although *dadih* powder supplementation did not significantly affect food intake, energy intake or body weight, there was a tendency for energy intake to normalize toward the control group levels without associated weight changes (Fig. 2). This suggests that *dadih* powder helps maintain normal energy intake without affecting body weight. This consumption pattern aligns with previous studies indicating that animals with energy density in their diets tend to regulate their intake based on caloric needs rather than food volume (Burchfield et al., 2018). The absence of significant obesity symptoms may be explained by mechanisms such as eating rate regulation affecting actual intake, increased non-exercise activity thermogenesis (NEAT), and maintained leptin sensitivity involved in satiety regulation and energy balance (Teo, Van Dam, Whitton, Tan & Forde, 2021).

Blood glucose and lipid profile

The blood glucose, triglyceride, total cholesterol, and LDL cholesterol levels was observed in the *dadih*-treated groups, as shown in Fig. 3. The initial data showed that there was not significantly difference in blood glucose, triglyceride, total cholesterol, and LDL cholesterol between groups ($p>0.05$).

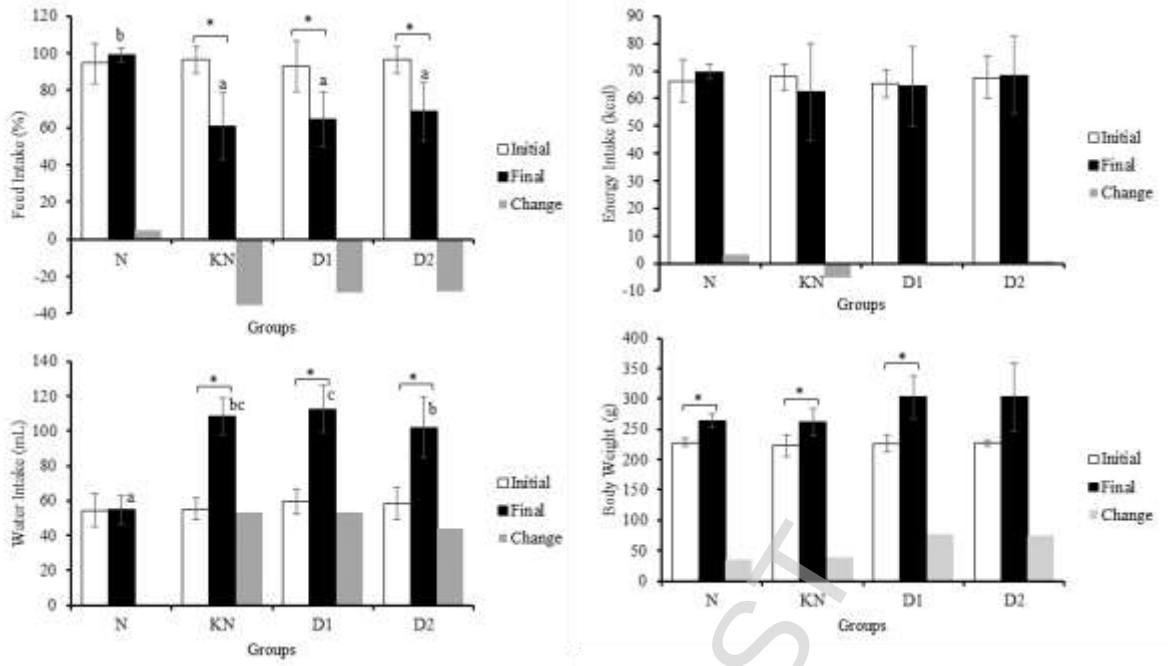

Figure 2. Feed intake, water consumption, energy intake, and body weight of *dadih* powder N: standard diet; KN: high-fat high-sucrose diet; D1: high-fat high-sucrose diet + 0.335 mg/g *dadih* powder; D2: high-fat high-sucrose diet + 0.67 mg/g *dadih* powder. Data are presented as mean±SD (n=4). Different superscript letters (a, b, c) indicate significant differences among groups at the same time point ($p<0.05$). *indicates a significant difference within the same group over time ($p<0.05$)

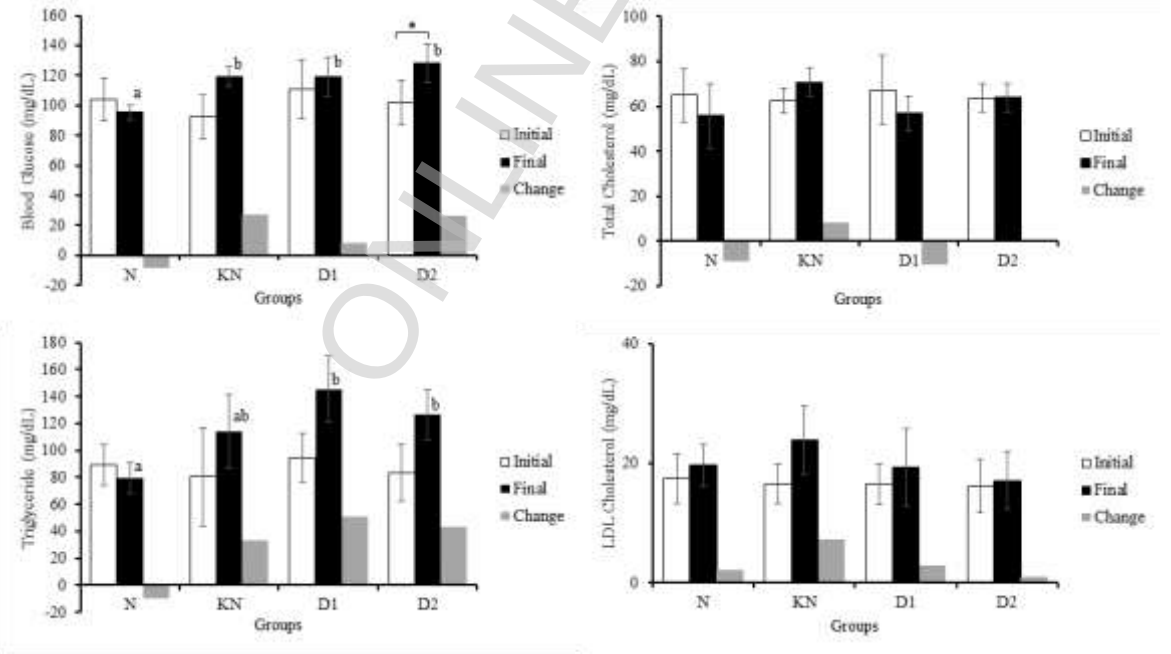

Figure 3. Blood analysis results of experimental animals fed high-fat high-sucrose diet and dadih powder. N: standard diet; KN: high-fat high-sucrose diet; D1: high-fat high-sucrose diet + 0.335 mg/g *dadih* powder; D2: high-fat high-sucrose diet + 0.67 mg/g *dadih* powder. Different superscript letters (a, b) indicate significant differences among groups at the same time point ($p<0.05$). *indicate a significant difference within the same group over time ($p<0.05$)

After 8 weeks of *dadih* powder intervention, blood glucose level were significantly affected ($p<0.05$). For triglyceride, significant differrences were observed between the *dadih*-treated groups (D1 and D2) and the normal control group (N) ($p<0.05$), whereas no significant differences were found between the *dadih*-treated groups and high-high-sucrose control group (KN), nor between the N and KN groups ($p>0.05$). Total cholesterol and LDL cholesterol levels were not significantly affected by *dadih* powder supplementation ($p>0.05$). The KN group showed significantly elevated blood glucose and triglyceride level compared to the N group (standard diet). *Dadih* powder supplementation in groups D1 and D2 did not significantly affect the measured parameters compared to the KN group. However, a decreasing trend in total cholesterol levels was observed in the *dadih*-treated groups, while LDL cholesterol levels ware not significantly changed, as shown in Fig. 3.

In this study, *dadih* powder supplementation did not result in a significant reduction in total cholesterol levels or LDL cholesterol levels. Although no significant changes were observed, a modest tendency toward lower total cholesterol levels was noted in *dadih*-treated groups. Probiotic *Lactobacillus* has been reported to influences cholesterol metabolism through bile salt deconjugation and short-chain fatty acids production that inhibit cholesterol synthesis in the liver (Reis, Conceicao, Rosa, Siqueira & Peluzio, 2017). The consumption of *dadih* powder did not show a significant effect on changes in blood glucose and lipid profiles in the D1 and D2 groups, presumably because a high-fat and high-sugar diet can affect the efficacy of probiotics in controlling blood glucose and lipid levels (Peng et al., 2024).

Increased water consumption in the KN group correlated with higher blood glucose and triglyceride levels compared to group N. Hepatic sucrose metabolism via glycolysis pathways rapidly enhances triglyceride synthesis and secretion (Di Ciaula et al., 2017; Gugliucci, 2023), whereas short-term sucrose intake does not immediately elevate cholesterol and LDL levels (Bergwall, Johansson, Sonestedt & Acosta, 2022). At the tested doses, *dadih* powder intervention did not consistently yield significant effects on these metabolic parameters, although a decreasing trend in total cholesterol and LDL levels was observed. The *dadih* fermentation process produces secondary metabolites, including bioactive peptides and fatty acids. *Dadih*-derived bioactive peptides have demonstrated inhibitory effects on cholesterol synthesis and enhanced bile acid excretion, potentially contributing to LDL reduction. Both saturated (palmitic and stearic acids) and unsaturated (oleic, linoleic, and linolenic acids) fatty acids may lower cholesterol levels by inhibiting hepatic HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase activity, while antioxidant compounds help prevent LDL oxidation, thereby reducing the risk of atherosclerosis (Mohankumari, Naidu, Narasimhamurthy & Vijayalakshmi, 2021).

Malondialdehyde (MDA) levels

Malondialdehyde (MDA), an organic compound derived from lipid peroxidation, serves as a reliable indicator of oxidative stress. Figure 4 shows the MDA levels in each group of rats. *Dadih* powder supplementation significantly reduced serum MDA levels in rats fed a high-fat, high-sucrose diet. The standard diet group (N) showed MDA levels of 2.21 ± 0.41 nmol/dl, whereas the high-fat, high-sucrose group (KN) exhibited a significant increase to 3.66 ± 0.38 nmol/dL ($p < 0.05$). *Dadih* powder intervention demonstrated potent protective effects against oxidative stress in the present study. The D1 group (single-dose powdered *dadih*) displayed remarkably low MDA levels (0.68 ± 0.39 nmol/dl), which were statistically different from those of the other groups ($p < 0.05$). The D2 group (double-dose powdered *dadih*) showed MDA levels of 1.95 ± 0.61 nmol/dl, which were not significantly different from those of group N.

MDA is secondary product of lipid peroxidation, a process influenced by the presence of reactive oxygen species (ROS). Fat accumulation in adipose tissue contributes directly to an increase inflammation and the production of ROS. ROS generated from activation of the NADPH oxidase (NOX) enzyme in the cell membrane and in the mitochondria through the oxidative phosphorylation process. This process produces free radicals such as superoxide anion ($\bullet O_2^-$), which is rapidly converted into hydrogen peroxide (H_2O_2) by the enzyme superoxide dismutase (SOD), and can subsequently generate hydroxyl radicals ($\bullet OH$). The presence of these ROS plays a role in signaling processes (Ayala, Munoz & Arguelles, 2014).

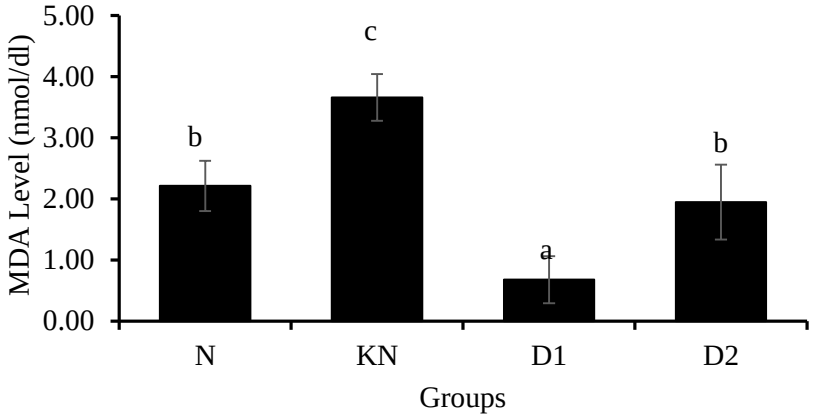

Figure 4. Serum malondialdehyde level in animals fed high-fat high-sucrose diet and powdered *dadih.* N: standard diet; KN: high-fat high-sucrose diet; D1: high-fat high-sucrose diet + 0.335 mg/g *dadih* powder; and D2: high-fat high-sucrose diet + 0.67 mg/g *dadih* powder

However, ROS are uncontrolled and may lead to oxidative stress. These ROS are synthesized during the oxidation of polyunsaturated fatty acid (PUFA), and their accumulation can cause cellular damage, including membrane lipid oxidation. The parabiotic of *dadih* powder exert as antioxidant properties to the suppression of ROS production, particularly by inhibiting enzymes such as lipoxygenase and cyclooxygenase (COX), which are involved in the oxidative processes leading to MDA formation. This is consistent with the significant reduction in MDA levels observed in the D1 group, suggesting that the bioactive components of *dadih* powder effectively mitigate oxidative damage. The reduction in MDA levels is attributed to the antioxidant constituents and is interpreted as evidence that lipid peroxidation of polyunsaturated fatty acids was suppressed, thereby contributing to the protection of cells and DNA from oxidative damage. This decrease may also be associated with the drying process of the product, which facilitated the encapsulation of its bioactive components and preserved their antioxidant activity (Naibaho, Safithri & Wijaya, 2019).

Fermentation of buffalo milk by LAB generates bioactive peptides through β-casein hydrolysis (Taha et al., 2017). These antioxidants neutralize free radicals produced during excessive lipid metabolism, thereby protecting heaptocyte cell membranes and organelles structures such as mitochondria from oxidative damage. Additionally, antioxidants inhibit free radical-induced inflammatory pathway activation, reducing proinflammatory cytokine release, which can exacerbate hepatic inflammation and fibrosis. Furthermore, antioxidants regulate the gene expression governing lipid synthesis and metabolism, contributing to the maintenance of hepatocyte lipid homeostasis (Mohammadian, Fakhar, Keramat & Stanek, 2024).

In the D2 group, there was an increase in MDA levels, although it remained within a balanced range compared to the control. This increase may be due to the higher concentrations of arachidonic acid (AA) and polyunsaturated fatty acids (PUFAs) in the powdered dadih product after the drying process. The drying process enhances the content of these fatty acids, which can facilitate MDA synthesis through enzymatic pathways involving cyclooxygenase (COX) and thromboxane A2 synthase. Despite the increase in MDA, the levels in the double-dose group remained balanced with the control, indicating that the lipid oxidation inhibition mechanism of the powdered dadih still functions, even with the additional effects of increased AA and PUFA (Masenga, Kebwe, Chakulya & Kirabo, 2023).

Hepatic histopathology

Histopathological examination revealed that high-fat and high-sucrose diet consumption induced hepatic damage, characterized by severe steatosis and mild inflammation in the KN group, as shown in Fig. 5. The KN group exhibited marked hepatic steatosis, with substantial lipid droplet accumulation within heaptocytes, cellular swelling, and sinusoidal compression.

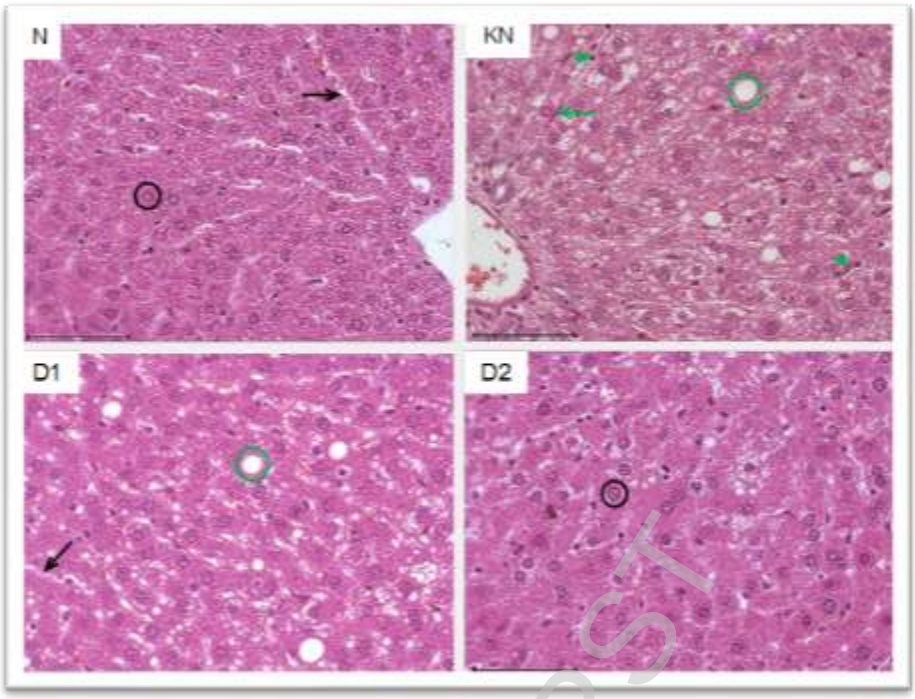

Figure 5. Hepatic histopathology using hematoxylin and eosin (H&E) staining. N: standard diet; KN: high-fat high-sucrose diet; D1: high-fat high-sucrose diet + 0.335 mg/g *dadih* powder; D2: high-fat high-sucrose diet + 0.67 mg/g *dadih* powder. Arrow indice hepatosit (○), steatosis (○), sinusoid (→), sinusoidal (→), Kupffer cell (►). Images were captured at 400x magnification. Scale bar = 75 µm

Kupffer cells in this group appeared enlarged and clustered in specific areas, indicating activation and potential amplification of pro-inflammatory cytokine production, which could contribute to inflammation, as previously described (Diehl et al., 2020).

The D1 group (0.335 mg/g *dadih* powder) showed notable hepatocyte structural improvement, with reduced damage and less pronounced hepatocyde degeneration compared to the KN group. However, mild steatosis and limited necrosis were still observed, suggesting partial protection. The D2 group (0.670 mg/g *dadih* powder) demonstrated near-normal hepatic architecture, with intact hepatocyte nuclei and uncompressed sinusoids. These finding suggest a stronger hepatoprotective effect at the higher dose of *dadih* powder. Normal liver tissue in the N group exhibited well-preserved architecture, with hepatocytes of regular size and distinct cellular boundaries (Fig. 5A). The sinusoidal architecture was intact, showing no evidence of steatosis, necrosis, or inflamemation (Hasan, Falah, Handharyani & Dwicesaria, 2023). Previous studies reported that high-fat diets induce metabolic disorders, including glucose intolerance,dyslipidemia, and hepatic oxidative stress (Lasker, Rahman, Parvez, Zamila & Miah, 2019). In line with these findings, our study showed that a high-fat and high-sucrose diet induced significant metabolic disturbances in rats, evidenced by elevated blood glucose, total cholesterol, LDL, triglycerides, and serum MDA levels, alongside hepatic tissue damage. *Dadih* powder supplementation provided protective effects against metabolic syndrome, particularly at the higher dose (D2), although it did not significantly affect body weight. This suggests that *dadih* powder may help alleviate hepatic damage and improve lipid metabolism without significantly influencing body weight, as also observed in previous studies (Ramos-García et al., 2021).

CONCLUSIONS

MOCAF at 10% addition level was shown to be effective filler in *dadih* powder production, as it contains LAB and valuable nutritional content. This study demonstrated that consumption of a high-fat, high-sucrose diet in rat models significantly increased blood glucose, triglycerides, and oxidative stress marker MDA, as well as induced hepatic steatosis and inflamemation. Although *dadih* powder inter-

vention did not produce statistically significant changes in glucose, triglyceride, total cholesterol, or LDL cholesterol levels, it showed potential protective effects against oxidative stress and liver damage, particularly at the higher dose (0.67 mg/g body weight). *Dadih* powder supplementation significantly reduced MDA levels, with effects approaching or potentially exceeding those observed in the normal control group. Histopathological improvements were most prominent in the high-dose group, where near-normal hepatocyte structures were observed. These beneficial effects are likely attributed to the antioxidant compounds present in *dadih*. These findings support the development of *dadih* powder as an innovative functional food, containing parabiotic, that may help in the prevention and management of metabolic syndrome through the consumption of fermented local food products.

AUTHOR CONTRIBUTIONS

Conceptualization, I.A.S, S.A.M., E.P., and E.H..; Methodology, I.A.S. and S.A.M.; Investigation, formal analysis, validation, writing-original draft preparation, I.A.S, S.A.M., E.P., and E.H.; Writing-review and editing, I.A.S. and E.P.; Supervision, S.A.M., E.P., and E.H.

DATA AVAILABILITY STATEMENT

Data contained within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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FERMENTISANO BIVOLJE MLEKO U PRAHU (DADIH) SA SMANJENOM AKTIVNOŠĆU VODE KAO PARABIOTIK ZA ZAŠTITU OD OKSIDATIVNOG STRESA I OŠTEĆENJA JETRE

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Sažetak: Praškasti dadih predstavlja osušeno fermentisano mleko bivola. Ova studija je imala za cilj da proceni efikasnost dadih praha obogaćenog modifikovanim brašnom od kasave (MOCAF) na parametre metaboličkog sindroma kod laboratorijskih pacova soja Sprague-Dawley. Formulacije koje su sadržale 10, 30 i 50% MOCAF-a analizirane su u pogledu fizičko-hemijskih svojstava i vijabilnosti bakterija mlečne kiseline (LAB). Optimalna formulacija određena je metodom deGarmo, na osnovu hemijskog sastava i ukupnog broja LAB, pri čemu je formulacija sa 10% MOCAF-a pokazala najviši indeks efikasnosti. Ova formulacija je zatim korišćena u interventnoj studiji. Pacovi su bili podeljeni u četiri grupe: normalna dijeta (N), dijeta sa visokim sadržajem masti i saharoze (KN), kao i dve eksperimentalne grupe koje su dobijale suplementaciju dadih prahom u dozama od 0,335 mg/g (D1) i 0,670 mg/g (D2) telesne mase tokom osam nedelja. Nakon toga, životinje su žrtvovane, a uzorci krvi i jetre prikupljeni su za biohemijske i histološke analize. Tradicionalno, svež dadih je visoko kvarljiv, sa rokom trajanja od približno tri dana. Proces sušenja smanjio je aktivnost vode sa 0,70 na 0,66, čime je poboljšana stabilnost i produžen vek trajanja. Sušenje je efikasno očuvalo vijabilnost LAB do nivoa od 10^2 cfu/g, što je omogućilo svrstavanje proizvoda u kategoriju parabiotika. Suplementacija dadih prahom značajno je smanjila nivo malondialdehida, ukazujući na antioksidativnu aktivnost, iako redukcije glukoze i lipidnog profila nisu bile statistički značajne. Histopatološka analiza pokazala je izraženu hepatičnu steatozu u KN grupi, dok je D2 grupa imala gotovo normalnu strukturu jetre. Ovi nalazi ukazuju da dadih prah može služiti kao parabiotička hrana koja štiti od oksidativnog stresa i oštećenja jetre povezanih sa metaboličkim sindromom.

Ključne reči: *antioksidativnost, ishrana bogata mastima i šećerom, metbolički sindrom, modifikovano kasava brašno, aktivnost vode*

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