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The effect of soybean tempeh and gembus tempeh on obesity parameters, blood glucose, and short-chain fatty acids

Efek tempe kedelai dan tempe gembus terhadap parameter obesitas, glukosa darah, dan short-chain fatty acids

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Abstract

Obesity contributes to hyperglycemia and metabolic dysfunction globally. Soybean and gembus tempeh, traditional Indonesian fermented foods, contain distinct dietary fiber compositions that may differentially influence metabolic outcomes through short-chain fatty acid (SCFA) production. However, comparative evidence regarding their metabolic effects is limited. This study aimed to compare the effects of soybean and gembus tempeh on obesity parameters, fasting blood glucose, and SCFA profiles in a rat model of hyperglycemic obesity. Methods: A randomized controlled pre–post trial was conducted at the Food and Nutrition Study Center, Universitas Gadjah Mada (April–June 2019). Forty-two rats were assigned to seven groups: normal, hyperglycemic-obese + AIN-93, AIN-93 + gembus tempeh, AIN-93 + soybean tempeh, high-fat high-fructose diet (HFFD), HFFD + gembus tempeh, and HFFD + soybean tempeh. The obesity index, fasting blood glucose, and SCFA concentrations were measured. Both types of tempeh significantly reduced the obesity index and fasting glucose levels in rats fed either the AIN-93 or HFFD diet, with greater reductions observed in the AIN-93 group ($p < 0.001$). Butyrate levels were significantly higher in the tempeh-supplemented groups than in the control group ($p < 0.01$), whereas propionate levels did not differ significantly ($p = 0.079$). In conclusion, soybean tempeh and gembus tempeh improved fasting glucose levels, obesity index, and SCFA production in hyperglycemic-obese rats. Gembus tempeh demonstrated a greater effect on reducing obesity index and fasting blood glucose than soybean tempeh.

Keywords: Dietary fiber, fermented foods, hyperglycemia, obesity, short-chain fatty acids

Abstrak

Obesitas menjadi penyebab penting munculnya hiperglikemia dan gangguan metabolik di seluruh dunia. Tempe kedelai dan tempe gembus, sebagai pangan fermentasi khas Indonesia, memiliki komposisi serat yang berbeda sehingga berpotensi memberikan efek metabolik yang tidak sama melalui produksi asam lemak rantai pendek (short-chain fatty acids/SCFA). Namun, bukti yang membandingkan pengaruh keduanya masih terbatas. Penelitian bertujuan untuk menilai perbedaan efek tempe kedelai dan tempe gembus terhadap parameter obesitas, kadar glukosa darah puasa, serta profil SCFA pada model tikus obesitas dengan hiperglikemia. Metode, penelitian uji coba terkontrol acak dengan desain pre–post dilakukan di Pusat Studi Pangan dan Gizi Universitas Gadjah Mada (April–Juni 2019). Sebanyak 42 tikus dibagi ke dalam tujuh kelompok: normal, obesitas-hiperglikemik + AIN-93, AIN-93 + tempe gembus, AIN-93 + tempe kedelai, HFFD, HFFD + tempe gembus, dan HFFD + tempe kedelai. Indeks obesitas, kadar glukosa, serta konsentrasi

SCFA dianalisis. Hasil, kedua jenis tempe menurunkan indeks obesitas dan glukosa darah puasa secara signifikan pada diet AIN-93 maupun HFFD, dengan penurunan lebih besar pada AIN-93 ($p < 0.001$). Kadar butirat meningkat secara signifikan pada kelompok suplementasi tempe dibanding kontrol ($p < 0.01$), sementara kadar propionat tidak menunjukkan perbedaan bermakna ($p = 0.079$). Kesimpulan, **t**empe kedelai dan tempe gembus terbukti meningkatkan profil metabolik melalui penurunan glukosa darah puasa, indeks obesitas, dan peningkatan SCFA. Tempe gembus menunjukkan efek yang lebih kuat dibanding tempe kedelai dalam menurunkan obesitas dan glukosa darah.

Kata Kunci: Fermentasi pangan, hiperglikemia, indeks obesitas, serat pangan

Introduction

Obesity remains a global health challenge in both developed and developing countries (Ijazah 2019). It is a multifactorial disease involving complex interactions between genetic and environmental factors (Młynarska et al., 2025). Lifestyle, behavior, sociocultural influences, physiological conditions, and metabolic disturbances contribute to the rising prevalence of obesity (Saraswati et al., 2021). Obesity has a substantial impact on morbidity and mortality, as it increases the risk of degenerative diseases such as cardiovascular disease, cancer, respiratory disorders, and type 2 diabetes mellitus (T2DM) (Ullah & Tamanna, 2025).

The World Obesity Federation estimates that by 2030, obesity will affect approximately 50% of the global adult population (World Obesity Federation 2025). In Indonesia, obesity has become a national concern for all age groups. Among adults aged >18 years, the prevalence increased from 14.8% in 2013 to 21.8% in 2018 (Indonesian Ministry of Health 2018). Based on the 2023 SKI report, the prevalence decreased to 15.7%; however, it remains high, coinciding with increases in sedentary lifestyles and high-calorie diets (Indonesian Ministry of Health, 2023; Al Rahmad, 2021). These trends indicate that obesity is a serious public health issue that requires effective intervention strategies. One such approach is lifestyle modification, particularly improvements in dietary patterns (Ministry of Health, Republic of Indonesia, 2018).

Obesity results from an imbalance between energy intake and expenditure, with weight gain occurring when the energy intake exceeds the energy expenditure (Bechard, 2024). High-fat and high-fructose diets contribute to excessive energy intake, promote obesity, and induce insulin resistance through mechanisms involving chronic inflammation and oxidative stress (Sah et al., 2016). Obesity increases the risk of T2DM, cancer, cardiovascular disease, and stroke (Parums, 2025).

A high-fiber diet is widely recommended for preventing obesity. Soluble dietary fiber forms a viscous gel in the gastrointestinal tract, prolonging satiety and promoting reduced calorie intake (Sunarti, 2017). Dietary fiber has been extensively studied for its role in glycemic control in individuals with T2DM (O'Kennedy et al., 2021). Its antidiabetic effects are attributed to delayed digestion and gastric emptying, as well as the production of short-chain fatty acids (SCFAs) through fermentation by the gut microbiota. SCFAs stimulate the secretion of glucagon-like peptide-1 (GLP-1), gastric inhibitory polypeptide, and peptide YY, thereby enhancing insulin sensitivity (Sunarti, 2017).

Soybean and gembus tempeh are traditional fermented Indonesian foods. Soybean tempeh is made from whole soybeans, whereas gembus tempeh is produced from tofu byproducts (Sunarti et al. 2019). Both are fermented using Rhizopus spp. Differences in raw materials result in distinct nutrient profiles and bioactive component contents. Soybean tempeh contains higher levels of peptide compounds, whereas gembus tempeh has a higher dietary fiber content. Both types of tempeh have been explored as functional foods for preventing or managing degenerative diseases, including T2DM. Soybean tempeh has been studied for its antidiabetic potential through antioxidant mechanisms associated with isoflavones (Astawan et al., 2017); however, other bioactive components may also contribute to glycemic regulation. Tempeh, with its high dietary fiber content, is believed to support obesity and hyperglycemia management through SCFA-mediated pathways.

This study aimed to evaluate the effects of soybean and gembus tempeh on obesity, blood glucose levels, and SCFA composition in rats fed a high-fat, high-fructose diet. Comparative data on the metabolic effects of these two tempeh types remain limited; therefore, the findings of this study provide important empirical evidence of the functional benefits of soybean and gembus tempeh.

Methods

This study employed a randomized controlled pre–post design with two groups. This study was conducted at the Center for Food and Nutrition Studies, Gadjah Mada University, from April to June 2019.

Sprague–Dawley rats were obtained from the Animal Laboratory of the Center for Food and Nutrition Studies, Gadjah Mada University. The sample size was based on the WHO criteria, which require a minimum of five rats per treatment group. To anticipate potential dropouts, one additional rat was included in each group of the study. Random sampling was performed. The inclusion criteria were as follows: 7-week-old rats weighing 150–200 g, healthy and active, with post-induction fasting blood glucose levels >126 mg/dL and a Lee index >300 (Bast & Serra, 2020). The exclusion criteria included rats that were already obese prior to induction or exhibited signs of stress.

The research participants were assigned to seven groups:

K1 (positive control): healthy rats fed a standard AIN-93 diet;
K2 : obese and hyperglycemic rats fed AIN-93;
K3 : obese and hyperglycemic rats fed AIN-93 with 30% gembus tempeh substitution;
K4 : obese and hyperglycemic rats fed AIN-93 with 30% soybean tempeh substitution;
K5 (negative control): obese and hyperglycemic rats fed a high-fat, high-fructose diet (HFFD);
K6 : obese and hyperglycemic rats fed HFFD with 30% soybean tempeh substitution;
K7 : obese and hyperglycemic rats fed HFFD with 30% gembus tempeh substitution.

The intervention was administered for 28 days. Body weight, body length, and blood glucose levels were measured before and after the treatment. At the end of the intervention, digesta from the cecum was collected for short-chain fatty acid (SCFA) analysis (Primec et al., 2017).

Soybean and gembus tempeh flours were prepared using tempeh obtained from a home industry in Sukoharjo. Tempeh was stabilized at 100°C for 5 min, dried in an oven at 55°C for 8–9 h, and ground using a 60-mesh sieve (Astuti & Sulistiani, 2025). Seven-week-old rats (150–200 g) were used in this study. Obesity and hyperglycemia were induced using HFFD containing 31% fat and 25% fructose. Induction was performed for seven weeks (Lozano et al., 2016). The normal control group received a standard AIN-93 diet (Wahyuni, 2017).

Data collection of the obesity index was determined using the Lee index, calculated as (body weight [g] / nose–anus length [cm]) × 1000, with values greater than 300 classified as obese (Bast & Serra, 2020). Fasting blood glucose was assessed using serum collected via orbital vein puncture following a 10-hour fasting period. Blood glucose concentrations were analyzed using the glucose oxidase–phenol aminoantipyrine (GOD-PAP) method, as described by Madi et al. (2018). Short-chain fatty acids (SCFAs) were quantified from cecal digesta samples using gas chromatography, following the analytical procedures outlined (Gao et al., 2023).

Data were inspected for normality and homogeneity of variance using the Shapiro–Wilk and Levene's tests, respectively. Parametric data that met these assumptions were analyzed using one-way analysis of variance (ANOVA) to evaluate the differences among the treatment groups. A significant main effect was observed. For variables that violated normality or variance assumptions, equivalent non-parametric tests were used. All statistical analyses were conducted with a significance level of $p < 0.05$, and the results are presented as mean ± standard deviation (SD).

All experimental procedures involving animals were conducted in accordance with the internationally accepted ethical standards for laboratory animal care and use. Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Veterinary Medicine, Gadjah Mada University (approval no. 0024/EC-FKH/Eks/2019). This study adhered to the principles of the 3Rs (Replacement, Reduction, and Refinement) and followed institutional guidelines for minimizing animal discomfort, stress, and pain. All handling, fasting procedures, blood collection, and euthanasia techniques were performed by trained personnel under veterinary supervision to ensure the welfare of the animals throughout the study.

Result and Discussion

Characteristics of Test Animals

As shown in Table 1, groups K2, K3, K4, K5, K6, and K7 were classified as obese and hyperglycemic, as indicated by a mean Lee index greater than 300 and fasting blood glucose levels exceeding 126 mg/dL. These findings confirmed the success of the induction procedure.

Table 1. Description of body weight, body length, obesity index, and fasting blood glucose levels before treatment

Group	n	BW (grams)	BL (cm)	Obesity Index	Fasting Blood Glucose (mg/dl)
K1	6	211.33 ± 4.589	20.71 ± 0.232	287.5017 ± 2.209	90.12 ± 42.260
K2	6	228.83 ± 3.970	19.47 ± 0.371	314.1517 ± 7.474	179.16 ± 2.903
K3	6	222.33 ± 3.880	19.54 ± 0.313	309.2233 ± 5.363	159.22 ± 42.89
K4	6	223.33 ± 5.316	19.68 ± 0.361	308.2700 ± 4.503	174.93 ± 1.628
K5	6	222.16 ± 5.269	19.36 ± 0.260	314.2000 ± 5.666	176.91 ± 2.852
K6	6	221.83 ± 4.355	19.75 ± 0.320	306.5133 ± 3.237	174.86 ± 2.852
K7	6	223.5 ± 4.037	19.72 ± 0.316	307.6950 ± 5.343	174.86 ± 3.193

K1: healthy control; K2: obese and hyperglycemic rats fed AIN-93; K3: obese and hyperglycemic rats fed AIN-93 + gembus tempeh; K4: obese and hyperglycemic rats fed AIN-93 + soybean tempeh; K5: obese and hyperglycemic rats fed HFFD; K6: obese and hyperglycemic rats fed HFFD + gembus tempeh; and K7: obese and hyperglycemic rats fed HFFD + soybean tempeh.

Effect of Gembus Tempeh and Soybean Tempeh on Obesity Status, Fasting Blood Glucose, and SCFA Profiles

Obesity Status

Table 2 shows a significant difference in the obesity index among the treatment groups after the intervention ($p < 0.001$). Rats receiving AIN-93 supplemented with gembus tempeh or soybean tempeh (groups K3 and K4) exhibited a greater reduction in the obesity index than those receiving HFFD supplemented with tempeh (groups K6 and K7). These results indicate that both gembus and soybean tempeh contributed to a reduction in the obesity index, with the strongest effect observed when administered alongside the AIN-93 diet.

The smaller reduction in the HFFD groups may be attributable to the higher caloric density of HFFD than that of AIN-93, thereby diminishing the impact of tempeh substitution on obesity status.

Table 2. Changes in obesity status after administration of gembus tempeh and soybean tempeh

Group	n	Pre-obesity index	Obesity Index post intervention	p-value	Δobesity index
K1	6	287.5017 ± 2.209	290.335 ± 4.349^{a}	0.440	2.833 ± 2.593^{e}
K2	6	314.1517 ± 7.474	323.360 ± 5.209^{b}	0.028	9.28 ± 5.483^{f}
K3	6	309.2233 ± 5.363	290,770 ± 4,211^{a}	0.000	-18,450 ± 1,315^{a}
K4	6	308,270 ± 4,503	293,110 ± 3,975^{a}	0.000	-15.120 ± 1.011^{b}
K5	6	314,200 ± 5,666	311.171 ± 5.016^{c}	0.001	-2.628 ± 1.032^{c}
K6	6	306.5133 ± 3.237	293.46 ± 3.227^{a}	0.000	-13.053 ± 0.574^{b}
K7	6	307.6950 ± 5.343	300.791 ± 5.413^{d}	0.000	-6.903 ± 0.226^{d}
Anova			F-value: 18.96 p-value < 0.001		F-value: 103.73 p-value < 0.001

Different superscript letters indicate significant differences between the groups.

The study confirmed significant differences in the obesity index across the treatment groups ($p < 0.001$). Post hoc analysis showed that the groups receiving tempeh supplementation (K2, K3, K4, K6, and K7) differed significantly from the negative control group. These findings suggest that soybean and gembus tempeh have potential as functional foods for obesity management. Greater reductions were observed when tempeh was combined with the AIN-93 diet, reflecting the influence of energy intakes. HFFD is energy dense; thus, substitution with tempeh resulted in higher caloric levels than AIN-93.

Soybean tempeh contains bioactive compounds such as isoflavones, proteins, peptides, and dietary fiber (Astawan et al., 2018), whereas gembus tempeh contains high levels of dietary fiber and peptides. The reported dietary fiber content in soybean tempeh is 57.93% (14.14% soluble; 43.78% insoluble), whereas gembus tempeh contains 79.93% fiber (28.94% soluble; 50.07% insoluble) (Sunarti et al., 2019). Dietary fiber contributes to satiety, delays gastric emptying, and reduces calorie intake (Li & Komarek, 2017).

Fasting Blood Glucose Levels

Based on Table 3, the greatest reduction in fasting blood levels occurred in the AIN-93 groups substituted with gembus tempeh and soybean tempeh (K3 and K4). The HFFD groups (K6 and K7) also experienced reductions, although to a lesser extent than the LFD groups. In both dietary combinations, gembus tempeh resulted in a greater decrease in fasting glucose levels than soybean tempeh. This is likely related to the higher dietary fiber content in gembus tempeh, which may enhance glucose-lowering effects through SCFA production and improved insulin sensitivity.

Additionally, total energy intake may influence glucose reduction, as gembus tempeh has a lower energy content than soybean tempeh, resulting in a lower caloric intake in the gembus-substituted feed.

Table 3. Changes in fasting blood glucose levels after administration of soybean tempeh and gembus tempeh

Group	n	Blood glucose before intervention (mg/dl)	Blood glucose after intervention (mg/dl)	p-value	Δfasting blood glucose level
K1	6	72.74 ± 4.040	75.0 ± 3.772^{a}	0.002	15.563 ± 0.508^{a}
K2	6	179.16 ± 2.900	181.281 ± 2.58^{f}	0.005	15.731 ± 1.247^{a}
K3	6	176,640 ± 3.196	104,478 ± 3.138^{b}	0.000	-58,730 ± 2,418^{b}
K4	6	174,931 ± 1,628	118.53 ± 2.107^{c}	0.000	-42.595 ± 1.787^{c}
K5	6	176.91 ± 2.812	180.133 ± 2.443^{e}	0.000	22.755 ± 0.778^{d}
K6	6	174.59 ± 2.852	119.465 ± 2.610^{c}	0.000	-41.755 ± 1.524^{c}
K7	6	174,863 ± 3,193	130,471 ± 2,391^{d}	0.000	-31.146 ± 1.554^{e}
Anova			F-value: 1281.89 p-value < 0.001		F-value: 3207.827 p-value < 0.001

Different superscript letters indicate significant differences between groups.

Both types of tempeh significantly lowered fasting blood glucose levels compared to the negative control. Greater reductions were observed in the AIN-93 groups (K3 and K4) than in the HFFD group (K6 and K7). This hypoglycemic effect is likely related to improvements in the obesity status. Obesity increases the levels of pro-inflammatory cytokines, such as TNF-α, which promote insulin resistance (Azeez, (2023). A reduction in obesity may improve the insulin sensitivity.

Isoflavones in soybean tempeh have been reported to increase insulin sensitivity (Astawan et al., 2017). These findings align with those of previous studies showing the glucose-lowering effects of okara in individuals with T2DM (Nguyen et al., 2019; Ismaiel et al., 2017).

Digesta Weight, Moisture Content, and pH

Table 4 demonstrates that digesta weight in groups K3 and K4 was higher than that in the control group, and a similar pattern was observed for K6 and K7. This suggests that the dietary fiber in both types of tempeh contributes to an increase in fecal volume. Conversely, the HFFD group showed the lowest digesta weight, likely because high-fat diets reduce fluid retention and disrupt the normal gut microbiota, thereby decreasing fecal mass.

Table 4. Digesta weight, moisture content, and ph in rats induced with HFFD by treatment group

Group	Digesta weight (grams)		Moisture Content (%)		pH	
	Mean ± SD		Mean ± SD		Mean ± SD	
K1	1.35^{a}	± 0.503	63.48^{d}	± 1.942	6.90^{a}	± 0.032
K2	1.70^{ab}	± 0.413	32.85^{a}	± 5.418	6.46^{b}	± 0.040
K3	2.69^{c}	± 1.011	65.86^{d}	± 3.378	6.72^{c}	± 0.683
K4	2.30^{bc}	± 0.380	56.21^{c}	± 2.999	6.70^{c}	± 0.125
K5	1.31^{a}	± 0.242	31.55^{a}	± 0.999	6.52^{d}	± 0.063
K6	2.95^{6}	± 0.106	53.19^{b}	± 1.781	6.74^{c}	± 0.542
K7	2.51^{bc}	± 0.894	50.51^{b}	±2.802	6.50^{d}	± 0.498
Anova	F-value: 5.02 p <0.001					
Kruskal-Wallis			F-value: 119.2 p-value < 0.001		F-value: 33.98 p-value < 0.001	

Different superscript letters indicate significant differences between groups.

The highest fecal pH was observed in the normal control group (K1), which received a fiber-rich diet. Groups K3 and K4 also showed relatively high pH values that were significantly different from those of the negative control. These findings support the theory that dietary fiber influences stool characteristics and fecal pH.

Significant differences were observed in digesta weight, moisture content, and pH across the treatment groups. Tempeh supplementation increased fecal volume, consistent with the known function of dietary fiber to bind water in the colon (Astawan et al., 2017; He et al., 2018). The highest moisture content was observed in the AIN + gembus tempeh group (K3), likely due to its high-fiber content.

Effect of Soybean Tempeh and Gembus Tempeh on SCFA Levels

The results in Table 5 show that acetate levels were significantly higher (p = 0.037) in rats fed AIN-93 with tempeh substitution (K3 and K4) than in the negative control (K5). Acetate levels in the HFFD groups receiving tempeh (K6 and K7) were lower than those in K3 and K4 but remained higher than those in the negative control, indicating that tempeh increases acetate production despite the attenuating effect of HFFD.

Propionate levels were higher in the tempeh substitution groups than in the control group, although the difference was not statistically significant (p = 0.079). This may be due to propionate production being influenced not only by fiber but also by the abundance of *Bacteroides* spp., which were not evaluated in this study.

Table 5. SCFA levels by treatment group

Group	Acetate (%M)	Propionate (%M)	Butyrate (%M)
	Mean ± SD	Mean ± SD	Mean ± SD
K1	96.24 ± 44.084^{b}	39.84 ± 18.07^{a}	12.87 ± 5.589^{a}
K2	65.91 ± 22.050^{ab}	46.59 ± 12.336^{ab}	16.30 ± 3.923^{ab}
K3	97.79 ± 9.737^{b}	61.85 ± 5.118^{b}	21.14 ± 1.450^{bc}
K4	95.48 ± 24.490^{b}	61.56 ± 22.079^{b}	23.13 ± 8.631^{bc}
K5	57.39 ± 16.311^{a}	48.88 ± 11.284^{ab}	18.24 ± 3.535^{abc}
K6	76.60 ± 19.473^{ab}	60.20 ± 15.869^{b}	22.41 ± 7.389^{bc}
K7	71.21 ± 25.887^{ab}	60.90 ± 15.980^{b}	24.96 ± 5.922^{c}
F value	2.552	2.091	3.305
p-value	0.037	0.079	0.010

Different superscript letters indicate significant differences between groups.

Butyrate levels were significantly higher in K3 and K4 than in K5 (p = 0.010). As butyrate is primarily produced from undigested carbohydrates, the dietary fiber in tempeh likely contributes to increased butyrate production. Overall, both soybean tempeh and gembus tempeh increased SCFA levels, although the effects were weaker in the HFFD combinations.

Both soybean and gembus tempeh increased acetate and butyrate levels. Increased SCFA levels, particularly butyrate, were associated with metabolic improvements, such as a lower obesity index and lower fasting glucose levels. Soluble fiber supports SCFA production by serving as a substrate for fermentation, whereas insoluble fiber supports gut motility and promotes microbial diversity (Li & Komarek, 2017). Tempeh also contains lactic acid bacteria that may function as probiotics (Aritonang et al., 2017; Suliantari et al., 2015).

These findings support the potential of soybean and gembus tempeh as functional foods for improving metabolic health. These results are consistent with evidence showing that okara increases SCFA production (Cela et al., 2016) and that soybean residue–based snacks improve SCFA profiles (Pei et al., 2023; Louis & Flint, 2017). Further research should isolate the fiber components of tempeh and examine their interactions with the gut microbiota to better understand the mechanisms underlying SCFA modulation and metabolic outcomes.

Conclusion

The administration of soybean and gembus tempeh at a 30% substitution level in the diet contributed to reductions in obesity and fasting blood glucose levels in experimental rats, with gembus tempeh demonstrating a more significant effect. Both types of tempeh increased digesta weight, moisture content, and pH, and elevated acetic acid and butyrate concentrations. These effects were more pronounced when tempeh was substituted into the AIN-93 diet rather than the high-fat high-fructose diet (HFFD).

These findings suggest that incorporating soybean tempeh or gembus tempeh into a balanced diet may help with weight and glycemic control. Future applications should consider integrating tempeh as a functional food component in nutritional programs aimed at improving metabolic health outcomes.

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