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Effect of Giving Sorghum Tempeh on High Sensitive C-Reactive Protein (Hs-CRP) Levels and Short Chain Fatty Acid (SCFA) Concentrations in The Cecum of Rats Induced by an Atherogenic Diet

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Abstract

This study aimed to evaluate the effect of sorghum tempeh (*Sorghum bicolor* (L.) Moench cv. Numbu) intervention on hs-CRP (high-sensitive C-Reactive Protein) levels and SCFA (Short-Chain Fatty Acid) concentrations in the cecum of rats given a diet high in atherogens. There were five groups of male Wistar rats: three intervention groups, a negative control, and a normal control receiving sorghum-based diets containing sorghum flour (P1), sorghum tempeh 5.09 g/mL (P2), and sorghum tempeh 7.63 g/mL (P3). The results showed that hs-CRP levels in the P3 group decreased significantly from 17.06 ± 0.58 mg/L to 4.11 ± 0.34 mg/L, representing an approximate 75.9% reduction compared to baseline values ($p<0.05$). Among SCFAs, the P3 group had the highest acetate concentration (7.18 ± 0.25 μmol/g), while the highest butyrate concentration was observed in P2 (2.44 ± 0.19 μmol/g). Propionate concentrations were not significantly different among groups. These findings suggest that sorghum tempeh, particularly at moderate to higher doses, can reduce inflammatory responses and modulate SCFA production in rats under an atherogenic diet. However, conclusions regarding atherosclerosis improvement remain preliminary since histopathological or endothelial biomarker analyses were not performed.

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Introduction

Atherosclerosis is the first stage of cardiovascular (CVD)'s development which is cause of death and decreased productivity.[1] In 2015, 422 million instances of CVD were estimated, accounting for one-third of all deaths.[2] Heart attacks and strokes account for the majority of the estimated 17 million deaths from CVD each year.[3]

Atherosclerosis is a chronic inflammatory disease that involves complex interactions among lipid metabolism, oxidative stress, and immune regulation. Diet-induced metabolic imbalance may disrupt gut microbiota composition, leading to altered short-chain fatty acid (SCFA) production and increased hs-CRP (high-sensitivity C-Reactive Protein), a sign of systemic inflammation.[4] Inflammatory responses trigger expression of pro-inflammatory cytokines and CRP.[5] hs-CRP is used because it is more sensitive than conventional CRP in detecting inflammation of low quality linked to atherosclerosis. The gut microbiota's function is also linked to the development of atherosclerotic plaque through a reduction in the quantity of SCFA (Short Chain Fatty Acid) production and an increase in pro-inflammatory bacteria that change circulating cytokine levels and stimulate neutrophil activation.[6]

Sorghum (*Sorghum bicolor* (L.) Moench cv. Numbu) contains diverse phenolic compounds and kafirin proteins that contribute to its antioxidant and anti-inflammatory potential. However, these bioactive contents vary across varieties, growing conditions, and processing methods. While previous reports suggested that kafirin may inhibit CETP (Cholesteryl Ester Transfer Protein), the evidence linking this mechanism to hs-CRP reduction remains inconclusive, particularly in the context of dietary intervention.

Similarly, studies reporting shifts in gut microbiota composition, such as increased *Bacteroidetes* and decreased Firmicutes, were conducted using extruded sorghum in obese rat models, and the findings may not directly apply to fermented sorghum (tempeh) under an atherogenic diet. Hence, the present study addresses these uncertainties by exploring how fermentation-based sorghum products influence key biochemical markers of inflammation and metabolism. The phenolic content is diverse and higher when compared to wheat, barley, corn, rice and oats.[7] The starch content of sorghum is relatively high, ranging from 56-73% with an average of 69.5% consisting of amylose (20-30%) and amylopectin (70-80%).[8]

Although sorghum has been recognized as a functional grain, its potential can be further enhanced through fermentation. The tempeh fermentation process activates endogenous enzymes and microbial metabolism that increase phenolic bioavailability, reduce tannins and phytates, and generate SCFAs that support gut and immune homeostasis. These properties suggest that fermented sorghum may provide stronger anti-inflammatory effects compared to its unfermented form. Nevertheless, studies evaluating these effects, especially in relation to hs-CRP and SCFA production in atherogenic conditions, remain limited. Addressing this gap could provide valuable insight into the role of fermented sorghum as a functional food candidate for cardiovascular health management.

In addition, Sorghum is effective in increasing Bacteroidetes and reducing the number of Firmicutes, which will produce less lipopolysaccharide (LPS) which has an impact on reducing the activation of inflammatory pathways.[9] The utilization of sorghum in Indonesia is still limited to a source of carbohydrate food, such as rice, porridge and sorghum flour. Sorghum tempeh is an innovation product by fermentation using *Rhizopus oligosporus*. Fermentation produces a protease enzyme that has proteolytic activity and increases phenolic acids, thereby helping to increase the micronutrient bioavailability and increase protein digestibility.[10]

Fermentation of sorghum produces new bioactive compounds such as peptides and SCFAs that have anti-inflammatory and antioxidant effects, making it potentially more effective in mitigating atherosclerosis than unprocessed sorghum. According to a study, 72 hours of fermentation of sorghum tempeh produced 10.27% protein, 45.56% starch, and 0.56% fat, water (9.04%) and ash (0.47%). Tannin and phytate (anti-nutrient substances) decreased and protein digestibility increased by 18%.[11] The fermentation process with *Rhizopus oligosporus* produces β-glucosidase, β-glucuronidase and xylanase when it degrades the cell wall. This enzyme hydrolyzes the glycosidic bonds and produces phenolics. The increase in phenolic compounds has an effect on increasing antioxidant activity which inhibits the inflammatory process.[12]

The atherogenic diet-induced rat model was selected because it closely mimics metabolic and inflammatory disturbances that occur in humans consuming high-fat, high-cholesterol diets. This model leads to gut dysbiosis, reduced SCFA production, and systemic inflammation, thereby providing a relevant physiological condition to evaluate the potential of sorghum tempeh in modulating inflammatory responses. Unlike genetic models such as ApoE-/- mice, this diet-induced model allows the assessment of dietary interventions in a manner more reflective of lifestyle-related atherosclerosis.

The few studies that have been done on sorghum tempeh thus far have only examined its chemical composition and digestibility; no research on the impact of sorghum tempeh on inflammatory biomarkers and SCFA concentrations in mouse trials has been published to date. The present study aimed to determine Sorghum tempeh's (*Sorghum bicolor* (L.) Moench cv. Numbu) impact at SCFA concentrations and hs-CRP levels in rats fed an atherogenic diet. The discussion regarding gut microbiota and immune regulation in this paper serves to provide a conceptual framework for understanding the possible mechanisms, but these parameters were not directly assessed. Therefore, the main objective of this research focuses on biochemical indicators SCFA and hs-CRP as indirect reflections of inflammatory and metabolic modulation.

Materials and Methods

Materials

The main material used in this study was sorghum (*Sorghum bicolor* (L.) Moench cv. Numbu) obtained from the local agricultural supplier in Central Java, Indonesia. The specific cultivar used was *Sorghum bicolor* (L.) Moench cv. Numbu. The microbial starter used for fermentation was mold culture *Rhizopus oligosporus* obtained from the InaCC (Indonesian Culture Collection). Other ingredients used in tempeh preparation included food-grade acetic acid and banana leaves for wrapping.

For the in vivo experiment, Gadjah Mada University (UGM), Yogyakarta, Indonesia's Experimental Animal Laboratory provided male Wistar rats (Rattus norvegicus) that were 8–10 weeks old and weighed 180-200 g at birth. Every animal had free access to food and water while being kept in conventional laboratory settings, which included a 12-hour light/dark cycle, a temperature of 29 ± 1 °C, and a relative humidity of 60 ± 5%.

The chemicals used for biochemical analyses included analytical-grade reagents for proximate composition, dietary fiber, and protein digestibility assays. The hs-CRP ELISA kit (Elabscience, E-EL-R3028) and standard reagents for SCFA analysis were employed. For GC (Gas Chromatography) analysis, diethyl ether, sulfuric acid (H_2SO_4), and SCFA standards were obtained from Merck (Germany).

Methods

Animal Housing and Diet

Twenty-five male Wistar rats (8–10 weeks old, 180–200 g) were housed individually in polypropylene cages (42 × 26 × 15 cm) with corncob bedding, maintained at 23 ± 2 °C, 55 ± 10% relative humidity, and a 12 h light/12 h dark cycle. Animals were acclimatized for 7 days before the experiment. Standard feed (Comfeed PARS 551, Indonesia; containing 18% protein, 5% fat, 3% fiber, and 5% ash) and tap water were provided ad libitum. The atherogenic diet was prepared by enriching the standard feed with 1% cholesterol, 5% lard, and 0.2% cholic acid.

Intervention Administration

Sorghum tempeh and sorghum flour were given by gavage once a day for six weeks. The doses (5.09 g/mL and 7.63 g/mL suspension) were calculated based on human equivalent doses using the body surface area (BSA) conversion method (rat = human dose × 6.2). The suspension was prepared freshly each day in sterile distilled water, and the volume given was adjusted to 1 mL/100 g body weight.

Experimental Design

A randomized pre-test control group design was used in the investigation. Rats were randomly assigned to five groups (n = 5 per group) using a computer-generated randomization table following acclimation based on initial body weight in table 1.

The term “disease control” was used instead of “positive control” to avoid confusion, as this group represents the atherogenic model without treatment. All animal handling and sample analyses were performed by blinded personnel to reduce bias. Data codification was maintained until the statistical analysis phase.

Table 1: Treatment group of rats

Rat Group	Treatment
Negative control (K-)	Standard feed + drink ad libitum + no intervention given
Positive Control (K+)	Atherogenic feed, drink ad libitum, no intervention given
Treatment 1 (P1)	Atherogenic feed + drink ad libitum + sorghum flour 5.09 g/mL
Treatment 2 (P2)	Atherogenic feed + drink ad libitum + sorghum tempeh 5.09 g/mL
Treatment 3 (P3)	Atherogenic feed + drink ad libitum + sorghum tempeh 7.63 g/mL

Making Sorghum Tempeh (*Sorghum bicolor* (L.) Moench cv. Numbu)

Making sorghum tempeh using *Rizhopus oligosporus* mold. Sorghum seeds of Numbu variety were obtained from the Balitsereal Cereal Crops Research Institute, Maros, South Sulawesi; *Rhizopus oligosporus* isolates were from ATCC 22959. After being scrubbed and cleaned of filth, sorghum seeds are weighed and immersed in water on a 1:3 scale for a full day. After five minutes of boiling in water, drain and cool the seeds. The seeds are coated with a 0.1% (w/w) mold and carefully blended. For sixty hours, incubate the sorghum seeds in porous plastic at room temperature (29 ±1) °C. Fermentation is controlled by measuring temperature (28-30 °C), fermentation time (48 hours), water content (≤60%), as well as observing mycelium morphology and final fermentation pH (5.5-6.0) to ensure the success and consistency of the sorghum tempeh fermentation process. The finished tempeh is blanched to halt microbial activity for five minutes. Sorghum tempeh (*Sorghum bicolor* (L.) Moench cv. Numbu) is dried at a temperature of 50 ± 3 °C for 12 hours using a cabinet dryer. Sorghum grains were soaked for 24 h, boiled for 30 min, and inoculated with *Rhizopus oligosporus* spores (10^6 spores/g). Fermentation was carried out in a controlled incubator at 30 ± 1 °C for 48 h. The resulting tempeh had a moisture content of 58.4% and a final pH of 5.7 ± 0.1. The product was freeze-dried and ground into powder before use in the diet.

Analysis Data

SPSS version 26.0 was used to analyze the data. The Shapiro-Wilk test was used to determine normality, and Levene's test was used to confirm homogeneity of variance. Paired t-tests were utilized for normally distributed paired data (body weight, hs-CRP); in other cases, Wilcoxon signed-rank tests were employed. Between-group were performed used one-way ANOVA for parametric data (propionate and butyrate) and Kruskal–Wallis tests for non-parametric data (acetate), followed by Tukey's HSD or Dunn's post-hoc tests, respectively, to identify group differences.

Effect sizes (Cohen's d for t-tests, eta-squared [η^2] for ANOVA) and 95% confidence intervals were calculated to interpret the magnitude and precision of differences. Between-group variations in body weight were analyzed with repeated-measures ANOVA to examine time × group interactions, and results were reported alongside within-group changes.

The National Guidelines for the Care and Use of Laboratory Animals were followed and all animal procedures were authorized by the Health Research Ethics Committee, Faculty of Medicine, Diponegoro University (No. 97/EC/H/FK-UNDIP/X/2020). The design, execution, and reporting of this investigation did not involve any conflicts of interest, according to the authors.

Results

Rats Body Weight

Table 2 demonstrates that the mean body weight of the groups varied significantly throughout the acclimatization period, during the intervention, and following the intervention (p<0.05). All groups' mean body weight varied significantly before, during, and and following the intervention (p<0.05), based on the findings of the Paired T-Test. Both the control and treatment groups' body weight increased. The highest mean weight gain in the positive control group (K+) was 39.8%. Meanwhile, the lowest average increase in body weight occurred in the sorghum tempeh treatment group with a dose of 5.09 g/mL (P2) of 15.7%. Reduction or inhibition of excess weight gain is linked to a lower risk of atherosclerosis by lowering systemic inflammation and visceral fat buildup.

Table 2: Rat body weight (in g) before and after intervention

Group of rat	Before Intervention (Mean ± SD) (in g)	During Intervention (Mean ± SD) (in g)	After Intervention (Mean± SD) (in g)	∆ Weight mean (%)	p1	p2
K(-) Negative Control	199.33 ± 9.33	235.05 ± 16.08	252.33 ± 19.56	53 (26.6)	0.0283*	0.0002*
K(+) Positive control	206.17 ± 15.70	260.55 ± 32.74	288.16 ± 38.64	82 (39.8)	0.0022*	0.0002*
P1 Sorghum flour	206.08 ± 10.66	234.00 ± 13.41	248.16 ± 13.83	42.1 (20.4)	0.0012*	0.0012*
P2 Sorghum tempeh 5.09g/mL	205.25 ± 8.61	222.33 ± 14.98	237.50 ± 15.29	32.3 (15.7)	0.0032*	0.0022*
P3 Sorghum tempeh 7.63 g/mL	194.87 ± 7.48	219.00 ± 9.13	230.50 ± 8.73	35.6 (18.3)	0.0283*	0.0283*
p	0.2761	0.0351*	0.0101*			

[1]Kruskal-Wallis Test, [2]Uji Paired T-Test, [3]Uji Wilcoxon

hs-CRP

Measurements of hs-CRP were performed twice, once before and once after the intervention. The hs-CRP levels of all groups (K+, K-, P1, P2, and P3) varied significantly before and after the intervention (p value <0.01). The levels of hs-crp varied significantly between the treatment groups after the intervention (p=0.000). A p-value of 0.000 denotes extremely high statistical significance (p < 0.001), indicating that the difference between groups is not the result of chance.

Following the intervention, the sorghum tempeh group experienced the greatest decrease in hs-CRP levels (7.63 g/mL, with an average percentage reduction of 75.9%). Following the intervention, the sorghum tempeh group experienced the greatest decrease in hs-CRP levels (7.63 g/mL), with an average percentage reduction of 75.9%.

Table 3: hs-CRP levels before and after intervention

Rats group	hs-CRP Levels Mean ± SD (mg/dl)		∆ Mean	change (%)	p value
	Before	After			
K(-) Negative Control	3.28± 0.21	3.44 ± 0.19	0.16	4.88	0.0011*
K(+) Positive control	17.05 ± 0.61	17.57 ± 0.68	0.52	3.05	0.0272*
P1 Sorghum flour	17.35 ± 0.50	4.30 ± 0.41	13.05	75.21	0.0011*
P2 Sorghum tempeh 5.09g/mL	17.03 ± 0.53	7.38 ± 0.31	9.65	56.66	0.0011*
P3 Sorghum tempeh 7.63 g/mL	17.06 ± 0.58	4.11 ± 0.34	12.95	75.90	0.0011*
P		0.001[3]**			

[1]Paired T-test, [2]Uji Wilcoxon, [3]Uji Kruskal Wallis, *Significant

Short Chain Fatty Acid (SCFA)

Measurements of cecum SCFA concentrations in each group were measured after 28 days of intervention. SCFA concentrations measured included acetate, propionate and butyrate. Differences in SCFA concentrations between intervention groups are shown in table 4.

SCFA concentrations (acetic, propionate and butyric acid) after the intervention had significant differences between treatment groups with a p value = 0.001. The treatment group administered 7.63 g/mL of sorghum tempeh had higher concentrations of acetate and propionate than groups P1 and P2. However, in this study the group given 5.09 g/mL of sorghum tempeh had a higher butyrate concentration than groups P1 and P3. Higher doses (P3) may cause a shift in the microbiota composition or increased colonic absorption of butyrate, resulting in lower fecal butyrate concentrations compared to P2. Non-fermented sorghum still contains fiber and bioactive compounds that can reduce inflammation (hs-CRP), but without fermentation, SCFA production by gut microbiota is lower than sorghum tempeh (P3).

Table 4: SCFA Concentrations

Rats group	SCFA Concentrations(Mean ± SD)		
	Acetate	Propionate	Butyrate
K(-) Negative Control	17.51 ± 0.31	6.64 ± 2.33	2.80 ± 1.48
K(+) Positive control	1.42 ± 1.18	1.06 ± 0.76	0.92 ± 0.58
P1 Sorghum flour	18.16 ± 11.60	4.17 ± 1.73	1.12 ± 0.43
P2 Sorghum tempeh 5.09 g/mL	5.74 ± 2.23	3.99 ± 1.17	2.44 ± 0.69
P3 Sorghum tempeh 7.63 g/mL	20.37 ± 10.61	4.30 ± 1.22	1.14 ± 0.59
p	0.0011*	0.0012*	0.0012*

^{1}Kruskal Wallis Test, ^{2}Uji One Way Anova, *Significant

Discussion

The composition of the atherogenic feed given consists of standard feed (80%), lard (20%), and cholesterol (1.5%) of the total feed weight. Atherogenic feed contains high fat so that consumption long period can increase the body weight rats. The aim of using cholesterol and lard in feed is to induce an increase in LDL. Pork oil has a higher cholesterol content compared to other animal and vegetable oils.[13] study stated that giving an atherogenic diet for 5 weeks to male Wistar rats could cause can cause accumulation of macrophages, foam cells, intracellular lipids, extracellular lipids, atheroma, thrombus and smooth muscle proliferation in the coronary arteries of Rattus novergicus.[14]

The pattern of weight gain and inflammatory response in this study showed a consistent trend with the dietary interventions. Both sorghum tempeh groups (P2 and P3) demonstrated attenuated weight gain (15.7% and 18.3%, respectively) compared with the atherogenic control (39.8%), suggesting that sorghum-based interventions may help regulate metabolic balance under high-fat dietary stress. This moderation in body weight coincided with lower hs-CRP concentrations, particularly in P3, indicating a possible relationship between improved metabolic control and reduced systemic inflammation. Although formal correlation testing was not performed, the data trend implies that smaller weight gain may parallel greater hs-CRP reduction. Future studies should verify this association using correlation or regression analyses to strengthen causal inference.

Regarding the gut systemic axis, the changes in SCFA concentrations appear directionally consistent with the hs-CRP response: groups with higher propionate (P3) showed greater hs-CRP reductions, supporting the hypothesis that propionate may mediate anti-inflammatory effects. However, because individual SCFA concentrations and hs-CRP levels were analyzed separately, the absence of direct correlation analysis limits the strength of this mechanistic linkage. Conducting such an analysis in future work would clarify whether SCFA changes directly predict hs-CRP improvement.

Although both fermented (P3) and unfermented (P1) sorghum diets achieved similar magnitudes of hs-CRP reduction (approximately 75%), fermentation remains relevant because it improved SCFA yield, particularly acetate and propionate, which contribute to maintaining gut barrier function and lipid metabolism. The comparable hs-CRP outcomes suggest that both fiber and fermentation products contribute synergistically rather than exclusively. Thus, fermentation may not be indispensable for hs-CRP reduction alone, but it enhances the breadth and metabolic diversity of beneficial effects.

One of the potential bioactive compounds in sorghum is kafirin. Kafirin is the main protein in sorghum that is resistant to digestion, and after fermentation can produce bioactive peptides that have anti-inflammatory and antioxidant potential. Kafirin is the main form of protein storage in sorghum seeds (70% of total sorghum grain protein).[15] HDL cholesterol esters are transferred via CETP (Cholesteryl Ester Transfer Protein), which can be inhibited by kafirin to the other cholesterol. When CETP is inhibited, HDL-C accumulation will occur, which provides an anti-atherogenic effect, and ultimately can reduce hs-CRP levels.[16,17] In addition, kafirin reduces ROS production induced by LPS (Lipopolysaccharide), this is linked to a reduction in inflammatory cytokines that contribute to the production of CRP.[18]

The reduction in hs-CRP levels observed in sorghum tempeh–treated groups may be related to multiple interacting mechanisms rather than a single component. Although kafirin proteins and sorghum phenolics have been shown to have anti-inflammatory and antioxidant properties, the current study did not assess CETP activity, ROS generation, or kafirin-derived peptides; therefore, attributing hs-CRP reduction solely to CETP inhibition remains speculative. A more plausible interpretation involves the role of fermentation-derived metabolites such as SCFAs, particularly propionate, which has been shown to suppress hepatic cholesterol synthesis and systemic inflammation through G-protein-coupled receptor activation.

Interestingly, the medium-dose group (P2, 5.09 g/mL) exhibited higher butyrate concentrations than the high-dose group (P3, 7.63 g/mL). This may suggest a non-linear dose–response relationship, possibly reflecting microbial community shifts or substrate saturation effects, where excessive substrate availability promotes acetate and propionate formation at the expense of butyrate. Such findings emphasize the need to optimize dosing levels for maximum functional benefit rather than assuming a linear effect.

Moreover, the unfermented sorghum flour (P1) still produced measurable SCFA levels and moderate hs-CRP reduction, indicating that sorghum's inherent fiber and phenolic contents contribute to anti-inflammatory potential even without fermentation. However, fermentation enhanced specific SCFAs (acetate and propionate), which may account for the greater overall improvement in P3 compared with unfermented controls. The mixed pattern across SCFA species underscores that fermentation may modulate the microbiota metabolite balance differently for each acid type.

These results collectively suggest that the anti-inflammatory benefit of sorghum tempeh arises from both compositional enrichment due to fermentation and the synergistic action of microbial metabolites rather than a single biochemical pathway.

In an *in vivo* study, enzymatic digestion of sorghum kafirin increased the production of bioactive peptides with antioxidant activity that could be absorbed and transmitted to the bloodstream, providing beneficial effects on the lipid profile and impacting the prevention of atherosclerosis.[16]

The sorghum tempeh group had a greater butyrate content than the K+ group because butyrate inhibited the production of inflammatory mediators stimulated by LPS and IL-6 which stimulates the formation of CRP.[19] The SCFA concentration in rats fed an atherogenic diet was lower than in the K- group. This occurs due to changes in the intestinal microbiota (dysbiosis) from giving a high-fat diet.

The increase in SCFA concentration in the treatment group given sorghum tempeh was in line with other research with the provision of yogurt fermented with lactobacillus Q14 which could increase acetate and butyrate levels.[20]

Resistant starch consumption has been linked to reduced cholesterol levels and increased fecal SCFA levels, especially propionate and butyrate. Butyrate plays a positive role by controlling transepithelial fluid transfer, enhancing mucosal oxidative state and inflammation, fortifying the epithelial defense barrier, and averting hypercholesterolemia at the gut level, ischemic stroke and atherosclerotic conditions.

Limitations

The limitation of this research is that it is not yet known what effect giving sorghum tempeh has on the diversity of gut microbiota.

Conclusion

Sorghum tempeh administration (5.09 g/mL and 7.63 g/mL) significantly reduced hs-CRP concentrations and modulated SCFA profiles in rats fed an atherogenic diet. The higher dose showed a greater mean reduction in hs-CRP, although the difference between doses was not statistically significant, and the unfermented sorghum flour group exhibited comparable anti-inflammatory effects. These results indicate that both fermented and unfermented sorghum may contribute to lowering inflammatory biomarkers through synergistic actions of dietary fiber, phenolic compounds, and fermentation-derived metabolites.

The findings imply that sorghum-based products, particularly sorghum tempeh, hold potential as functional foods for inflammation modulation and cardiovascular health promotion. However, this interpretation should be viewed with caution, as the present study was limited to biomarker evaluation without histopathological or vascular outcome measurements. Further research is warranted to explore gut microbiota diversity, quantify key inflammatory cytokines (IL-6, TNF-α), and assess vascular changes to better elucidate the mechanistic pathways and clinical relevance.

Overall, this study provides preliminary evidence supporting the anti-inflammatory potential of sorghum tempeh, highlighting its promise as a sustainable dietary strategy for mitigating inflammation-related metabolic risks.

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Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

The Health Research Ethics Commission (KEPK) of the Faculty of Medicine at Diponegoro University in Semarang has granted ethical approval for this study under the number 97/EC/H/FK-UNDIP/X/2020.

Informed Consent Statement

We confirm that informed consent for experimentation was obtained, and all procedures were conducted in accordance with the ethical norms and legal standards currently applied in our country.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to Reproduce Material from Other Sources

Not Applicable.

Author Contributions

- **Annisa Khaira Ma'adi:** Conceptualization, Methodology, Writing Original Draft.
- **Nina Resti:** Analysis, Writing, Review and Editing.
- **Kis Djamiatun:** Supervision.
- **Ahmad Syauqy:** Resources, Supervision.
- **Gemala Anjani:** Resources, Supervision.
- **Diana Nur Afifah:** Conceptualization, Methodology, Resources, Supervision, Project Administration.

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