Journal of Health and Nutrition Research
Vol. 4, No. 2, 2025, pg. 641-652, https://doi.org/10.56303/jhnresearch.v4i2.443
Journal homepage: https://journalmpci.com/index.php/jhnr/index

e-ISSN: **2829-9760**

Selenium-Fortified Red Dragon Fruit *Dadih* Improves Obesity Markers in Rats Induced by a High-Fat, High-Fructose Diet

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ARTICLE

Submitted: 16 May 2025

Accepted: 2 June 2025

Keywords:
Obesity, Probiotics, Antioxidant Activity, Selenium, Red Dragon Fruit Dadih

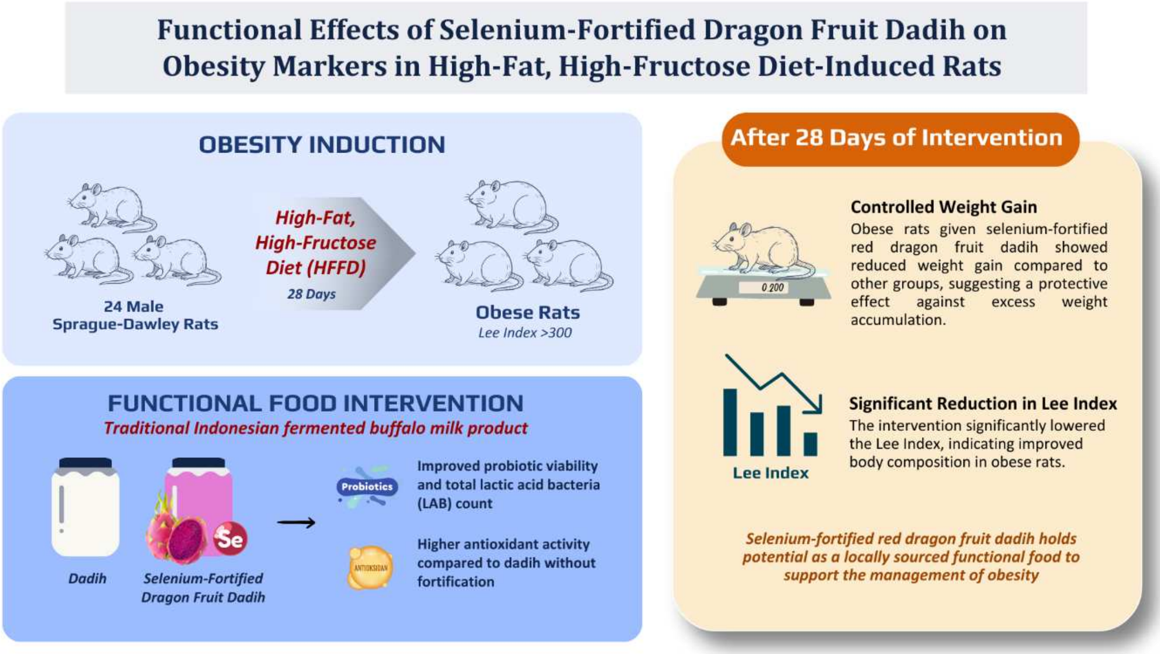
ABSTRACT

Obesity is a growing global health problem linked to metabolic disorders. Functional foods have emerged as a potential non-pharmacological approach to support obesity management. Dadih, a traditional Indonesian fermented buffalo milk product, contains probiotics but has limited antioxidant capacity. This study aimed to evaluate the effects of selenium-fortified red dragon fruit dadih on obesity markers namely body weight and Lee Index and its functional characteristics in rats induced with a high-fat, high-fructose diet (HFFD). A true experimental design with randomized controlled groups was conducted using 24 male Sprague-Dawley rats divided into four groups: healthy control (K−), obesity control (K+), dadih (Di), and selenium-fortified red dragon fruit dadih (Di+). After 28 days of HFFD induction, Di and Di+ groups received 2 mL /200 g BW/day of dadih for 28 days. At the end of the intervention, the Di+ group exhibited significantly moderated body weight gain (from 278.4 ± 3.36 g to 316.6 ± 2.40 g, p = 0.000**)** and the lowest Lee Index (296.1), compared to Di (329.8 ± 2.58 g to 316.6 ± 2.40 g, Lee Index: 299.0) and K+ (281.2 ± 2.58 g to 364.4 ± 3.50 g, Lee Index: 343.4). Functional analysis showed that Di+ had higher lactic acid bacteria (7.7 × 10^8 CFU/g) and antioxidant activity (19.54%) than Di. These findings demonstrate that the combination of probiotics, red dragon fruit bioactives, and selenium synergistically improved metabolic regulation. In conclusion, selenium-fortified red dragon fruit dadih improved the functional quality of the product and showed potential to regulate obesity markers. This formulation offers a promising functional food innovation for obesity management and warrants further clinical investigation.

Key Messages:

- Selenium-fortified red dragon fruit dadih improving probiotic activity and antioxidant capacity.
- This formulation mitigates obesity markers and offers potential as a locally sourced functional food for obesity management.
- The synergy between lactic acid bacteria, red dragon fruit bioactives, and selenium contributed to better metabolic regulation.

GRAPHICAL ABSTRACT



INTRODUCTION

Obesity has become one of the serious global health issues as it is associated with various chronic diseases, such as cardiovascular disorders, diabetes mellitus, and non-alcoholic fatty liver disease(1,2). In addition to its clinical impacts, obesity also imposes a significant economic and social burden on both individuals and healthcare systems (3). In Indonesia, the rising prevalence of obesity, reaching 23.4% among individuals over 18 years of age in 2023 and 36.8% for central obesity among those over 15, underscores the urgent need for preventive strategies that emphasize nutrition and lifestyle modifications. In this context, functional foods are increasingly gaining attention as a supportive intervention that may help regulate metabolism and reduce body fat accumulation (4).

Fermented dairy products containing probiotics, particularly lactic acid bacteria (LAB), have attracted attention due to their potential health benefits, including antiobesity effects (5). Probiotics, such as Lactobacillus and Bifidobacterium strains, modulate the gut microbiota, which is critical for energy metabolism, appetite regulation, and fat storage. They produce short-chain fatty acids (SCFAs) that improve gut barrier integrity and modulate appetite-regulating hormones, thereby reducing visceral fat and improving glucose metabolism (6–8). Probiotics also help reduce inflammation and oxidative stress by downregulating proinflammatory cytokines and upregulating antioxidant enzymes such as catalase and superoxide dismutase (8).

Additionally, bioactive polyphenols, found in fruits, vegetables, and other plant-based foods, exhibit antioxidant and anti-inflammatory properties that are beneficial in managing obesity. Polyphenols enhance gut barrier integrity and reduce gut leakiness, which is associated with lower inflammation and improved metabolic health (9). In addition, they affect metabolic pathways by reducing oxidative stress and improving lipid metabolism, which aids in weight management and the prevention of obesity-related complications (10). The combined effects of probiotics and polyphenols on gut microbiota and oxidative stress highlight their potential as complementary strategies in obesity management (11,12).

Among the various fermented foods with functional potential, traditional products such as dadih offer a culturally relevant and microbiologically rich option that aligns with local dietary habits. Dadih is a traditional Indonesian fermented dairy product originating from West Sumatra, made from buffalo milk through spontaneous fermentation without the addition of starter cultures. This product is rich in lactic acid bacteria (LAB), which are known to contribute to digestive health, enhance the immune system, and reduce blood lipid levels (13–15). However, conventional dadih has limitations in antioxidant activity, which is crucial for addressing metabolic disturbances in obesity, necessitating innovations to enhance the

https://doi.org/10.56303/jhnresearch.v4i2.443

product's functional quality. Previous research showed that the antioxidant activity of dadih was 1177.637 ppm (low) (16).

One innovative approach that has been developed is the addition of red dragon fruit (*Hylocereus polyrhizus*) into dadih*.* This tropical fruit contains various bioactive compounds such as betalains, flavonoids, phenolic compounds, and vitamin C, which possess antioxidant and anti-inflammatory activities (17,18). Several studies have demonstrated that the addition of red dragon fruit can increase the total LAB count and antioxidant capacity of fermented products while also improving organoleptic characteristics (19–21).

Furthermore, selenium (Se) fortification has emerged as a promising strategy for enhancing the functional effects of fermented foods (22,23). Selenium is an essential mineral that plays a key role in the body's antioxidant defence system, particularly through the enzyme glutathione peroxidase(24,25). Additionally, selenium is involved in regulating lipid metabolism and insulin sensitivity (23). Probiotics fortified with selenium have previously shown benefits in reducing body fat and improving metabolic profiles (22).

Although the individual benefits of red dragon fruit and selenium have been widely reported, research on their combination in dairy fermentation products, such as dadih, is still limited, particularly in the context of obesity management. No study has specifically evaluated the effects of dadih fortified with red dragon fruit and selenium on obesity status indicators such as body weight and the Lee index in animal models with high-fat, high-fructose diets.

This study aims to evaluate the functional effects of selenium-fortified red dragon fruit dadih on body weight and the Lee index in rats induced to become obese through a high-fat, high-fructose diet, and to characterize the functional and microbial properties of the product. By addressing this research gap, this study aims to provide scientific evidence for the potential of an innovative, locally based dadih formulation as a functional food intervention supporting the nutritional management of obesity.

METHODS

This study employed a proper experimental design with a pre-post test and a randomized control design. The study involved 24 male Sprague-Dawley rats, aged eight weeks, with an initial body weight ranging from 150 to 200 grams. Following a seven-day acclimatization period, the rats were assigned to four groups (n = 6 per group) using simple randomization, generated with Microsoft Excel to ensure an equal and unbiased distribution. The sample size was determined using the Federer formula, which is commonly applied in preliminary animal studies to ensure minimum statistical requirements are met. The negative control group (K−) received a standard diet and water ad libitum. The obesity control group (K+), the dadih group (Di), and the selenium-fortified dragon fruit dadih group (Di+) were induced with obesity by feeding a high-fat, high-fructose diet (HFFD) for 28 days. The HFFD consisted of 3 g pork fat (15%), 2 g duck egg yolk (10%), 15 g standard chow (75%), and an additional 2 mL of fructose solution.

An overview of the experimental design is illustrated in Figure 1. Once obesity was established, the Di group received 2 mL of dadih per 200 g of body weight per day. In contrast, the Di+ group was administered selenium-fortified dragon fruit dadih at the exact dosage. The interventions were administered orally using a gavage tube for 28 consecutive days. The selected dose was adapted from Makwana et al. (2023), who demonstrated that probiotic fermented milk supplementation at 2 mL/day for 4 weeks significantly reduced body weight and metabolic markers in obese rats (26).

Dadih was prepared traditionally by heating 1000 mL of buffalo milk to 72°C for 15 seconds, followed by cooling to 30°C. For the fortified formulation, 10% red dragon fruit juice and 0.4 ppm of selenium (Na_2SeO_3) were added to the milk. Both the plain and fortified milk were poured into clean bamboo tubes, sealed with banana leaves, and secured with rubber bands. Fermentation was conducted at room temperature for 48 hours. After fermentation, the dadih products were stored at −18°C until they were used.

Equipment used for production included bamboo tubes, banana leaves, a stove, stirring spoons, a thermometer, and a digital scale. For animal handling and treatment, cages, feeding and drinking containers (ad libitum), gavage tubes, masks, and gloves were utilized. The nutritional properties and functions of

https://doi.org/10.56303/jhnresearch.v4i2.443

dadih were evaluated using proximate analysis (AOAC 2012: gravimetric for moisture and ash content, Kjeldahl for protein, Soxhlet with Weibull modification for fat, difference for carbohydrate, and Atwater factor for energy), antioxidant activity test (DPPH method by Yen & Cheng, 1995), LAB count (Total Plate Count on PGY agar), and pH measurement (two-point calibrated pH meter, HANNA Instruments HI9813-6).

The data collected included rat body weight before and after HFFD induction, body weight before and after the dadih intervention, and Lee's index before and after the intervention. All statistical analyses were performed using SPSS version 26 for Windows (IBM Analytics, Armonk, NY, USA). Data were presented as mean ± standard deviation (SD). The Shapiro–Wilk test was used to assess the normality of the data. Statistical comparisons between groups were conducted using one-way ANOVA, followed by the Bonferroni post hoc test for normally distributed data, and the Kruskal–Wallis test, followed by the Mann–Whitney U test for non-normally distributed data. A p-value of <0.05 was considered statistically significant.

This study has received ethical approval from the Health Research Ethics Committee of the Faculty of Medicine, Diponegoro University, with approval number 090/EC-H/KEPK/FK-UNDIP/IX/2024, dated September 4, 2024. It adheres to international guidelines for the care and use of laboratory animals.

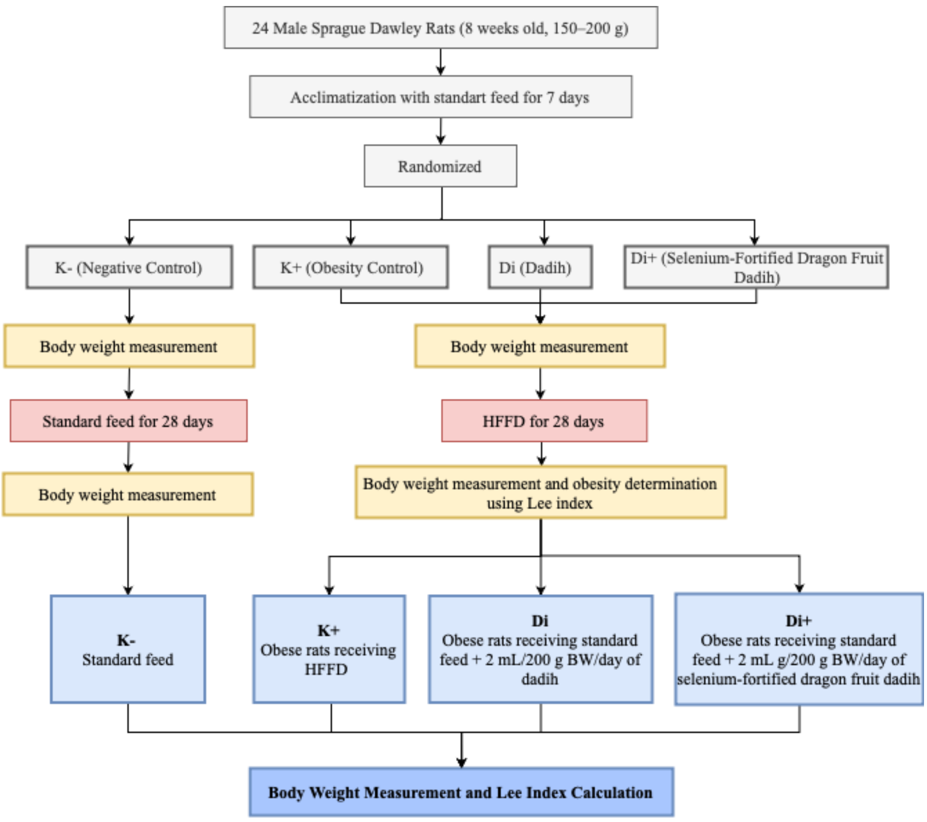


Figure 1. Experimental Design

RESULTS

The nutritional content of Dadih and Selenium-Fortified Dragon Fruit Dadih is presented in Table 1, showing differences in composition between the non-fortified product (Di) and the product fortified with dragon fruit and selenium (Di+). The Di+ exhibited a higher moisture content (78.09%) compared to Di (72.09%). The fat and protein contents were higher in Di, recorded at 13.18% and 7.40% respectively, compared to 10.52% and 4.68% in Di+. Carbohydrate content was also slightly higher in Di (6.46%) than in Di+ (4.85%), while ash content remained relatively similar between the two.

The total lactic acid bacteria (LAB) count in Di+ was 7.7×10^8 CFU/g, higher than in Di (9.5×10^7 CFU/g). Antioxidant activity was also significantly higher in Di+ (19.54%) compared to Di (11.58%). The pH value of Di+ was lower (4.4) than that of Di (4.8), indicating a higher level of acidity. These findings

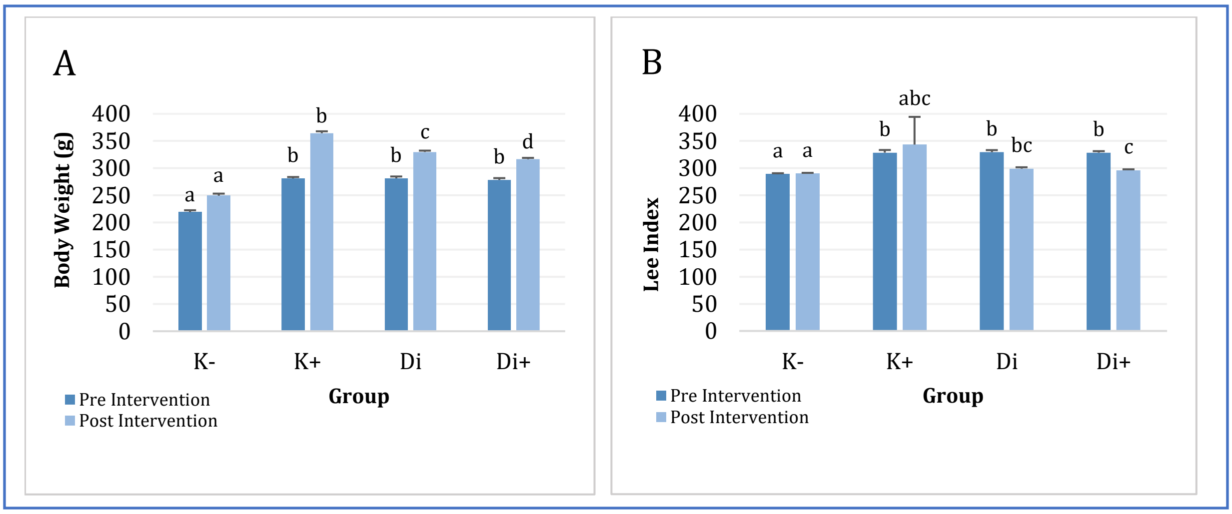
https://doi.org/10.56303/jhnresearch.v4i2.443

suggest that fortification with dragon fruit and selenium affects the nutritional composition of the product, which will be further discussed in the following section. The observed differences highlight the benefits of nutrient modification in traditional fermented products, enhancing their functional benefits.

Table 1. The nutritional content of Dadih and Selenium-Fortified Dragon Fruit Dadih

Content	Dadih	Selenium-Fortified Dragon Fruit Dadih
Moisture (%)	72.09	78.09
Ash (%)	0.87	0.86
Fat (%)	13.18	10.52
Protein (%)	7.40	4.68
Carbohydrates (%)	6.46	4.85
Total LAB (Cfu/g)	9.5×10^7	7.7×10^8
Antioxidants (%)	11.58	19.54
pH	4.8	4.4

Figure 2 illustrates the changes in body weight and Lee index of rats during the 28-day intervention following obesity induction. Body weight changes were observed in all rat groups throughout the intervention period (Figure 2A). The healthy control group (K-) exhibited a lower weight gain compared to the obesity control group (K+), indicating the impact of a high-fat, high-fructose diet on body weight increase. The groups receiving dadih (Di) and selenium-fortified dragon fruit dadih (Di+) exhibited a more moderate weight gain, with Di+ showing the most favorable outcome, suggesting a potential protective effect of selenium-fortified dragon fruit dadih.



Different superscript letters (a,b) indicate significant differences between groups ($p < 0.001$).

Different superscript letters (a,b,c) indicate significant differences between groups ($p < 0.001$).

Figure 2. Changes in Body Weight and Lee Index during The Intervention

Figure 2B illustrates the changes in the average Lee Index of rats throughout the intervention in each group. Before the intervention, a significant difference was observed among the groups, with the K-group showing the lowest Lee Index (289.2), while the groups receiving the high-fat, high-fructose diet (HFFD) exhibited a Lee Index greater than 300, indicating the onset of obesity in these rats.

Following the intervention, significant differences were observed between groups in both body weight and Lee Index. The K+ group exhibited the most significant increase in body weight, gaining an average of +83.20 g (from 281.20 ± 2.58 g to 364.40 ± 3.50 g), which reflects the obesogenic effect of the HFFD. The K-group showed a smaller weight gain of +30.20 g (from 219.20 ± 3.42 g to 249.40 ± 3.84 g). Among the intervention groups, the Di group gained +48.40 g (from 281.40 ± 3.36 g to 329.80 ± 2.58 g),

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while the Di+ group gained +38.20 g (from 278.40 ± 3.36 g to 316.60 ± 2.40 g). Correspondingly, the Lee Index increased significantly in the K+ group to 343.4, confirming the obesogenic impact of the diet. In contrast, the intervention groups showed significant reductions in the Lee Index after dadih administration, with the most significant decrease observed in the Di+ group (296.1) compared to the Di group (299.0). This outcome may be attributed to the synergistic effects of probiotics in dadih, antioxidants from dragon fruit, and the essential micronutrient selenium, which collectively contribute to improved metabolic regulation, reduced oxidative stress, and inflammation

Table 2. Weight Changes during The Intervention

Group	Initial Body Weight (Mean ± SD)	Final Body Weight (Mean ± SD)	*p*	Weight Change (g)	Δ Lee Index
K-	219.20 ± 3.42[a]	249.40 ± 3.84[a]	0.000	+30.20	+0.75
K+	281.20 ± 2.58[b]	364.40 ± 3.50[b]	0.000	+83.20	+14.78
Di	281.40 ± 3.36[b]	329.80 ± 2.58[c]	0.000	+48.40	-30.56
Di+	278.40 ± 3.36[b]	316.60 ± 2.40[d]	0.000	+38.20	-32.47
p^1	0.000	0.000			

p = Uji Paired T-Test
p^1 = Uji One-Way ANOVA
[a,b,c] = Significant differences between groups (p < 0.05) based on Bonferroni test

DISCUSSION

The addition of red dragon fruit and selenium fortification to dadih significantly increased the antioxidant activity and viability of lactic acid bacteria (LAB). The observed increase in LAB population was mainly due to the prebiotic components naturally present in red dragon fruit, such as soluble dietary fiber, oligosaccharides, and polyphenols. These compounds serve as fermentable substrates that stimulate the proliferation and metabolic functions of LAB (21). This finding is aligned with previous studies, which have shown that the inclusion of red dragon fruit in fermented dairy products such as kefir and yogurt increases the total LAB count, likely due to its prebiotic properties (21,27). In addition, antioxidant-rich phytochemicals, especially polyphenols, present in red dragon fruit contribute to the observed increased antioxidant capacity in fortified dadih (28).

In parallel, selenium fortification further supports LAB viability by enhancing its resistance to oxidative stress during fermentation and storage. Several LAB strains have demonstrated the capacity to convert inorganic selenium (e.g., sodium selenite) into bioabsorbable organic or nano-selenium forms, which have potent antioxidant activity (29). In addition, selenium-enriched LAB strains exhibit increased intracellular glutathione levels and enhanced glutathione reductase activity, which facilitates improved selenite reduction and adaptation to oxidative stress (30).

Increased LAB abundance in enriched products may offer systemic functional benefits beyond the food matrix. Increased intestinal colonization by lactic acid bacteria (LAB) plays a crucial role in modulating host metabolism, particularly through the fermentation of nondigestible substrates into short-chain fatty acids (SCFAs), including butyrate, propionate, and acetate. These SCFAs function not only as energy substrates but also as signaling molecules that regulate lipid metabolism, enhance insulin sensitivity, and modulate appetite via the brain-gut axis (33–36).

Furthermore, increased antioxidant capacity supported by selenium and phytochemicals may help reduce systemic oxidative stress and chronic low-grade inflammation, both of which are major contributors to the pathophysiology of obesity (22,36). Selenium-enriched LAB may further enhance endogenous antioxidant defenses, reduce oxidative damage to metabolic tissues, and support improved metabolic homeostasis (22,37).

In addition to improving gut-related functional outcomes, the physicochemical properties of fortified dadih also reflect better fermentation dynamics and product quality. The lower pH observed in selenium-enriched dragon fruit dadih (4.4) compared to unenriched dadih (4.8) indicates more active bacterial fermentation, which enhances the production of microbial metabolites such as organic acids and

https://doi.org/10.56303/jhnresearch.v4i2.443

bioactive peptides (38). The fermentation process causes a decrease in pH, which inhibits pathogenic microorganisms and contributes to product stability (39). Fortification with selenium nanoparticles has also been shown to stimulate the growth of LAB and enhance their metabolic activity without negatively affecting product quality or sensory attributes (40). Additionally, the higher moisture content and lower fat content in the fortified dadih reflect a product matrix that may support improved energy balance and body mass regulation.

Changes in body weight and Lee Index over the 28-day intervention period reflect the physiological responses of rats to a high-fat, high-fructose diet and the potential modulatory effects of fermented product intervention. As shown in Figure 2A, the healthy control group (K−) exhibited the least weight gain. In contrast, the obesity control group (K+) showed a significant increase in body weight, confirming the obesogenic effects of the high-fat, high-fructose diet. This finding is consistent with previous studies reporting that HFFD induces weight gain through excess energy intake and altered lipid metabolism (41). Notably, obese rats receiving interventions with plain dadih (Di) and selenium-fortified dragon fruit dadih (Di+) experienced more controlled weight gain. Specifically, the Di+ group showed the lowest weight gain profile among all obese groups.

Several biological mechanisms may underlie these findings, particularly the role of probiotics in modulating gut microbiota, improving metabolic function, and reducing systemic inflammation(42). Probiotics in dadih, primarily species from the genera Lactobacillus and Bifidobacterium, help maintain a balanced gut microbiota by promoting beneficial bacteria and suppressing pathogenic strains. A healthy microbiota produces metabolites such as short-chain fatty acids (SCFAs), notably butyrate, acetate, and propionate, which exert anti-inflammatory effects and enhance gut barrier integrity. Improved mucosal integrity reduces the translocation of endotoxins such as lipopolysaccharide (LPS) into the systemic circulation, thereby attenuating TLR4/NF-κB-mediated inflammatory pathways and decreasing the production of pro-inflammatory cytokines such as TNF-α and IL-6, which contribute to alleviating endoplasmic reticulum (ER) stress in hepatocytes (6,34,43). By mitigating ER stress, probiotics can downregulate the expression of lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), ultimately reducing lipid synthesis (44). This result aligns with the findings of Kobayashi et al., who reported that fermented milk products containing LAB can regulate lipid metabolism and reduce fat accumulation through gut microbiota modulation and gene expression changes associated with lipogenesis (45,46).

Further evaluation of the Lee Index (Figure 2B), a sensitive anthropometric parameter for assessing obesity in animal models, reinforced these findings. Before the intervention, all obese groups exhibited Lee Index values greater than 300, indicating successful induction of obesity. Post-intervention analysis revealed significant differences among groups ($p = 0.010$), with the K+ group demonstrating a further increase in the Lee Index to 343.4, confirming progression of obesity in the absence of treatment. In contrast, both Di and Di+ groups showed a decrease in the Lee Index, with the Di+ group displaying a more substantial reduction, indicating the effectiveness of the intervention in improving adiposity status.

These differences correspond to the nutritional composition of the respective products, particularly the LAB content and antioxidant capacity. Selenium-fortified dragon fruit dadih contained a higher LAB count and antioxidant activity compared to the unfortified version, likely exerting more potent effects on gut microbiota balance and systemic inflammation, thereby influencing body fat accumulation (47,48).

Furthermore, selenium is known for its anti-inflammatory properties and its regulatory effects on gut microbiota, potentially preventing or reducing fat deposition and thus influencing body weight (49). Selenium is an essential micronutrient that functions as a cofactor for the antioxidant enzyme glutathione peroxidase (GPx), which neutralizes lipid peroxides and alleviates oxidative stress. By reducing reactive oxygen species (ROS) and preventing lipid membrane damage, selenium contributes to hepatocyte stability and inhibits ER stress signaling that promotes lipogenesis. The combination of dragon fruit and selenium demonstrates a synergistic potential in reducing lipogenesis and improving metabolic conditions (50,51). Selenium has also been shown to inhibit adipogenesis via the peroxisome proliferator-activated receptor gamma (PPARγ) pathway, mediated by selenoenzyme antioxidant activity (52). Selenium supplementation

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has been shown to be effective in alleviating lipid-related metabolic disorders, such as hypertriglyceridemia (23).

Taken together, these findings support the use of selenium-fortified dragon fruit dadih as a functional fermented food to reduce the risk or manage the condition of obesity through a multifactorial approach involving antioxidants, probiotics, and essential micronutrients. The combination of red dragon fruit and selenium in the dadih formulation not only enhances nutritional quality but also strengthens the product's functional characteristics by improving probiotic activity and antioxidant capacity. Similar findings have been reported in studies. The addition of dragon fruit extract or juice to yogurt and kefir has been shown to increase antioxidant content, affect color parameters (L*, a*, b*), and influence physicochemical characteristics such as viscosity, pH, and total dissolved solids (21,27). Similarly, selenium-enriched yogurt has been shown to increase antioxidant enzyme activity, reduce inflammation, and modulate gut microbiota composition, resulting in decreased weight gain and improved lipid profiles (53). These results support the development of dadih as a fermentation-based functional food with broader metabolic benefits.

However, this study has several limitations that need to be considered. First, formal power calculations were not performed. Sample sizes were determined using the Federer formula, a widely used method in preliminary animal experiments to meet minimum statistical requirements. Although appropriate for pilot studies, this approach may limit statistical power and the generalizability of findings. Second, nutrient composition analyses of curd were performed in duplicate due to limited sample availability and laboratory resources. Consequently, statistical comparisons between groups for macronutrient parameters were not performed, and only descriptive values are reported. Future studies should incorporate formal power analyses, larger sample sizes, and more extensive replications to enable a more rigorous statistical evaluation and increase the reliability of observed effects.

CONCLUSION

This study demonstrated that selenium-fortified red dragon fruit dadih exhibited enhanced functional and nutritional properties compared to unenriched dadih, as reflected by higher lactic acid bacteria (LAB) count (7.7×10^8 CFU/g), more potent antioxidant activity (19.54%), and lower pH (4.4). These characteristics contributed to improved physiological responses in obese mice, including lower body weight gain (+38.2 g) and a 9.9% decrease in Lee Index (from 328.6 to 296.1) after 28 days of intervention. The synergistic combination of probiotics, red dragon fruit bioactives, and selenium effectively modulated obesity-related parameters, supporting the functional potential of this formulation in improving metabolic health.

These findings highlight the efficacy of selenium-enriched red dragon fruit as a culturally relevant functional food for obesity management. The effects are mediated through modulation of gut microbiota, enhancement of antioxidant defense, and attenuation of systemic inflammation and lipogenesis. Future research should explore the long-term effects and molecular mechanisms in clinical settings, as well as comparative studies with other fermented functional products such as kefir or yogurt enriched with similar bioactive components.

FUNDING

This research was funded by the Indonesia Endowment Fund for Education (LPDP), the Ministry of Finance, Indonesia. The scholarship was awarded to a US student.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Lembaga Pengelola Dana Pendidikan (LPDP) for funding support, as well as to Diponegoro University for providing research facilities.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

https://doi.org/10.56303/jhnresearch.v4i2.443

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