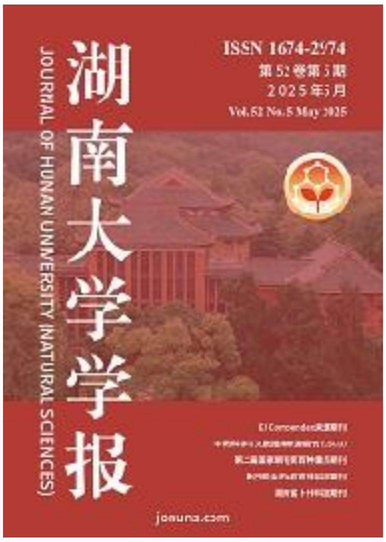

Journal of Hunan University

(Natural Sciences)

Vol. 52 No. 5

May 2025

Available online at

https://jonuns.com

ELSEVIER
Scopus
Clarivate
WEB OF SCIENCE

Open Access Article https://doi.org/10.55463/issn.1674-2974.52.5.4

Effects of Pomegranate, Lactobacillus, and Tempeh Combination on Metabolism, Oxidative Stress, and Intestinal Health in High-Fat Diet Mice

Diniwati Mukhtar[1]*, Nunung Ainur Rahmah[2], Aan Royhan[3], Linda Weni[4], Andrea Ivana Allicia Hutabarat[5], Hanifah Hafsari[6]

[1] Department of Physiology, Faculty of Medicine, YARSI University, Jakarta, Indonesia

[2] Department of Anatomical Pathology, Faculty of Medicine, YARSI University, Jakarta, Indonesia

[3] Doctoral Candidate, Department of Biomedical Science Doctoral, Graduate School, YARSI University, Jakarta, Indonesia

[4] Department of Biomedical Science Doctoral, Graduate School, YARSI University, Jakarta, Indonesia

[5] Universitas Padjadjaran, Indonesia

[6] Tangerang Regional General Hospital, Indonesia

* Corresponding author: diniwati.mukhtar@yarsi.ac.id

Article History:
Received: *March 25, 2025*
Revised: *April 29, 2025*
Accepted: *May 16, 2025*
Published online: *June 30, 2025*

Abstract: Pomegranate polyphenols, particularly ellagic acid, regulate glucose and lipid metabolism, while reducing oxidative stress through antioxidant and anti-inflammatory mechanisms. Lactobacillus supports gut health by enhancing the intestinal barrier function and modulating microbial populations. This study aimed to examine the effect of pomegranate juice, either alone or in combination with Lactobacillus or Tempeh, on the metabolic health of Wistar rats. Rats were divided into six treatment groups: K1 (normal control), K2 (high-fat diet control), K3 (pomegranate seeds), K4 (pomegranate seeds and peel), K5 (pomegranate seeds + Lactobacillus), and K6 (pomegranate seeds + tempeh). The outcomes assessed included the lipid profile, glucose levels, oxidative stress markers, fecal pH, and histological analysis. Significant differences were found across the treatment groups for lipid profile, blood glucose, superoxide dismutase (SOD), malondialdehyde (MDA), fecal pH, and intestinal histological parameters ($p<0.01$). Among the pomegranate-treated groups, K5 exhibited the lowest total cholesterol (92.70±4.29), triglycerides (84.28±2.31), LDL (28.00±2.92), and blood glucose (85.41±2.09), while showing the highest HDL (79.12±1.64) and SOD (79.67±3.40). The lowest MDA level was found in K6 (2.58±0.13), whereas K3 had the lowest fecal pH (6.94±0.05). Mucosal thickness and villus length differed significantly ($p<0.01$), whereas the goblet cell count did not ($p=0.19$). K3 exhibited the greatest mucosal thickness (577.34±76.39) and villus length (296.13±39.42), K5 had the lowest villus destruction (60% intact), and K6 had the highest goblet cell count (143.40±32.75). Pomegranate juice, with Lactobacillus or Tempeh, improves lipid and glucose metabolism, reduces oxidative stress, and enhances gut health in rats.

Keywords: Pomegranate Juice, Lactobacillus, Tempeh, Metabolic Health, Oxidative Stress, Gut Microbiota, High-Fat Diet, Wistar Rats, Lipid Profile, Blood Glucose.

石榴、乳酸菌和豆豉组合对高脂饮食小鼠代谢、氧化应激和肠道健康的影响

摘要：石榴多酚，尤其是鞣花酸，能够调节血糖和脂质代谢，并通过抗氧化和抗炎机制减轻氧化应激。乳酸杆菌通过增强肠道屏障功能和调节微生物群落来维护肠道健康。本研究旨在探讨石榴汁（单独饮用或与乳酸杆菌或豆豉联合饮用）对Wistar**大鼠代**谢健康的影响。大鼠被分为六个治疗组：K1（**正常**对照组）、K2（**高脂**饮食对照组）、K3（**石榴籽**组）、K4（**石榴籽和果皮**组）、K5（**石榴籽+乳酸杆菌**组）和K6（**石榴籽+豆豉**组）。评估结果包括血脂水平、血糖、氧化应激指标、粪便pH值和组织学分析。各治疗组血脂水平、血糖、超氧化物歧化酶（SOD)、**丙二醛**（MDA)、粪便 pH 值和肠道组织学参数均存在显著差异($p<0.01$)。**在石榴治**疗组中，K5 **的**总胆固醇(92.70±4.29)、**甘油三**酯(84.28±2.31)、LDL(28.00±2.92)**和血糖**(85.41±2.09)**最低，而** HDL(79.12±1.64)**和** SOD(79.67±3.40)**最高**。K6 **的** MDA **水平最低**(2.58±0.13)，**而** K3 **的**粪便 pH 值最低(6.94±0.05)**粘膜厚度和**绒毛长度差异显著($p<0.01$)，**而杯状**细胞数量差异无统计学意义($p=0.19$)K3组肠黏膜厚度(577.34±76.39)**和**绒毛长度(296.13±39.42)**均最大**，K5组肠绒毛破坏程度最低(60%**完好**)，K6组杯状细胞数量最多(143.40±32.75)**。添加乳酸杆菌或豆豉的石榴汁可改善大鼠的脂**质和葡萄糖代谢，降低氧化应激，并增强肠道健康。

关键词：石榴汁、乳酸杆菌、豆豉、代谢健康、氧化应激、肠道菌群、高脂饮食、Wistar **大鼠、血脂**谱、血糖。

1. Introduction

Pomegranates contain abundant polyphenols, with ellagitannins (ETs) being the predominant type [1]. These bioactive compounds possess strong antioxidant and anti-inflammatory properties [2]. One key ellagitannin, ellagic acid (EA), plays a crucial role in glucose regulation by stimulating GLUT2, IGF-1, IRS-1, and IRS-2 expression. It mitigates oxidative stress and glycation by inhibiting lipid peroxidation. EA also modulates carbohydrate metabolism by inhibiting α-glucosidase through α-amylase suppression, restricting glycogen breakdown via reduced glycogen phosphorylase secretion, and enhancing glucose uptake and storage while lowering blood glucose levels.

Additionally, EA contributes to lipid homeostasis by suppressing iodothyronine deiodinase 2 and Nr4a1 in brown fat and by activating PPARG coactivator 1 alpha, which promotes insulin sensitivity, limits white fat accumulation, and supports lipid metabolism. It further aids in fat regulation by decreasing liver-derived TG and TC, reducing leptin and resistin, boosting adiponectin levels, and preventing fat cell differentiation [3].

Certain Lactobacillus species are commonly used as probiotics [4]. Specific strains of Lactobacillus plantarum, CCFM1286, CCFM1290, and CCFM1291, facilitate the conversion of ellagitannins into urolithin A [5]. This metabolite promotes intestinal mucus secretion by activating the Nrf-2 and AhR pathways, which play vital roles in preserving intestinal barrier function [6]. Tempeh is a valuable source of probiotics, with Lactobacillus species being the most prevalent [7]. It contains a diverse range of probiotic bacteria, including Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii, Lactobacillus agilis, Lactobacillus mucosae, Lactobacillus paralimentarius, and Lactobacillus zeae, along with Lactococcus sp., Weissella confusa, W. paramesenteroides, Enterococcus faecalis, E. faecium, and Pediococcus pentosaceus [8]. In addition to its probiotic content, tempeh is a rich source of isoflavones with concentrations surpassing those found in other soy products such as tofu and soymilk [9]. Isoflavones contribute to gut health by fostering beneficial microbiota, inhibiting pathogenic bacteria, and influencing microbial populations linked to obesity [10]. They also support the proliferation of probiotics and enhance their antibacterial activity. Recognized for their broad health benefits, isoflavones have been associated with a reduced risk of cardiovascular disease, osteoporosis, hormone-dependent cancers (such as breast, prostate, and colon cancer), and menopausal symptoms. These protective effects are largely attributed to their antioxidant activity, which includes neutralizing free radicals, minimizing oxidative damage to DNA and LDL, and stimulating antioxidant enzyme activity [11].

This study aimed to investigate the effects of pomegranate juice, both alone and in combination with Lactobacillus or Tempeh, on various metabolic health parameters in rats. Specifically, it will assess lipid profiles (total cholesterol, LDL, HDL, and triglycerides), fasting blood glucose levels, antioxidant activity, and oxidative stress markers. Additionally, we evaluated the impact of these treatments on gut microbiota activity through fecal pH measurements and histological changes in intestinal tissues. Our findings are expected to provide scientific evidence of the potential benefits of combining pomegranate juice with probiotics or prebiotics, contributing to the development of natural formulations for metabolic health support.

2. Method

This experimental study utilized a post-test-only control group design to evaluate the effects of pomegranate juice, pomegranate juice with Lactobacillus, and pomegranate juice with tempeh extract on metabolic health in Wistar rats. A total of 24 male Wistar rats (8 weeks old, weighing 200–250 g) were divided into six groups, each containing four rats. The groups were as follows: K1 (normal control) received standard feed and water; K2 (HFD control) received a high-fat diet; K3 received pomegranate seed extract (3 mL/200 g); K4 received pomegranate seed and skin extract (3 mL/200 g), K5 received pomegranate seed extract (3 mL/200 g) with Lactobacillus (36 mg/200 g), and K6 received pomegranate seed extract (3 mL/200 g) with tempeh (70% incorporated into feed + soy milk). The assessed variables included lipid profile (total cholesterol, LDL, HDL, triglycerides), fasting blood glucose, superoxide dismutase (SOD) as a marker of antioxidant activity, malondialdehyde (MDA) as a marker of oxidative stress, fecal pH, and histological analysis of the liver, muscle, and intestinal tissues. All treatments were administered orally for four weeks, with blood samples taken via retro-orbital venous collection for lipid and glucose analysis, fecal samples collected for pH measurement, and tissue samples of the liver, muscle, and intestine obtained for histological examination. Data analysis included enzymatic colorimetric assays for lipid profiles and glucose, spectrophotometry for SOD, the TBARS method for MDA, a portable pH meter for fecal pH, and hematoxylin-eosin staining for histological analysis. The study protocol was approved by the Ethics Committee of the YARSI University (number 019/KEP-UY/EA.10/I/2025.

3. Results and Discution

One-way ANOVA was conducted to analyze differences in lipid profile, blood glucose, superoxide dismutase (SOD), malondialdehyde (MDA), and fecal pH among the treatment groups. Tukey's post hoc test was performed to further assess group differences.

Our findings revealed significant variation in lipid profile parameters, including total cholesterol (TC), triglycerides (TG), HDL, and LDL, across the treatment groups ($p<0.01$), as shown in Table 1. The highest total cholesterol levels were observed in the K2 group (208.81 ± 3.51), while the lowest levels were recorded in the control group (K1, 89.24 ± 2.78). Among the pomegranate-treated groups, K5 exhibited the lowest total cholesterol levels (92.70 ± 4.29). Tukey's post-hoc test revealed significant differences in total cholesterol levels between K1 vs K2, K3, K4, and K6; K2 vs K3, K4, K5, and K6; K3 vs K5 and K6; and K4 vs K5 ($p<0.01$), as shown in Table 2.

One-way ANOVA revealed a significant difference in triglyceride levels among the treatment groups ($p<0.01$). The highest triglyceride levels were observed in the K2 group (139.72±4.04), whereas the lowest levels were found in the control group (K1, 72.00±1.86). Among the groups receiving pomegranate treatment, K5 exhibited the lowest triglyceride levels (84.28±2.31). The Tukey post-hoc test further confirmed significant differences in triglyceride levels between K1 and K2; K3, K4, K5, and K6; K2 and K3; K4, K5, and K6; K3 and K5 and K6; and K4 and K5 ($p<0.01$), as presented in Table 2.

A significant difference in LDL levels was observed between the treatment groups ($p<0.01$). The highest LDL levels were observed in the K2 group (139.72±4.04), while the lowest levels were recorded in the control group (K1, 27.48±1.80). Among the pomegranate-treated groups, K5 exhibited the lowest LDL levels (28.00±2.92). The Tukey post-hoc test further confirmed significant differences between K1 and K2, K3 and K4; K2 and K3, K4, K5 and K6; K3 and K5 and K6; and K4 and K5 and K6 ($p<0.01$).

The analysis revealed a statistically significant variation in the HDL levels across the different treatment groups ($p<0.01$). The K2 group exhibited the lowest HDL levels (23.15±2.11), whereas the control group (K1) had the highest recorded levels (80.73±2.76). Among the groups receiving pomegranate treatment, K5 showed the most notable increase in HDL levels (79.12±1.64). Further post-hoc Tukey analysis confirmed significant pairwise differences between K1 and K2, K3 and K4; K2 and K3, K4, K5 and K6; K3 and K4, K5 and K6; K4 and K5 and K6 ($p<0.01$).

Table 1. One-Way ANOVA Results for Lipid Profile Parameters

Group	Lipid Profile			
	Total cholesterol (Mean±SD, mg/dL)	**Triglyceride** (Mean±SD, mg/dL)	**LDL** (Mean±SD, mg/dL)	**HDL** (Mean±SD, mg/dL)
K1	89.24±2.78	72.00±1.86	27.48±1.80	80.73±2.76
K2	208.81±3.51	139.72±4.04	78.96±1.62	23.15±2.11
K3	107.44±2.25	95.59±2.95	46.13±1.61	60.51±1.68
K4	105.27±3.12	93.10±2.44	38.90±2.40	67.98±3.37
K5	92.70±4.29	84.28±2.31	28.00±2.92	79.12±1.64
K6	101.66±4.83	86.48±1.59	30.80±3.11	75.61±1.41
p-value	**0.00****	**0.00****	**0.00****	**0.00****

**) $p<0.01$
*) $p<0.05$

Table 2. Tukey Post-Hoc Results

Group	Total Cholesterol	Triglyceride	LDL	HDL	Blood Glucose	Antioxidant Activity	Oxidative Stress	Fecal pH
K1 vs K2	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
K1 vs K3	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
K1 vs K4	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
K1 vs K5	0.648	0.00**	0.999	0.867	0.00**	0.856	0.00**	0.998
K1 vs K6	0.00**	0.00**	0.250	0.017	0.00**	0.017	0.00**	0.126
K2 vs K3	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
K2 vs K4	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
K2 vs K5	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
K2 vs K6	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
K3 vs K4	0.930	0.680	0.001	0.00**	0.462	0.349	0.138	0.051
K3 vs K5	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
K3 vs K6	0.00**	0.00**	0.00**	0.00**	0.007	0.013	0.00**	0.007
K4 vs K5	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
K4 vs K6	0.607	0.007	0.00**	0.00**	0.00**	0.569	0.00**	0.00**
K5 vs K6	0.007	0.774	0.424	0.181	0.041	0.187	0.00**	0.274

**) $p<0.01$
*) $p<0.05$

One-way ANOVA demonstrated a significant variation in blood glucose levels among the different treatment groups (p<0.001), as shown in Table 3. The highest average blood glucose level was found in the K2 group (158.65±1.80), while the lowest was recorded in the control group (K1, 74.52±1.30). Among the groups receiving pomegranate treatment, the K5 group exhibited the lowest blood glucose level (85.41±2.09). Subsequent Tukey post-hoc analysis confirmed significant pairwise differences between K1 and K2, K3, K4, K5, and K6; K2 and K3, K4, K5, and K6; K3 and K5; and K4 and K5 and K6 (p<0.01).

Table 3. Blood Glucose Levels in Across Treatment Groups

Group	**Blood Glucose** (Mean±SD, mg/dL)	**p-value**
K1	74.52±1.30	0.00**
K2	158.65±1.80	
K3	98.29±1.32	
K4	95.66±3.16	
K5	85.41±2.09	
K6	89.96±3.13	

**) p<0.01
*) p<0.05

The one-way ANOVA test assessing antioxidant activity in blood samples revealed a significant difference in superoxide dismutase (SOD) activity among the treatment groups (p<0.01), as shown in Table 4. The highest mean SOD activity was observed in the control group (K1, 79.67±3.40), whereas the lowest was recorded in the K2 group (25.25±3.40). Among the pomegranate-treated groups, K5 exhibited the highest SOD activity (79.67±3.40). Further Tukey post-hoc analysis confirmed significant pairwise differences between K1 and K2, K3 and K4; K2 and K3, K4, K5 and K6; K3 and K5; and K4 and K5 (p<0.01), as presented in Table 2.

Table 4. Superoxide Dismutase Activity across Treatment Groups

Group	**Superoxide Dismutase (SOD)** (Mean±SD, %)	**p-value**
K1	82.62±3.40	0.00**
K2	25.25±3.40	
K3	63.93±4.49	
K4	69.18±5.84	
K5	79.67±3.40	
K6	73.44±3.15	

**) p<0.01
*) p<0.05

One-way ANOVA for oxidative stress markers in blood samples revealed a significant variation in malondialdehyde (MDA) levels among the treatment groups (p<0.01), as shown in Table 5. The highest MDA levels were observed in the K2 group (10.19±0.16), whereas the lowest levels were recorded in the control group (K1, 1.62±0.13). Among the groups receiving pomegranate treatment, the highest MDA level was found in the K3 group (3.98±0.12), while the lowest was in the K6 group (2.58±0.13). A detailed summary of these findings is presented in Table 4. Tukey post-hoc analysis revealed significant pairwise differences between K1 and K2, K3, K4, K5, and K6; K2 and K3, K4, K5, and K6; K3 and K5 and K6; K4 and K5 and K6; and K5 and K6 (p<0.01), as shown in Table 2.

Table 5. Malondialdehyde Levels Across the Treatment Groups

Group	**Malondialdehyde (MDA)** (Mean±SD, mmol/mL)	**p-value**
K1	1.62±0.13	0.00**
K2	10.19±0.16	
K3	3.98±0.12	
K4	3.71±0.16	
K5	3.17±0.24	
K6	2.58±0.13	

**) p<0.01
*) p<0.05

One-way ANOVA for fecal pH revealed a significant difference between the treatment groups. The highest fecal pH was observed in the K1 group (7.38±0.04), while the lowest was recorded in the K2 group (6.66±0.11). Among the pomegranate-treated groups, K3 had the lowest fecal pH (6.94±0.05), whereas K5 had the highest (7.36±0.05). Table 5 presents the results. Further Tukey post-hoc analysis confirmed significant differences between K1 and K2, K3 and K4; K2 and K3, K4, K5 and K6; K3 and K5; and K4 and K5 and K6, as shown in Table 2.

Table 6. Fecal pH Across Treatment Groups

Group	**Fecal pH Mean±SD**	**p-value**
K1	7.38±0.04	0.00**
K2	6.66±0.11	
K3	6.94±0.05	
K4	7.08±0.08	
K5	7.36±0.05	
K6	7.26±0.05	

The results of the one-way ANOVA for intestinal histopathology parameters, including mucosal thickness, villus length, and goblet cell count, are presented in Table 7. To identify the groups that differed the most, a post-hoc Tukey test was performed, as shown in Table 8.

Table 7. One-Way ANOVA Results for Intestinal Histopathological Parameters

Group	Mucosal Thickness (Mean±SD, μm)	Villi Length (Mean±SD, μm)	Goblet Cell Count (Mean±SD)
K1	654.86±79.37	396.89±20.17	140.00±36.62
K2	516.91±125.39	266.03±105.99	99.00±51.50
K3	577.34±76.39	296.13±39.42	107.40±31.26
K4	492.35±147.83	252.72±95.87	85.40±31.13
K5	376.49±63.58	229.22±34.43	118.80±51.79
K6	430.69±98.87	284 20±75.37	143.40±32.75
p-value	**0.004****	**0.016***	**0.190**

**) p<0.01
*) p<0.05

Table 8. Tukey's Post-Hoc Analysis of Intestinal Histopathological Parameters

Group	Mucosal Thickness	Villi Length
K1 vs K2	0.311	0.066
K1 vs K3	0.837	0.240
K1 vs K4	0.164	0.034**
K1 vs K5	0.003**	0.010*
K1 vs K6	0.023	0.149
K2 vs K3	0.935	0.982
K2 vs K4	0.999	0.00**
K2 vs K5	0.293	0.958
K2 vs K6	0.769	0.998
K3 vs K4	0.779	0.919
K3 vs K5	0.051	0.658
K3 vs K6	0.251	1.000
K4 vs K5	0.496	0.994
K4 vs K6	0.930	0.978
K5 vs K6	0.958	0.810

**) p<0.01
*) p<0.05

A statistically significant difference in mucosal thickness was observed between treatment groups (p=0.004). Our findings revealed that the greatest mucosal thickness was observed in the control group. Among the pomegranate-treated groups, K3 exhibited the greatest mean mucosal thickness (577.34±76.39). In contrast, the lowest mucosal thickness was recorded in K5 (376.49±63.58). Tukey's post hoc test confirmed a statistically significant difference in mucosal thickness between K1 and K5 (p=0.003).

Our study revealed significant differences in villus length between the treatment groups (p= 0.016). Among the pomegranate-treated groups, K3 exhibited the longest mean villus length (296.13±39.42), whereas the shortest was observed in K5 (229.22±34.43). Tukey's post-hoc test indicated significant differences between K1 and K4 (p=0.034), K2 and K4 (p=0.00), and K1 and K5 (p= 0.01).

In this study, no significant differences in goblet cell counts were observed between the treatment groups (p=0.19). Among the pomegranate-treated groups, K6 exhibited the highest mean goblet cell count (143.40±32.75). In contrast, the lowest goblet cell count was recorded in K4 measuring (85.40±31.13)

In this study, a Kruskal-Wallis test was conducted to assess the differences in villus destruction percentages, and the results are presented in Table 9. A significant variation in villus destruction was observed among the treatment groups (p=0.016). Our findings indicated that groups K3 and K4 predominantly exhibited mild villus destruction, with a prevalence of 60%. In contrast, the majority of the samples in K5 showed no villus destruction (60%). K6 exhibited moderate villus destruction (60%). To further analyze group differences, a Mann-Whitney post hoc test was performed, and the results are presented in Table 10. The most significant differences were observed between K1 and K2 (P = 0.009), K1 and K3 (P = 0.014), and K1 and K6 (P = 0.009).

Table 9. Kruskal-Wallis Results of Villi Destruction

Group		**Villi Destruction**				**Total**	**p-value**
		None	**Mild**	**Moderate**	**Severe**		
K1	n	4	1	0	0	5	0.016*
	%	80.0%	20.0%	0.0%	0.0%	100.0%	
K2	n	0	1	1	3	5	
	%	0.0%	20.0%	20.0%	60.0%	100.0%	
K3	n	0	3	1	1	5	
	%	0.0%	60.0%	20.0%	20.0%	100.0%	
K4	n	0	3	0	2	5	
	%	0.0%	60.0%	0.0%	40.0%	100.0%	
K5	n	3	1	0	1	5	
	%	60.0%	20.0%	0.0%	20.0%	100.0%	
K6	n	0	1	3	1	5	
	%	0.0%	20.0%	60.0%	20.0%	100.0%	

Table 10. Mann–Whitney U test Results of Villi Destruction

Group	K1	K2	K3	K4	K5	K6
K1		0.009**	0.014*	0.014	0.439	0.009**
K2			0.180	0.356	0.063	0.371
K3				0.811	0.154	0.371
K4					0.121	0.658
K5						0.106
K6						

3.1 Discussion

In this study, we found significant differences in the lipid profile parameters among the treatment groups. Total cholesterol (TC), triglyceride (TG), and LDL levels were highest in the K2 group (high-fat diet), whereas the lowest levels were observed in the control group (K1). Among the pomegranate-treated groups, the lowest levels were detected for K5 (pomegranate seed + Lactobacillus). These findings suggest that the combination of pomegranate and probiotics may have a synergistic effect in lowering TC and TG levels, potentially owing to the role of Lactobacillus in lipid metabolism. This aligns with the findings of Sohrab et al. [12] and Kojadinovic et al. [13], who reported significant reductions in TC and LDL-C levels following pomegranate consumption. Additionally, a systematic review by Momin et al. [14] highlighted that probiotics significantly reduce triglyceride, LDL, and overall cholesterol levels in the blood.

The highest HDL-c levels were observed in the K5 group, indicating that the combination of pomegranate seed extract and Lactobacillus may have a beneficial effect on HDL-c levels. This contrasts with the findings of Mustafa et al., who reported that pomegranate juice did not significantly influence HDL-c levels [15]. However, a meta-analysis by Bahari et al. [16] concluded that pomegranate consumption could enhance HDL-c levels, which aligns with our results. These inconsistencies may stem from variations in the study design, dosage, or the presence of other bioactive compounds in the interventions. Additionally, a study by Andriani et al. on broiler chickens found that probiotics could effectively increase HDL levels, further supporting our findings [17].

In this study, we observed a significant difference in the blood glucose levels among the treatment groups. The highest blood glucose levels were recorded in the K2 group (high-fat diet control), whereas the lowest were found in the control group (K1). Among the pomegranate-treated groups, the K5 group exhibited the lowest blood glucose levels. This finding aligns with the study by Kerimi et al., who reported that polyphenols in pomegranate can reduce the postprandial glycemic response [18]. Similarly, our results are consistent with a meta-analysis by Zhong et al., who found that Lactobacillus plantarum enhanced glucose metabolism in patients with type 2 diabetes mellitus [19].

A significant difference in superoxide dismutase (SOD) levels, an indicator of antioxidant activity, was observed among the treatment groups. The highest

SOD levels were observed in the control group, whereas the lowest levels were recorded at K2. Among the pomegranate-treated groups, K5 exhibited the highest SOD level. These findings align with those of Naghizadeh-Baghi et al., who reported that pomegranate juice significantly modulated oxidative stress [20]. Additionally, Mu et al. found that Lactobacillus plantarum enhances free radical scavenging by increasing superoxide dismutase levels [21].

Our study revealed a significant difference in malondialdehyde (MDA) levels, a key marker of oxidative stress, across the treatment groups. The highest MDA levels were observed at K2, whereas the lowest levels were observed in the control group. Among the pomegranate-treated groups, K6 (pomegranate + tempeh) exhibited the lowest MDA level. These findings align with those of Naghizadeh-Baghi et al., who reported a reduction in MDA levels following pomegranate juice consumption compared with the control [20]. Similarly, Rachmawati et al. demonstrated that tempeh gembus effectively lowered MDA levels [22]. Additionally, Mu et al. found that Lactobacillus plantarum contributes to free radical scavenging by reducing MDA levels [21].

In this study, a significant difference was observed in the fecal pH among the treatment groups. The lowest fecal pH was observed in the K2 group, while the highest was recorded in the control group. Among the pomegranate-treated groups, K3 (pomegranate seed) exhibited the lowest fecal pH. These findings are consistent with the study by Varma et al., who reported that calves fed pomegranate peel extract had lower fecal pH than the control group [23]. Similarly, our results align with the findings of Alcon-Giner et al., who demonstrated that probiotic supplementation with Bifidobacterium and Lactobacillus (Bif/Lacto) reduced fecal pH in preterm infants [24].

In our study, significant differences in mucosal thickness were observed between the treatment groups. The control group (K1) exhibited the thickest mucosa, whereas among the pomegranate-treated groups, K3 showed the highest thickness and K5 the lowest. Rizzo et al. demonstrated that pomegranate extract supports intestinal repair by stabilizing barrier integrity, influencing the mucus layer, and regulating paracellular permeability [25]. Similarly, Ahl et al. reported that Lactobacillus reuteri protects against colitis by enhancing mucus thickness and reinforcing epithelial structure [26]. However, in this study, the combined Lactobacillus-pomegranate treatment did not yield the thickest mucosa, potentially because of strain differences or the bactericidal effects of pomegranate [27], which may interfere with the probiotic efficacy of Lactobacillus.

Variations in villus length were also evident across treatment groups. The longest villi were observed in the control group (K1), whereas among the pomegranate-treated groups, K3 exhibited the greatest villi length and K5 the shortest. Abozahra et al. similarly reported a significant increase in villus length in fish supplemented with pomegranate peel extract (PPE). Shonyela et al. found that Lactobacillus rhamnosus GG (LGG) enhances villi growth [28]. However, despite these findings, our study did not show the longest villi in the Lactobacillus pomegranate group, possibly due to strain-specific responses or the antimicrobial properties of pomegranate [27], which may limit the effectiveness of Lactobacillus as a probiotic.

The severity of villus destruction varied among different treatment groups. Mild destruction was most frequently observed in the K3 group, whereas the majority of the samples in the K5 group remained intact. Using a mouse model mimicking inflammatory bowel disease (IBD), Zhang et al. reported that PPE alleviates intestinal damage, potentially by inhibiting the MAPK and NF-κB signaling pathways [29]. Similarly, Cheng et al. found that Lacticaseibacillus rhamnosus VHProbi M15 and polyphenols effectively prevented intestinal villus destruction [30].

Unlike other parameters, goblet cell count did not differ significantly between the treatment groups in this study. This contrasts with the findings of Abozahra et al., who reported a significantly higher number of goblet cells in fish supplemented with PPE than in the control [31]. Likewise, Xie et al. revealed that Lactobacillus reuteri 22 suppresses the Notch signaling pathway, thereby promoting intestinal stem cell differentiation into goblet cells [32]. The discrepancies between these studies and our findings may be due to strain-specific responses or the antimicrobial effects of pomegranate [27], which could compromise the probiotic function of Lactobacillus.

4. Conclusion

Pomegranate juice, particularly when combined with Lactobacillus or Tempeh, enhances lipid and glucose metabolism, reduces oxidative stress, and improves gut health in rats.

This study has several limitations that need to be considered. First, the relatively small sample size (only four mice per group) may limit the generalizability of the results. Second, the short duration of the intervention (four weeks) may not be sufficient to capture the long-term effects of the combination of pomegranate juice, Lactobacillus, and tempeh on metabolic and gut health. In addition, variability in the type and dosage of probiotics and the nutrient content of tempeh were not strictly controlled, which could have affected the consistency of the results.

Future studies should explore its long-term effects, optimal dosages, and clinical applications in humans to develop potential dietary interventions for metabolic disorders and to improve gut health.

References

[1] N. P. Seeram *et al.*, "Pomegranate juice and extracts provide similar levels of plasma and urinary ellagitannin metabolites in human subjects," *J. Med. Food*, vol. 11, no. 2, pp. 390–394, 2008.

[2] D. Heber, "Multitargeted therapy of cancer by ellagitannins," *Cancer Lett.*, vol. 269, no. 2, pp. 262–268, 2008.

[3] X. Wang, X. Zhou, and X. Zhang, "Effects of Ellagic Acid on Glucose and Lipid Metabolism: A Systematic Review and Meta-Analysis," *J. Nutr. Metab.*, vol. 2024, no. 1, p. 5558665, 2024.

[4] P. A. Bron, S. Tomita, A. Mercenier, and M. Kleerebezem, "Cell surface-associated compounds of probiotic lactobacilli sustain the strain-specificity dogma," *Curr. Opin. Microbiol.*, vol. 16, no. 3, pp. 262–269, 2013.

[5] M. Zhang *et al.*, "Urolithin A produced by novel microbial fermentation possesses anti-aging effects by improving mitophagy and reducing reactive oxygen species in Caenorhabditis elegans," *J. Agric. Food Chem.*, vol. 71, no. 16, pp. 6348–6357, 2023.

[6] T. Yasuda *et al.*, "Urolithin A-mediated augmentation of intestinal barrier function through elevated secretory mucin synthesis," *Sci. Rep.*, vol. 14, no. 1, p. 15706, 2024.

[7] A. Pangastuti *et al.*, "Metagenomic analysis of microbial community in over-fermented tempeh," *Biodiversitas*, vol. 20, no. 4, pp. 1106–1114, 2019.

[8] D. P. Virgianti and R. Suhartati, "Tempeh's Probiotic Bacteria Content can Boost Its Value as a Functional Food in the COVID-19 Era," *Heal. Notions*, vol. 7, no. 4, pp. 80–88, 2023.

[9] W.-T. Liu *et al.*, "Changes in isoflavone profile, antioxidant activity, and phenolic contents in Taiwanese and Canadian soybeans during tempeh processing," *Lwt*, vol. 186, p. 115207, 2023.

[10] P. Chen *et al.*, "The bioavailability of soy isoflavones in vitro and their effects on gut microbiota in the simulator of the human intestinal microbial ecosystem," *Food Res. Int.*, vol. 152, p. 110868, 2022.

[11] G.-A. Yoon and S. Park, "Antioxidant action of soy isoflavones on oxidative stress and antioxidant enzyme activities in exercised rats," *Nutr. Res. Pract.*, vol. 8, no. 6, pp. 618–624, 2014.

[12] G. Sohrab *et al.*, "Effects of pomegranate juice consumption on oxidative stress in patients with type 2 diabetes: a single-blind, randomized clinical trial," *Int. J. Food Sci. Nutr.*, vol. 68, no. 2, pp. 249–255, 2017.

[13] M. Kojadinovic *et al.*, "Short-term consumption of pomegranate juice alleviates some metabolic disturbances in overweight patients with dyslipidemia," *J. Med. Food*, vol. 24, no. 9, pp. 925–933, 2021.

[14] E. S. Momin *et al.*, "The effects of probiotics on cholesterol levels in patients with metabolic syndrome: a systematic review," *Cureus*, vol. 15, no. 4, 2023.

[15] H. A. Mustafa and N. H. AbdElrahman, "Effect of punicagranatum juice on lipid profile of hypercholesterolemic albino rats," *EC Ophthalmol.*, vol. 2, pp. 178–184, 2015.

[16] H. Bahari *et al.*, "The effects of pomegranate consumption on lipid profile in adults: A systematic review and meta-analysis," *J. Funct. Foods*, vol. 108, p. 105727, 2023.

[17] A. D. Andriani *et al.*, "Efektifitas probiotik lactobacillus casei dan Lactobacillus rhamnosus sebagai pengganti antibiotic growth promoter terhadap total kolesterol, low density lipoprotein dan high density lipoprotein Ayam Broiler," *J. Med. Vet.*, vol. 3, no. 1, pp. 114–122, 2020.

[18] A. Kerimi, H. Nyambe-Silavwe, J. S. Gauer, F. A. Tomás-Barberán, and G. Williamson, "Pomegranate juice, but not an extract, confers a lower glycemic response on a high–glycemic index food: Randomized, crossover, controlled trials in healthy subjects," *Am. J. Clin. Nutr.*, vol. 106, no. 6, pp. 1384–1393, 2017.

[19] H. Zhong *et al.*, "Effects of Lactobacillus plantarum supplementation on glucose and lipid metabolism in type 2 diabetes mellitus and prediabetes: a systematic review and meta-analysis of randomized controlled trials," *Clin. Nutr. ESPEN*, 2024.

[20] A. Naghizadeh-Baghi, M. Mazani, A. Shadman-Fard, and A. Nemati, "Punica granatum juice effects on oxidative stress in severe physical activity," *Mater. Sociomed.*, vol. 27, no. 1, p. 48, 2015.

[21] G. Mu *et al.*, "Assessing and comparing antioxidant activities of lactobacilli strains by using different chemical and cellular antioxidant methods," *J. Dairy Sci.*, vol. 101, no. 12, pp. 10792–10806, 2018.

[22] P. A. Rachmawati, D. N. Afifah, N. Rustanti, G. Anjani, and A. Z. Juniarto, "The Effect of Tempeh gembus on Malondialdehyde and Superoxide Dismutase Enzyme Levels in Rats with Diet-Induced Metabolic Syndrome," *J. Biomed. Transl. Res.*, vol. 8, no. 2, pp. 91–98, 2022.

[23] V. S. Varma *et al.*, "Diet Supplementation With Pomegranate Peel Extract Altered Odorants Emission From Fresh and Incubated Calves' Feces," *Front. Sustain. Food Syst.*, vol. 2, p. 33, 2018.

[24] C. Alcon-Giner *et al.*, "Microbiota supplementation with Bifidobacterium and

Lactobacillus modifies the preterm infant gut microbiota and metabolome: an observational study," *Cell Reports Med.*, vol. 1, no. 5, 2020.
[25] G. Rizzo *et al.*, "Pomegranate extract affects gut biofilm forming bacteria and promotes intestinal mucosal healing regulating the crosstalk between epithelial cells and intestinal fibroblasts," *Nutrients*, vol. 15, no. 7, p. 1771, 2023.
[26] D. Ahl, H. Liu, O. Schreiber, S. Roos, M. Phillipson, and L. Holm, "Lactobacillus reuteri increases mucus thickness and ameliorates dextran sulfate sodium-induced colitis in mice," *Acta Physiol.*, vol. 217, no. 4, pp. 300–310, 2016.
[27] A. B. Howell and D. H. D′ Souza, "The pomegranate: effects on bacteria and viruses that influence human health," *Evidence-Based Complement. Altern. Med.*, vol. 2013, no. 1, p. 606212, 2013.
[28] S. M. Shonyela, B. Feng, W. Yang, G. Yang, and C. Wang, "The regulatory effect of Lactobacillus rhamnosus GG on T lymphocyte and the development of intestinal villi in piglets of different periods," *Amb Express*, vol. 10, pp. 1–11, 2020.
[29] Y. Zhang, S. Huang, S. Zhang, Z. Hao, and J. Shen, "Pomegranate peel extract mitigates diarrhea-predominant irritable bowel syndromes via MAPK and NF-κB pathway modulation in rats," *Nutrients*, vol. 16, no. 22, p. 3854, 2024.
[30] S. Cheng, H. Cui, J. Zhang, Q. Wang, and Z. Duan, "Probiotic potential of Lacticaseibacillus rhamnosus VHProbi M15 on sucralfate-induced constipation in mice," *Sci. Rep.*, vol. 14, no. 1, p. 1131, 2024.
[31] N. A. Abozahra, A. Albalawi, N. Althobaiti, R. Mohamed, and A. Diab, "Effects of Pomegranate (Punica granatum) Peel Methanolic Extract Dietary Supplementation on Oreochromis Niloticus Performance, Blood Health, Intestine Morphometry and Immunity," *Egypt. J. Vet. Sci.*, vol. 54, no. 7, pp. 221–236, 2023.
[32] S. Xie, S. Zhao, L. Jiang, L. Lu, Q. Yang, and Q. Yu, "Lactobacillus reuteri stimulates intestinal epithelial proliferation and induces differentiation into goblet cells in young chickens," *J. Agric. Food Chem.*, vol. 67, no. 49, pp. 13758–13766, 2019.

参考文:

[1] N. P. Seeram 等人，"石榴汁和提取物在人类体内产生相似水平的血浆和尿液鞣花单宁代谢物"，《医学食品杂志》，第11卷，第2期，第390–394页，2008年。
[2] D. Heber，"鞣花单宁对癌症的多靶点治疗"，《癌症通讯》，第269卷，第2期，第262–268页，2008年。
[3] X. Wang、X. Zhou 和 X. Zhang，"鞣花酸对葡萄糖和脂质代谢的影响：系统综述与荟萃分析"，《营养与代谢杂志》，2024年，第1期，第5558665页。
[4] P. A. Bron、S. Tomita、A. Mercenier 和 M. Kleerebezem，"益生乳酸杆菌细胞表面相关化合物维持菌株特异性学说"，《当前微生物学观点》，第16卷，第3期，第262–269页，2013年。
[5] M. Zhang 等人，"通过新型微生物发酵产生的Urolithin A通过改善线粒体自噬和减少氧化应激在秀丽隐杆线虫中具有抗衰老作用"，《农业与食品化学杂志》，第71卷，第16期，第6348–6357页，2023年。
[6] T. Yasuda 等人，"Urolithin A通过增加分泌性粘蛋白合成增强肠道屏障功能"，《科学报告》，第14卷，第1期，第15706页，2024年。
[7] A. Pangastuti 等人，"过度发酵天贝的微生物群落的宏基因组分析"，《生物多样性》，第20卷，第4期，第1106–1114页，2019年。
[8] D. P. Virgianti 和 R. Suhartati，"天贝中的益生菌含量可提升其在新冠时代作为功能性食品的价值"，《健康观点》，第7卷，第4期，第80–88页，2023年。
[9] W.-T. Liu 等人，"天贝加工过程中台湾和加拿大大豆异黄酮构成、抗氧化活性和酚类物质含量的变化"，《食品科学与技术》，第186期，第115207页，2023年。
[10] P. Chen 等人，"大豆异黄酮的体外生物利用度及其对肠道微生物群的影响：人体肠道模拟系统研究"，《食品研究国际》，第152期，第110868页，2022年。
[11] G.-A. Yoon 和 S. Park，"大豆异黄酮在运动大鼠中对氧化应激及抗氧化酶活性的抗氧化作用"，《营养研究与实践》，第8卷，第6期，第618–624页，2014年。
[12] G. Sohrab 等人，"饮用石榴汁对2型糖尿病患者氧化应激的影响：单盲随机临床试验"，《国际食品科学与营养杂志》，第68卷，第2期，第249–255页，2017年。
[13] M. Kojadinovic 等人，"短期饮用石榴汁缓解超重伴血脂异常患者的某些代谢障碍"，《医学食品杂志》，第24卷，第9期，第925–933页，2021年。
[14] E. S. Momin 等人，"益生菌对代谢综合征患者胆固醇水平的影响：系统综述"，《Cureus》，第15卷，第4期，2023年。
[15] H. A. Mustafa 和 N. H. AbdElrahman，"石榴汁对高胆固醇白化大鼠脂质水平的影响"，《眼科学前沿》，第2卷，第178–184页，2015年。
[16] H. Bahari 等人，"石榴摄入对成年人血脂水平的影响：系统综述与荟萃分析"，《功能食品杂志》，第108卷，第105727页，2023年。
[17] A. D. Andriani 等人，"乳酸杆菌对肉鸡血清总胆固醇、低密度脂蛋白和高密度脂蛋白的影响——

与抗生素生长促进剂的替代比较”，《兽医医学杂志》，第3卷，第1期，第114–122页，2020年。
[18] A. Kerimi 等人，“石榴汁而非提取物在高血糖指数食物中可降低血糖反应：健康人群中的随机交叉对照试验”，《美国临床营养杂志》，第106卷，第6期，第1384–1393页，2017年。
[19] H. Zhong 等人，“植物乳杆菌补充对2型糖尿病及糖尿病前期人群葡萄糖和脂质代谢的影响：随机对照试验的系统综述与荟萃分析”，《临床营养ESPEN》，2024年。
[20] A. Naghizadeh-Baghi 等人，“石榴汁对剧烈运动后氧化应激的影响”，《社会医学杂志》，第27卷，第1期，第48页，2015年。
[21] G. Mu 等人，“使用多种化学与细胞抗氧化方法评估与比较乳酸菌株的抗氧化活性”，《乳品科学杂志》，第101卷，第12期，第10792–10806页，2018年。
[22] P. A. Rachmawati 等人，“天贝对饮食诱导代谢综合征大鼠中丙二醛与超氧化物歧化酶水平的影响”，《生物医学转化研究杂志》，第8卷，第2期，第91–98页，2022年。
[23] V. S. Varma 等人，“补充石榴皮提取物可改变小牛粪便中新鲜和孵育期间的气味物质排放”，《可持续食品系统前沿》，第2卷，第33页，2018年。
[24] C. Alcon-Giner 等人，“补充双歧杆菌与乳酸杆菌可改变早产儿肠道微生物群与代谢组：观察性研究”，《细胞报告医学》，第1卷，第5期，2020年。
[25] G. Rizzo 等人，“石榴提取物影响肠道生物膜形成菌群，并通过调节上皮细胞与肠道成纤维细胞的信号传导促进肠黏膜愈合”，《营养素》，第15卷，第7期，第1771页，2023年。
[26] D. Ahl 等人，“鼠李糖乳杆菌可增加粘液层厚度并缓解硫酸葡聚糖钠诱导的结肠炎”，《生理学学报》，第217卷，第4期，第300–310页，2016年。
[27] A. B. Howell 和 D. H. D′Souza，“石榴对影响人类健康的细菌和病毒的作用”，《循证补充与替代医学》，2013年，第1期，第606212页。
[28] S. M. Shonyela 等人，“乳酸乳杆菌 GG 对不同阶段仔猪肠绒毛发育及T细胞的调控作用”，《Amb Express》，第10卷，第1–11页，2020年。
[29] Y. Zhang 等人，“石榴皮提取物通过调节MAPK和NF-κB通路缓解大鼠腹泻型肠易激综合征”，《营养素》，第16卷，第22期，第3854页，2024年。
[30] S. Cheng 等人，“Lacticaseibacillus rhamnosus VHProbi M15 在小鼠硫糖铝诱导的便秘模型中的益生潜力”，《科学报告》，第14卷，第1期，第1131页，2024年。
[31] N. A. Abozahra 等人，“膳食补充石榴皮甲醇提取物对尼罗罗非鱼生长表现、血液健康、肠道形态和免疫力的影响”，《埃及兽医科学杂志》，第54卷，第7期，第221–236页，2023年。
[32] S. Xie 等人，“鼠李糖乳杆菌可刺激幼鸡肠道上皮细胞增殖并诱导其分化为杯状细胞”，《农业与食品化学杂志》，第67卷，第49期，第13758–13766页，2019年。

Word count (excluding references): 7,796 words.

Peer-review record:
- ***Fast-track status:*** Not fast-tracked
- ***First-round reviews received:*** 3 reports
- ***Revision cycles completed:*** 3 rounds
- ***Final version submitted:*** May 18, 2025