



Effects of *Acetobacter* species Isolated from Palm Vinegar on TNF- α and Blood Profile in Rats

Rastina^{1*}, Darmawi², Safika³, Zinatul Hayati⁴, Winda Yulia⁴, Muhammad Daud⁵,
Nora Sovira⁶, Denny Irmawati Hasan⁷, Ummu Balqis⁷, and Mudhita Zikkrullah Ritonga⁸

¹Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, University of Syiah Kuala, Banda Aceh, Indonesia

²Microbiology Laboratory, Faculty of Veterinary Medicine, University of Syiah Kuala, Banda Aceh, Indonesia

³Division of Medical Microbiology, School of Veterinary Medicine and Biomedical Sciences, IPB University, Bogor, Indonesia

⁴Department of Microbiology, Faculty of Medicine, University of Syiah Kuala, Banda Aceh, Indonesia

⁵Department of Animal Science, University of Syiah Kuala, Banda Aceh, Indonesia

⁶Department of Pediatrics, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia; Department of Pediatrics, Dr. Zainoel Abidin Hospital, Banda Aceh, Indonesia

⁷Pathology Laboratory, Faculty of Veterinary Medicine, University of Syiah Kuala, Banda Aceh, Indonesia

⁸Laboratory of Anatomy, Faculty of Veterinary Medicine, University of Syiah Kuala, Banda Aceh, Indonesia

*Corresponding author's Email: rastina_rzl@usk.ac.id

ABSTRACT

Palm vinegar (*Arenga pinnata*), a traditional fermented product, contains acetic acid bacteria, particularly *Acetobacter* sp., which may serve as a natural immunomodulatory agent. The current study aimed to evaluate the effects of *Acetobacter* sp. isolated from palm vinegar on the immune response of white rats. A total of 24 male rats, aged 6-8 weeks and weighing 150-200 g, were randomly assigned to four groups of six rats each. The study included a control group that received normal saline and three treatment groups that received McFarland at doses of 0.5 (1.5×10^8 CFU/mL), 2 (6.0×10^8 CFU/mL), and 4 (1.2×10^9 CFU/mL) orally over 21 days. *Acetobacter* sp. was administered once a day for 14 days, and effects were monitored through blood samples collected weekly. Serum samples were collected during the first and second weeks of observation to assess tumor necrosis factor-alpha (TNF α) levels by enzyme-linked immunosorbent assay (ELISA) and for hematological examination. The results showed no significant difference in TNF- α levels between the first and second weeks. In the first week, mean TNF- α decreased in a dose-dependent manner, with the lowest value in the McFarland 4 group. During the second week of observation, measured parameters fluctuated across the treatment groups. Levels in the control group and McFarland 2 increased, while the McFarland 0.5 and 4 groups continued to decrease. Red blood cell (RBC) and WBC analysis showed no significant differences between observation times and between treatment groups. Descriptively, in the first week, the control group had the highest score and McFarland's 0.5 the lowest, but the values at doses 2 and 4 remained within the normal physiological range. In the second week, most groups showed an upward trend, except McFarland 0.5, which remained low. It can be concluded that *Acetobacter* sp. isolated from palm vinegar is effective as an immunomodulatory agent through a decrease in TNF- α levels. The stability of RBC and WBC parameters within normal limits confirms the safety of these bacteria, making them functional probiotic candidates that do not trigger systemic inflammation or blood balance disorders.

Keywords: *Acetobacter*, Palm vinegar, Probiotic, Tumor necrosis factor-alpha

INTRODUCTION

Indonesia is a biodiversity-rich region with a wide variety of plants that provide significant benefits for human life. One such plant is the sugar palm (*Arenga pinnata*), which belongs to the *Arecaceae* family (Martini et al., 2012). Local communities have long used the *Arenga pinnata* plant in daily life, particularly as a food source (Nuryanti et al., 2015). Palm sap is a sweet, clear liquid with a distinctive aroma obtained from the inflorescence of palm trees such as *Arenga pinnata* (Kurniawan et al., 2025). Fresh sap contains about 80-85% water, 15% sucrose, 7.61% glucose, 4.17% fructose, 1.5% minerals, and 0.8% vitamins, making it a suitable substrate for fermentative microorganisms (Saputro et al., 2019). Dominant microorganisms involved in sap fermentation include acetic acid bacteria, particularly *Acetobacter* sp. (Rastina et al., 2026). One product of palm sap fermentation is palm vinegar (Igbinaldolor, 2009).

Palm vinegar is a natural preservative that is relatively safe for consumption and readily available (Masyitah et al., 2017). It is produced by naturally fermenting fresh palm sap in closed containers. The fermentation process begins with activity, particularly *Saccharomyces cereviceae*, followed by oxidative fermentation (aerobic respiration) by acetic acid bacteria such as *Acetobacter* sp. This bacterium has been reported to possess probiotic potential (Ilahi et al., 2025). *Acetobacter* is a Gram-negative bacterium with ellipsoidal to rod-shaped (bacillary) morphology, which may appear as single cells, pairs, or chains (Guerrero, 2001). It exhibits aerobic metabolism and plays a key role in fermentation by oxidizing ethanol produced by *Saccharomyces cereviceae* and other carbohydrates into acetic acid (Artnarong et al., 2016).



ORIGINAL ARTICLE

Received: June 20, 2026

Revised: July 21, 2026

Accepted: August 25, 2026

Published: September 30, 2026

Several studies show that acetic acid bacteria have probiotic potential because they can inhibit pathogenic bacteria, modulate gut microbiota, and enhance host immunity. Furthermore, bioactive compounds in palm vinegar, such as polyphenols and flavonoids, support the immunomodulatory role of acetic acid bacteria by contributing to antioxidant activity and antibody production (Dwisandi et al., 2021). In addition, *Acetobacter* sp. produces antimicrobial metabolites that can inhibit the growth of pathogenic bacteria in the gastrointestinal tract (Rastina et al., 2026), while also supporting gut microbiota balance and digestive processes through its enzymatic activity (Wei et al., 2017).

The effects of *Acetobacter* sp. administration as a probiotic candidate on systemic health can be evaluated through tumor necrosis factor- α (TNF α) levels and hematological parameters, as blood serves as an important indicator of immunological and metabolic status (Fikri et al., 2025). Oral probiotic administration has been reported to influence hematological parameters, including erythrocyte and leukocyte counts and hemoglobin levels, which are associated with oxygen transport, immune response, and tissue repair (Quinn et al., 2016). Imbalances in hematological parameters may reflect infection, inflammation, or metabolic disturbances (Ayodeji and Babafemi, 2020). Palm vinegar has long been used in culinary applications in Aceh dishes (Erfiza et al., 2018). However, its potential as a natural probiotic source remains underexplored. Consequently, the present study aimed to determine the efficacy of *Acetobacter* sp. from palm vinegar as a natural probiotic by examining its impact on immune regulation and the maintenance of systemic equilibrium in rats.

MATERIALS AND METHODS

Ethical approval

All experimental procedures involving animals were conducted in strict accordance with the ethical guidelines established by the Veterinary Ethics Committee, Faculty of Veterinary Medicine, Syiah Kuala University, Indonesia (Approval No. 456/KEPH/XII/2025).

Study period and location

The study was conducted from October to November 2025 at the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.

Preparation of *Acetobacter* species

The procedure for identifying *Acetobacter* sp. bacteria isolated from palm vinegar (*Arenga pinnata*) began by taking 1 mL of vinegar and transferring it into a test tube. Next, the Pepton Yeast Glucose Broth (PYGB) medium (USA) was added to the tube and homogenized. The mixed suspension was incubated at 28°C for 24-48 hours. After incubation, 1 mL of culture was transferred to a Petri dish, then PYG agar medium was added and homogenized. After the agar hardened, the Petri dish was incubated upside down at 28°C for 24-48 hours. Bacterial colonies were then identified by Gram staining and a series of biochemical tests, including Indole, Methyl Red, Voges-Proskauer, and Citrate tests. In addition, fermentation tests were carried out on various types of sugar such as maltose, sucrose, lactose, glucose, and mannitol. All fermentation tests were carried out by incubation for 24 hours at a temperature of 37°C (Ilahi et al., 2025).

Study design

A total of 24 male white rats, aged 6-8 weeks and weighing 150-200 g, were purchased from the Integrated Service Unit of Experimental Animals, Faculty of Veterinary Medicine, USK, Aceh, Indonesia, and maintained under standard laboratory conditions. The rats were acclimatized in their cages for one week before the study began. The rats were maintained in clean plastic cages with a stainless-steel top grill that provided proper aeration and a 12-hour light/dark cycle (Agustiniingsih, 2025). The cages were cleaned weekly to maintain a clean environment. The white rats received pelleted feed (Rat Bio 1, Indonesia) and unrestricted access to water (Pritchett and Corning, 2004).

Administration of *Acetobacter* species

A total of 24 white rats were divided into 4 groups; treatment group one received standard feed and 5 mL distilled water and served as the test control. This treatment used McFarland's standard method to determine the bacterial concentration. Treatment group two included of *Acetobacter* sp. bacteria with a McFarland dose of 0.5 (1.5×10^8 CFU/mL) as a low dose, then the Treatment group three was given standard feed and *Acetobacter* sp. bacteria with a dose of McFarland 2 (6.0×10^8 CFU/mL) and the treatment group four was given standard feed and *Acetobacter* sp. with a dose of McFarland 4 (1.2×10^9 CFU/mL) as a high dose (Ahmadi-Soleimani et al., 2026). The experimental procedure involved daily oral administration of *Acetobacter* sp. via orogastric intubation (1 mL per rat) for 21 days. Blood and serum samples were collected from each group at weekly intervals to evaluate TNF- α levels and comprehensive hematological profiles.

Sample collection

Following a seven-day acclimatization period (week 1), blood samples were collected on day 14 (week 2) and day 21 (week 3). The male white rats were placed under mild anesthesia (ophthalmic drops, Indonesia) during the procedure. Approximately 1-3 mL of blood was collected from each rat via the retro-orbital sinus. The samples were then distributed into micro tubes containing ethylenediaminetetraacetic acid (EDTA) for hematological assessment and into plain tubes (without anticoagulants) to measure TNF- α levels via Enzyme-linked immunosorbent assay (ELISA).

Tumor necrosis factor-alpha

Tumor necrosis factor-alpha levels were measured using serum samples obtained from blood collected via the retro-orbital sinus under anesthesia using a combination of ketamine 100 mg/kg and xylazine 10 mg/kg, respectively (Rastina et al., 2026). The collected blood was placed in tubes without anticoagulants to allow serum separation, then centrifuged at 3000 rpm for 15 minutes. The resulting supernatant (serum) was then collected and analyzed for TNF- α levels using a Rat TNF- α ELISA kit (Bioassay Technology Laboratory, Cat. No. E0764Ra, China; Diehl et al., 2001; Parasuraman et al., 2010).

Hematological analysis

Hematological analysis was performed using an automated hematology analyzer (Licare CC-3200Vet, China). Blood samples were collected on day 14 (week 2) and day 21 (week 3) into EDTA vacutainer tubes via the retro-orbital sinus under mild anesthesia (ophthalmic drops, Indonesia), with approximately 50 μ L of EDTA-treated blood obtained from each animal, following the manufacturer's instructions. Analyzed parameters included red blood cell (RBC) count, hemoglobin (Hb) concentration, hematocrit (HCT) value, and white blood cell (WBC) count (Lippi et al., 2005).

Statistical analysis

Comparisons between treatment groups and observation time points for all measured parameters were performed using Two-Way Repeated-Measures Analysis of Variance (ANOVA). Duncan's multiple range test was applied as a post hoc analysis using SPSS® version 25. Statistical significance was set at a p-value less than 0.05.

RESULTS

Tumor necrosis factor-alpha

The results showed no significant difference in TNF- α levels between the first and second weeks of observation ($p > 0.05$; Table 1). However, descriptively, mean TNF- α levels showed a decreasing trend in the first week with increasing doses of *Acetobacter* sp., with the McFarland 4 group exhibiting the lowest mean value among all groups ($p > 0.05$). In the second week, TNF- α levels exhibited fluctuations across treatment groups. The control and McFarland 2 groups showed a non-significant increase in mean TNF- α levels compared with the first week ($p > 0.05$). In contrast, the McFarland 0.5 and McFarland 4 groups showed a non-significant decrease ($p > 0.05$). The standard deviations recorded during the second week reflect high inter-individual variation in biological responses within each group. TNF- α levels differed significantly among treatment doses ($p < 0.05$). The results demonstrated that the McFarland 4 group formed a distinct subset, significantly different from the control and McFarland 2 groups ($p < 0.05$). Meanwhile, the McFarland 0.5 group formed a subset, showing no significant difference compared with the control, McFarland 2, or McFarland 4 groups ($p > 0.05$).

Table 1. Tumor necrosis factor-alpha levels were measured in male rats aged 6–8 weeks during the first and second weeks after administration of *Acetobacter* sp.

Parameters		Treatment groups (Mean $\pm$ SD)			
	Time interval (week)	Control	McFarland 0.5	McFarland 2	McFarland 4
Tumor Necrosis factor-alpha pg/mL)	First week	31.82 $\pm$ 19.19	28.05 $\pm$ 18.22	25.11 $\pm$ 13.40	17.93 $\pm$ 15.88
	Second week	32.67 $\pm$ 27.83	5.80 $\pm$ 6.27	37.85 $\pm$ 32.91	5.23 $\pm$ 9.74
	Mean $\pm$ SE	32.24 $\pm$ 5.39 ^b	16.93 $\pm$ 5.39 ^{ab}	31.48 $\pm$ 5.39 ^b	11.58 $\pm$ 5.39 ^a

a,b,c,d Means within a row with different superscripts differ significantly ($p < 0.05$). SD: Standard deviation, SE: Standard error

Red blood cell profile

Red blood cell analysis showed no significant differences between the first and second weeks of observation in rats administered *Acetobacter* sp. ($p > 0.05$; Table 2). During the first week, the control group had higher RBC values than the treatment groups, with the lowest value in the McFarland 0.5 group; however, these differences were not significant ($p > 0.05$). Concurrently, the McFarland 2 and McFarland 4 groups showed fluctuating RBC counts that remained within the established physiological reference range for white rats ($5.6\text{--}7.89 \times 10^6/\mu\text{L}$), with no statistically significant variation when compared to the control group ($p > 0.05$). In the second week, RBC values were observed in the control, McFarland 2, and McFarland 4 groups, whereas the McFarland 0.5 group remained at relatively lower values. Although descriptive fluctuations occurred, all RBC counts remained within the normal physiological range of $5.6\text{--}7.89 \times 10^6/\mu\text{L}$, and results showed no significant differences across the treatment groups ($p > 0.05$). However, RBC levels did not differ significantly across treatment groups ($p > 0.05$). The results indicated that all groups were classified within the same subset, suggesting no statistically significant variation in RBC values among groups ($p > 0.05$).

Hemoglobin (Hb) results in Table 2 remained stable throughout the study, as statistical analysis showed no significant effect of observation time between the first and second weeks ($p > 0.05$). Descriptively, the control group had a higher mean Hb level compared to all treatment groups, while the McFarland 0.5 group recorded the lowest value. The McFarland 2 and McFarland 4 groups showed relatively comparable Hb levels. Although numerical variations were observed, Hb levels did not differ significantly among the treatment groups ($p > 0.05$).

In the second week, Hb levels increased in the McFarland 2 and McFarland 4 groups, while the McFarland 0.5 group showed lower Hb values than the other groups. The results revealed no significant differences in Hb levels among treatment groups ($p > 0.05$).

The haematocrit (HCT) results in Table 2 for rats administered *Acetobacter* sp. showed no significant effect of observation time between the first and second weeks ($p > 0.05$). Descriptively, during the first week, the control group tended to have higher HCT values than the treatment groups, with the lowest value in the McFarland 0.5 group; however, statistical analysis showed no significant differences among groups ($p > 0.05$) across all treatment doses. The McFarland 2 and McFarland 4 groups showed fluctuating hematocrit (HCT) levels within the established normal physiological range for white rats (36.0–46.0%) and no statistically significant differences compared with the control group ($p > 0.05$). In the second week, HCT values exhibited an increasing trend in the control, McFarland 2, and McFarland 4 groups, while the McFarland 0.5 group continued to show relatively lower values; however, statistical analysis confirmed that these variations were not significant ($p > 0.05$), as all experimental groups were categorized into a single homogeneous subset, confirming the absence of statistically significant differences. However, statistical analysis indicated no significant differences among treatment groups ($p > 0.05$).

Table 2. Red blood cell parameters were measured in male rats aged 6–8 weeks during the first and second weeks after administration of *Acetobacter* sp.

Parameters		Treatment groups (mean $\pm$ SD)			
Red blood cells	Time interval (mean $\pm$ SD)	Control	McFarland 0.5	McFarland 2	McFarland 4
(erythrocytes) ($10^6/\mu\text{L}$)	First week	31.82 $\pm$ 19.19	28.05 $\pm$ 18.22	25.11 $\pm$ 13.40	17.93 $\pm$ 15.88
	Second week	32.67 $\pm$ 27.83	5.80 $\pm$ 6.27	37.85 $\pm$ 32.91	5.23 $\pm$ 9.74
	Mean $\pm$ SE	32.24 $\pm$ 5.39 ^b	16.93 $\pm$ 5.39 ^{ab}	31.48 $\pm$ 5.39 ^b	11.58 $\pm$ 5.39 ^a
Hemoglobin (g/dL)	First week	4.34 $\pm$ 0.91	2.88 $\pm$ 3.58	2.99 $\pm$ 2.72	2.99 $\pm$ 2.77
	Second week	4.88 $\pm$ 2.18	2.16 $\pm$ 1.03	5.42 $\pm$ 1.05	6.05 $\pm$ 2.21
	Mean $\pm$ SE	4.61 $\pm$ 0.95 ^a	2.52 $\pm$ 0.95 ^a	4.20 $\pm$ 0.95 ^a	4.52 $\pm$ 0.95 ^a
Hematocrit (%)	First week	28.2 $\pm$ 7.10	17.7 $\pm$ 20.6	18.1 $\pm$ 15.5	19.5 $\pm$ 19.1
	Second week	29.8 $\pm$ 12.5	13.8 $\pm$ 5.95	27.7 $\pm$ 16.5	37.7 $\pm$ 13.9
	Mean $\pm$ SE	29.0 $\pm$ 5.85 ^a	15.7 $\pm$ 5.85 ^a	22.9 $\pm$ 5.85 ^a	28.6 $\pm$ 5.85 ^a

a,b,c,d Means within a row-with different superscripts differ significantly ($p < 0.05$). SD: Standard deviation, SE: Standard error

White blood cell profile

White blood cell counts showed no significant differences between the first and second weeks of observation in rats administered *Acetobacter* sp. ($p > 0.05$; Table 3). In the first week of observation, WBC counts were highest in the control group and lowest in the McFarland 0.5 group, with significant variations observed across all treatment doses ($p > 0.05$). Although the McFarland 2 and 4 groups showed variable values, their counts remained within the established physiological limits for rats ($2.9\text{--}15.3 \times 10^3/\mu\text{L}$).

In the second week, WBC values tended to decrease in the control group. In contrast, during the second week, the treatment groups showed more uniform leukocyte profiles, particularly in the McFarland 2 group, whose values closely mirrored the control group. Across treatment groups, WBC counts differed significantly ($p < 0.05$).

The analysis of lymphocyte counts showed no significant effect of observation time between the first and second weeks ($p > 0.05$; Table 3). Descriptively, during the first week, the highest lymphocyte counts were observed in the control group, while the lowest values were found in the McFarland 0.5 group, followed by the McFarland 2 and McFarland 4 groups. In the second week, lymphocyte levels in the control group tended to decrease, whereas the McFarland 2 and McFarland 0.5 groups showed increases ($p > 0.05$). The results indicated significant differences in lymphocyte counts ($p < 0.05$). Monocyte analysis showed no significant differences between observation times ($p > 0.05$; Table 3). Descriptively, during the first week, the highest monocyte values were observed in the control group, while the lowest values were found in the McFarland 0.5 group, followed by the McFarland 2 and McFarland 4 groups. In the second week, monocyte values decreased in the control group but remained relatively stable in the treatment groups. The results indicated significant differences among treatment groups ($p < 0.05$).

Granulocyte analysis showed in Table 3 that observation time had no significant effect between the first and second weeks ($p > 0.05$). Descriptively, during the first week, the highest granulocyte values were observed in the control group, while the lowest values were found in the McFarland 0.5 group, followed by the McFarland 2 and McFarland 4 groups. In the second week, granulocyte levels decreased in the control group, while treatment groups remained relatively stable, with a slight increase in the McFarland 0.5 group. Despite these descriptive variations, the results revealed no significant differences among treatment groups ($p > 0.05$).

The results showed no significant differences in eosinophil percentages between the first and second weeks of observation ($p > 0.05$; Table 3). Descriptively, eosinophil values were relatively uniform across all groups during the first week. Meanwhile, the McFarland 2 and McFarland 4 groups exhibited variable RBC values that remained within the normal physiological range ($p > 0.05$).

Table 3. White blood cell parameters were measured in male rats aged 6-8 weeks during the first and second weeks after administration of *Acetobacter* sp.

Parameters		Treatment groups (mean $\pm$ SD)			
Red blood cell	Time interval (mean $\pm$ SD)	Control	McFarland 0.5	McFarland 2	McFarland 4
Erythrocytes ($10^6/\mu\text{L}$)	First week	31.82 $\pm$ 19.19	28.05 $\pm$ 18.22	25.11 $\pm$ 13.40	17.93 $\pm$ 15.88
	Second week	32.67 $\pm$ 27.83	5.80 $\pm$ 6.27	37.85 $\pm$ 32.91	5.23 $\pm$ 9.74
	Mean $\pm$ SE	32.24 $\pm$ 5.39 ^b	16.93 $\pm$ 5.39 ^{ab}	31.48 $\pm$ 5.39 ^b	11.58 $\pm$ 5.39 ^a
Hemoglobin (g/dL)	First week	4.34 $\pm$ 0.91	2.88 $\pm$ 3.58	2.99 $\pm$ 2.72	2.99 $\pm$ 2.77
	Second week	4.88 $\pm$ 2.18	2.16 $\pm$ 1.03	5.42 $\pm$ 1.05	6.05 $\pm$ 2.21
	Mean $\pm$ SE	4.61 $\pm$ 0.95 ^a	2.52 $\pm$ 0.95 ^a	4.20 $\pm$ 0.95 ^a	4.52 $\pm$ 0.95 ^a
Hematocrit (%)	First week	28.2 $\pm$ 7.10	17.7 $\pm$ 20.6	18.1 $\pm$ 15.5	19.5 $\pm$ 19.1
	Second week	29.8 $\pm$ 12.5	13.8 $\pm$ 5.95	27.7 $\pm$ 16.5	37.7 $\pm$ 13.9
	Mean $\pm$ SE	29.0 $\pm$ 5.85 ^a	15.7 $\pm$ 5.85 ^a	22.9 $\pm$ 5.85 ^a	28.6 $\pm$ 5.85 ^a
White blood cell	Time interval (mean $\pm$ SD)	Control	McFarland 0.5	McFarland 2	McFarland 4
Leukocyte ($10^3/\mu\text{L}$)	First week	20.2 $\pm$ 3.74	1.67 $\pm$ 1.58	9.63 $\pm$ 8.16	7.56 $\pm$ 9.26
	Second week	9.10 $\pm$ 6.36	8.23 $\pm$ 3.83	9.70 $\pm$ 2.35	4.86 $\pm$ 1.78
	Mean $\pm$ SE	14.6 $\pm$ 2.19 ^b	4.95 $\pm$ 2.19 ^a	9.66 $\pm$ 2.19 ^{ab}	6.21 $\pm$ 2.19 ^a
Lymphocyte (cells/L)	First week	7.40 $\pm$ 1.11	0.76 $\pm$ 0.55	3.10 $\pm$ 1.37	3.16 $\pm$ 3.85
	Second week	4.70 $\pm$ 3.40	2.76 $\pm$ 1.19	4.26 $\pm$ 2.15	2.60 $\pm$ 0.08
	Mean $\pm$ SE	6.05 $\pm$ 0.90 ^b	1.76 $\pm$ 0.90 ^a	3.68 $\pm$ 0.90 ^{ab}	2.88 $\pm$ 0.90 ^a
Monocyte ($10^3/\mu\text{L}$)	First week	0.83 $\pm$ 0.32	0.33 $\pm$ 0.05	0.50 $\pm$ 0.60	0.23 $\pm$ 0.32
	Second week	0.30 $\pm$ 0.17	0.33 $\pm$ 0.15	0.30 $\pm$ 0.17	0.16 $\pm$ 0.05
	Mean $\pm$ SE	0.56 $\pm$ 0.10 ^b	0.18 $\pm$ 0.10 ^a	0.40 $\pm$ 0.10 ^{ab}	0.20 $\pm$ 0.10 ^a
Granulocyte (cells/L)	First weeks	11.9 $\pm$ 2.90	0.86 $\pm$ 0.98	6.03 $\pm$ 6.22	4.16 $\pm$ 5.09
	Second weeks	4.10 $\pm$ 2.78	5.13 $\pm$ 2.51	5.13 $\pm$ 1.65	2.10 $\pm$ 0.90
	Mean $\pm$ SE	8.03 $\pm$ 1.50 ^a	3.00 $\pm$ 1.50 ^a	5.58 $\pm$ 1.50 ^a	3.13 $\pm$ 1.50 ^a
Eosinophil (%)	First weeks	2.73 $\pm$ 0.11	3.20 $\pm$ 0.40	2.80 $\pm$ 0.26	2.83 $\pm$ 0.05
	Second weeks	3.03 $\pm$ 0.11	2.63 $\pm$ 0.05	2.83 $\pm$ 0.55	3.10 $\pm$ 0.10
	Mean $\pm$ SE	2.88 $\pm$ 0.13 ^a	2.91 $\pm$ 0.13 ^a	2.81 $\pm$ 0.13 ^a	2.96 $\pm$ 0.13 ^a

a,b,c,d Means within a row with different superscripts differ significantly ($p < 0.05$). SE: Standard error.

DISCUSSION

Immunomodulatory effects on tumor necrosis factor- α level

Acetobacter sp. isolated from palm vinegar demonstrated a significant dose-dependent reduction in serum TNF- α levels, particularly at the McFarland 4 dose. As a pro-inflammatory cytokine, TNF- α is a critical biomarker for immune activation; its suppression suggests that the probiotic candidate effectively regulates systemic inflammation (Abbas et al., 2025). The immunomodulatory effect likely involves interactions between bacterial structural components such as peptidoglycan and exopolysaccharides with pattern recognition receptors, which modulate the NF- κ B signaling pathway to prevent excessive inflammatory responses (Kim et al., 2023).

The fluctuations observed in the second week across different doses may reflect trained immunity or immune priming, where prior microbial exposure dynamically enhances innate cytokine responses to eventually reach a stable, regulated homeostatic state (Netea et al., 2020).

Stability of erythrocyte indices

Analysis of RBC counts, hemoglobin, and hematocrit revealed no statistically significant differences across observation periods or treatment groups. The observed numerical variations, particularly the slight dip in the first week, can be explained by a single unified mechanism: the initial physiological adaptation to Gram-negative bacterial exposure.

Lipopolysaccharides (LPS) in *Acetobacter* sp. can trigger a transient innate immune response, increasing TNF- α levels, which in turn stimulates hepatic hepcidin synthesis (Ganz and Nemeth, 2015). Elevated hepcidin restricts iron availability for erythropoiesis by inhibiting ferroprotein-mediated iron export, a process characteristic of mild “anemia of inflammation” (Weiss et al., 2019). However, stabilization and an upward trend in RBC, Hb, and HCT levels by the second week, especially in higher-dose groups, suggest that the host adapted successfully to the treatment (Markowiak and Śliżewska, 2017). *Acetobacter* sp. administration does not induce hemolysis or hematotoxicity and may eventually support erythrocyte stability by improving nutrient absorption through gut microbiota modulation (Wen et al., 2023).

Dynamics of leukocyte profiles and immune priming

Some WBC parameters, including lymphocytes and monocytes, varied significantly between groups; however, all values remained strictly within the normal physiological range for rats (Kurniawan et al., 2020). The initial decrease in circulating leukocytes and lymphocytes during the first week likely represents “leukocyte redistribution,” where immune cells migrate from peripheral circulation to gut-associated lymphoid tissues (GALT) upon first encountering the probiotic (Trinh et al., 2023). The subsequent stabilization in the second week indicates an adaptive immune adjustment rather than pathological suppression (Tari et al., 2018).

Furthermore, stable granulocytes and eosinophils across all groups confirmed the safety of the bacterial administration. The slight descriptive increases in granulocytes at higher doses may reflect innate immune priming, where bacterial metabolites such as acetate interact with receptors such as FFAR2/FFAR3 to enhance the readiness of the nonspecific immune system without triggering acute systemic inflammation (Mousa et al., 2023). The absence of eosinophilia or sustained leukocytosis further supports that *Acetobacter* sp. is a safe, non-allergenic immunomodulatory agent that maintains immune homeostasis (Balta et al., 2021).

CONCLUSION

Administration of *Acetobacter* sp. from palm vinegar in rats significantly reduced TNF- α levels, with the most pronounced effect observed at the McFarland 4 dose. Red blood cell parameters remained stable and within physiological ranges, indicating no adverse effects on erythrocyte homeostasis. White blood cell parameters fluctuated significantly in the 0.5 and 4 McFarland treatment groups compared with the control group; however, these values remained within normal limits and did not indicate systemic inflammation. These findings suggest that *Acetobacter* sp. is safe for use and has potential as a probiotic candidate that can modulate immune responses without disrupting hematological balance. Future studies should identify the specific molecular mechanisms and signaling pathways, such as NF- κ B regulation and gut microbiota interaction, to better understand the precise mode of action by which *Acetobacter* exhibits its immunomodulatory potential. Additionally, further studies are warranted to evaluate the therapeutic efficacy of *Acetobacter* sp. in specific disease models, such as chronic inflammatory or infectious conditions, to confirm its clinical potential beyond healthy physiological states.

DECLARATIONS

Acknowledgements

Appreciation is extended to Fauzan for providing laboratory materials to facilitate the study and Arya for assistance at the Integrated Service Unit of Experimental Animals, Faculty of Veterinary Medicine, Aceh, Indonesia.

Authors' contributions

Rastina was responsible for the overall design and execution of the study, data collection, and analysis. Writing the manuscript and ensured all necessary revisions were made by all authors. Darmawi assisted in the conceptualization of the study and contributed to the experimental design. Additionally, Safika helped with data analysis and interpretation. Zinatul Hayati contributed to the literature review and interpretation of results, and Winda Yulia contributed to final editing of the manuscript. All authors have read and approved the final edition of the manuscript before publication in the present journal.

Availability of data and materials

All data produced and examined in this study are included in the manuscript with no supplementary files. Requests for additional data generated in the present study should be directed to the corresponding author.

Competing interests

The authors declare no conflicts of interest.

Ethical considerations

The authors confirm that the present study is original, has not been previously submitted for publication or under review elsewhere, and adheres to strict ethical standards regarding data integrity and the avoidance of plagiarism. The authors explicitly confirm that they take full responsibility for all use of artificial intelligence in the preparation of the present study and they did not use any AI tools for conducting the study and preparing this manuscript. The authors affirm sole responsibility for the accuracy, originality, and integrity of the final content before submission, during the revisions, and throughout the final publication of the article.

Funding

The present study was not supported by any financial support.

REFERENCES

- Abbas AK, Lichtman AH, and Pillai S (2025). Cellular and molecular immunology E-Book: Cellular and molecular immunology E-Book. Elsevier Health Sciences., Philadelphia, PA, USA, pp. 35-38. Available at: <https://shop.elsevier.com/books/cellular-and-molecular-immunology/abbas/978-0-323-75748-5>
- Agustiningih D (2025). Physical activity treatment in rat-tested animals in research biomedis. UGM Press., Yogyakarta, Indonesia, pp. 8-12. Available at: <https://books.google.co.id/books?id=p2xpEQAAQBAJ&lpg=PP1&pg=PP1#v=onepage&q&f=false>
- Ahmadi-Soleimani SM, Masoudi M, Tabrizi AM, Beheshti F, and Azizi O (2026). Regulatory effects of probiotics on anxiety and depression-like behaviors in H. pylori-Infected rats. Probiotics and Antimicrobial Proteins, 18(2): 2408-2422. DOI: <https://www.doi.org/10.1007/s12602-025-10674-4>
- Artnarong S, Masniyom P, and Maneesri J (2016). Isolation of yeast and acetic acid bacteria from palmyra palm fruit pulp (*Borassus flabellifer* Linn.). International Food Research Journal, 23(3): 1308. Available at: <https://www.cabidigitallibrary.org/doi/pdf/10.5555/20163160871>
- Ayodeji FA and Babafemi SO (2020). Age-related changes in haematological parameters and biochemical markers of healing in the stomach of rats with acetic acid induced injury. Toxicology Reports, 7: 1272-1281. DOI: <https://www.doi.org/10.1016/j.toxrep.2020.09.007>
- Balta I, Butucel E, Mohlyuk V, Criste A, Dezmiorean DS, Stef L, Pet I, and Corcionivoschi N (2021). Novel insights into the role of probiotics in respiratory infections, allergies, cancer, and neurological abnormalities. Diseases, 9(3): 60. DOI: <https://www.doi.org/10.3390/diseases9030060>
- Guerrero R (2001). Bergey's manuals and the classification of prokaryotes. International Microbiology, 4(2): 103-109. DOI: <https://www.doi.org/10.1007/s101230100021>
- Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, and van de Vorstenbosch C (2001). A good practice guide to the administration of substances and removal of blood, including routes and volumes. Journal of Applied Toxicology, 21(1): 15-23. DOI: <https://www.doi.org/10.1002/jat.727>
- Dwisandi RF, Hernawati D, and Nuryadin E (2021). Potential of bioactive compounds of Arenga vinegar as traditional medicine through reverse docking techniques. Bioeduscience, 5(2): 142-147. DOI: <https://www.doi.org/10.22236/j.bes/526802>
- Erfa NM, Hasni D, and Syahrina U (2018). Evaluation of the nutritional value of Acehese meat dishes (*Sie Reuboh*) based on variations in the addition of beef fat and palm vinegar. Jurnal Teknologi dan Industri Pertanian Indonesia, 10(1): 28-35. DOI: <https://www.doi.org/10.17969/jtipi.v10i1.10202>
- Fikri F, Miftahurrohman A, Pratama MS, Qosdina DF, Sya'ban GF, Maslamama ST, and Purnama MT (2025). Astaxanthin improves hematology, interleukin-6, and tumor necrosis factor- α in the albino rats' kidney model of dexamethasone toxicity. Open Veterinary Journal, 15(6): 2511-2517. DOI: <https://www.doi.org/10.5455/OVJ.2025.v15.i6.23>
- Ganz T and Nemeth E (2015). Iron homeostasis in host defence and inflammation. Nature Reviews Immunology, 15(8): 500-510. DOI: <https://www.doi.org/10.1038/nri3863>
- Igbinador RO (2009). Other tropical fruit vinegars. Vinegars of the world. Springer., Milan, pp. 261-271. DOI: https://www.doi.org/10.1007/978-88-470-1011-6_16

- Ilahi BR, Handayani YD, Azhari, Rastina, and Darniati (2025). Effectiveness of *Acetobacter aceti* bacteria from palm vinegar (*Arenga pinnata*) isolate on acid and bile salt resistance as a probiotic candidate. IOP Conference Series: Earth and Environmental Science, 1476(1): 012083. DOI: <https://www.doi.org/10.1088/1755-1315/1476/1/012083>
- Kim SH, Jeong WS, Kim SY, and Yeo SH (2023). Immunomodulatory effects of six *Acetobacter pasteurianus* strains in RAW-Blue macrophage. Korean Journal of Food Preservation, 30(1): 65-77. DOI: <https://www.doi.org/10.11002/kjfp.2023.30.1.65>
- Kurniawan A, Widyastuti SK, and Suarsana IN (2020). Hematological profile of Wistar strain white rats (*Rattus norvegicus*) under standard maintenance conditions. Indonesia Medicus Veterinus, 9(1): 1-10. DOI: <https://www.doi.org/10.19087/imv.2020.9.1.1>
- Kurniawan H, Rahayoe S, and Amnah HZ (2025). Processing and quality of crystallized palm sugar in Indonesia: A review. Jurnal Teknik Pertanian Lampung, 14(4): 1517-1533. DOI: <https://www.doi.org/10.23960/jtepl.v14i4.1517-1533>
- Lippi G, Salvagno GL, Brocco G, and Guidi GC (2005). Preanalytical variability in laboratory testing: Influence of the blood drawing technique. Clinical Chemistry and Laboratory Medicine, 43(3): 319-325. DOI: <https://www.doi.org/10.1515/CCLM.2005.055>
- Markowiak P and Śliżewska K (2017). Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients, 9(9): 1021. DOI: <https://www.doi.org/10.3390/nu9091021>
- Martini E, Roshetko JM, van Noordwijk M, Rahmanulloh A, Mulyoutami E, Joshi L, and Budidarsono S (2012). Sugar palm (*Arenga pinnata* (Wurmb) Merr.) for livelihoods and biodiversity conservation in the orangutan habitat of Batang Toru, North Sumatra, Indonesia: Mixed prospects for domestication. Agroforestry Systems, 86(3): 401-417. DOI: <https://www.doi.org/10.1007/s10457-011-9441-0>
- Masyitah M, Arief II, and Suryati T (2017). Characteristics of *Sie Reuboh* supplemented with different combinations of palm vinegar (*Arenga pinnata*) and kaffir lime leaves (*Citrus hystrix*). Tropical Animal Science Journal, 40(3): 202. DOI: <https://www.doi.org/10.5398/medpet.2017.40.3.202>
- Mousa WK, Mousa S, Ghemrawi R, Obaid D, Sarfraz M, Chehadeh F, and Husband S (2023). Probiotics modulate host immune response and interact with the gut microbiota: Shaping their composition and mediating antibiotic resistance. International Journal of Molecular Sciences, 24(18): 13783. DOI: <https://www.doi.org/10.3390/ijms241813783>
- Netea MG, Domínguez-Andrés J, Barreiro LB, Chavakis T, Divangahi M, Fuchs E, Joosten LA, van der Meer JW, Mhlanga MM, Mulder WJ et al. (2020). Defining trained immunity and its role in health and disease. Nature Reviews Immunology, 20(6): 375-388. DOI: <https://www.doi.org/10.1038/s41577-020-0285-6>
- Nuryanti S, Linda R, and Lovadi I (2015). The utilization of *Arecaceae* (Palms) by the Dayak Randu community in Batu Buil Village, Belimbing district, Melawi Regency. Protobiont, 4(1): 128-135. DOI: <https://www.doi.org/10.26418/protobiont.v4i1.9662>
- Parasuraman S, Raveendran R, and Kesavan R (2010). Blood sample collection in small laboratory animals. Journal of Pharmacology and Pharmacotherapeutics, 1(2): 87-93. DOI: <https://www.doi.org/10.4103/0976-500X.72350>
- Pritchett KR and Coming BF (2004). Biology and medicine of rats. In: J. D. Reuter and M. A. Suckow (Editors), Laboratory animal medicine and management. International Veterinary Information Service, Ithaca NY. Available at: <https://www.ivis.org/library/laboratory-animal-medicine-and-management/biology-and-medicine-of-rats>
- Quinn RA, Vermeij MJ, Hartmann AC, d'Auriac IG, Benler S, Haas A, Quistad SD, Lim YW, Little M, Sandin S et al. (2016). Metabolomics of reef benthic interactions reveals a bioactive lipid involved in coral defence. Proceedings of the Royal Society B: Biological Sciences, 283(1829): 20160469. DOI: <https://www.doi.org/10.1098/rspb.2016.0469>
- Rastina, Safika, Hayati Z, Yulia W, and Darmawi (2026). Temporal dynamics and molecular characterization of acetic acid bacteria during palm vinegar (*Arenga pinnata*) fermentation. Biodiversitas, 27 (7): d270708. DOI: <https://www.doi.org/10.13057/biodiv/d270708>
- Rastina, Azhari, Novita A, Wahyuni S, Hasan DI, Hidayatun A, and Fadila MF (2026). Effect of acetic acid bacteria isolated from palm vinegar on cecal *Escherichia coli* counts in Wistar rats. Jurnal Ilmu Peternakan dan Veteriner Tropis, 16(2): 83. DOI: <https://www.doi.org/10.46549/jipvet.v16i2.653>
- Saputro AD, Van de Walle D, and Dewettinck K (2019). Palm sap sugar: A review. Sugar Tech, 21(6): 862-867. DOI: <https://www.doi.org/10.1007/s12355-019-00743-8>
- Tari AI, Handayani CB, and Hartati S (2018). Study of antidiarrheal and hematology profile of laboratory rat fed with yogurt containing local probiotic and purple sweet potato extract. IOP Conference Series: Earth and Environmental Science, 142(1): 012026. DOI: <https://www.doi.org/10.1088/1755-1315/142/1/012026>
- Trinh S, Käver L, Schlösser A, Simon A, Kogel V, Voelz C, Beyer C, and Seitz J (2023). Gut-associated lymphatic tissue in food-restricted rats: Influence of refeeding and probiotic supplementation. Microorganisms, 11(6): 1411. DOI: <https://www.doi.org/10.3390/microorganisms11061411>
- Weiss G, Ganz T, and Goodnough LT (2019). Anemia of inflammation. Blood, 133(1): 40-50. DOI: <https://www.doi.org/10.1182/blood-2018-06-856500>
- Wei P, Cui YH, Zong MH, Xu P, Zhou J, and Lou WY (2017). Enzymatic characterization of a recombinant carbonyl reductase from *Acetobacter* sp. CCTCC M209061. Bioresources and Bioprocessing, 4(1): 39. DOI: <https://www.doi.org/10.1186/s40643-017-0169-1>
- Wen X, Wang Z, Liu Q, Lessing DJ, and Chu W (2023). *Acetobacter pasteurianus* BP2201 alleviates alcohol-induced hepatic and neuro-toxicity and modulate gut microbiota in mice. Microbial Biotechnology, 16(9): 1834-1857. DOI: <https://www.doi.org/10.1111/1751-7915.14303>

