



The effect of *tempe semangit* cookies consumption on total and differential leukocyte counts in students of SMK karya teknologi 2 Jatilawang

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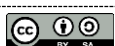
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Abstract

Background: The immune system plays an essential role in maintaining physiological homeostasis, which can be reflected through total and differential leukocyte counts. Functional foods containing bioactive compounds, such as over-fermented *tempeh* (*tempe semangit*), have attracted attention due to their potential immunomodulatory properties. **Objectives:** This study aimed to analyze the differences in total and differential leukocyte counts before and after the consumption of *tempe semangit* cookies among students of SMK Karya Teknologi 2 Jatilawang. **Materials and Methods:** This study employed a pretest-posttest control group design involving 32 participants who were equally divided into control and intervention groups. The intervention group consumed *tempe semangit* cookies at a dose of 40 g/day for 7 consecutive days, while the control group received no treatment. Total leukocyte count and differential leukocyte parameters, including band neutrophils, segmented neutrophils, lymphocytes, and monocytes, were measured before and after the intervention. Data were analyzed using paired t-test and Wilcoxon test with a significance level of 0.05. **Results:** The results showed no significant change in total leukocyte count in the control group ($p = 0.349$), whereas a significant reduction was observed in the intervention group ($p = 0.031$). No significant differences were found in differential leukocyte counts within each group ($p > 0.05$). However, significant differences in lymphocyte ($p = 0.029$) and monocyte levels ($p = 0.020$) were identified between groups after the intervention. **Conclusions:** In conclusion, short-term consumption of *tempe semangit* cookies may exert immunomodulatory effects by influencing total leukocyte count and selected immune cell parameters without altering the overall leukocyte differential profile.

Keywords

Adolescents, Functional Food, Immunomodulation, Leukocytes, *Tempe Semangit*.



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1. Introduction

The immune system plays a pivotal role in maintaining physiological homeostasis by protecting the body against pathogens and regulating inflammatory processes. One of the primary indicators used to assess immune status is the leukocyte profile, particularly total and differential leukocyte counts. These parameters are highly sensitive to various internal and external factors, including nutritional status, physical activity, and environmental exposure (Morales *et al.*, 2024). Adolescents represent a vulnerable group, as this developmental stage is characterized by rapid physiological changes that may influence immune function and hematological parameters (Bohn *et al.*, 2023).

Nutritional intake is a key determinant of immune competence. Inadequate consumption of macro- and micronutrients has been associated with impaired leukocyte production and reduced immune response. In recent years, functional foods have gained increasing attention as a nutritional approach to support immune function. These foods not only provide essential nutrients but also contain bioactive compounds that exert immunomodulatory effects (Fitsum *et al.*, 2025). Therefore, the development of locally available, nutrient-dense functional foods is particularly important for school-aged populations.

Tempeh, a traditional fermented soybean product, is widely recognized for its high nutritional value and affordability. The fermentation process enhances nutrient bioavailability and produces bioactive compounds such as peptides, isoflavones, and phenolic substances with antioxidant and immunomodulatory properties (Teoh *et al.*, 2024). A further stage of fermentation results in over-fermented *tempeh*, locally known as *tempe semangit*, which contains higher concentrations of free amino acids and bioactive peptides (Parhusip *et al.*, 2023). These compounds have been reported to exert immunomodulatory effects by modulating cytokine production, reducing oxidative stress, and enhancing immune cell activity (Fitsum *et al.*, 2025).

Despite its potential as a functional food, scientific evidence regarding the health effects of *tempe semangit* remains limited, particularly in human studies, as previous research has mainly focused on bioactive compound characterization and experimental animal models (Astawan *et al.*, 2025). In addition, the application of *tempe semangit* in acceptable and practical food forms remains underexplored. The incorporation of *tempe semangit* into

cookies (*tempe semangit cookies*) represents a feasible strategy to deliver functional nutrients in a form that is acceptable and convenient for adolescents.

Therefore, this study aimed to evaluate the effect of *tempe semangit cookies* consumption on total and differential leukocyte counts among adolescents using a pretest-posttest control group design.

2. Materials and Methods

2.1. Study Design

The study was conducted over two months, covering permit acquisition, sampling, laboratory testing, and report preparation. The research took place at the Hematology Laboratory, Department of Medical Laboratory Technology, SMK Karya Teknologi 2 Jatilawang. This study employed a true experimental design with a pretest-posttest control group approach to evaluate the effect of *tempe semangit cookies* on leukocyte counts under controlled laboratory conditions.

2.2. Etichal Approval

This study was approved by the Health Research Ethics Committee of Al-Irsyad University with ethical clearance number 2009/280/03.6.1. Written informed consent was obtained from all participants to participation in the study.

2.3. Participants

A total of 32 students from SMK Karya Teknologi 2 Jatilawang participated in this study. Participants were divided equally into control (n = 16) and intervention (n = 16) groups. Inclusion criteria were students aged 15-18 years who were willing to participate and provided informed consent. Exclusion criteria included current infection, chronic illness, use of immunomodulatory drugs, and absence during the intervention period.

2.4. Materials and Equipment

The primary material used in this study was *tempe semangit*, obtained from traditional fermented soybean (*Glycine max*) products purchased directly from local market vendors. The selection was based on typical household consumption characteristics to reflect real-world conditions. The cookie formulation consisted of low-protein wheat flour (Bogasari, Jakarta, Indonesia), powdered sugar (Dunia, Indonesia), margarine (Palmia Super Cake, Wilmar International, Indonesia), and butter (Anchor, Fonterra Co-operative Group, Auckland, New Zealand). All ingredients were food-grade and used without further purification.

For hematological analysis, blood samples were collected using EDTA anticoagulant tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, NJ, USA) to prevent coagulation and preserve cellular integrity. Leukocyte counts were determined manually using a hemocytometer (Neubauer chamber; Marienfeld, Lauda-Königshofen, Germany) and observed under a light microscope (Shinher, Ningbo, China).

2.5. Leukocyte Count Procedure

Total leukocyte counts were measured using the manual tube method. Whole blood was diluted with an appropriate leukocyte diluting fluid, homogenized, and loaded into the Neubauer counting chamber. Cells were counted under a microscope according to standard hematological procedures. Differential leukocyte counts were performed using peripheral blood smears stained with Giemsa stain (Merck, Darmstadt, Germany). A total of 100 leukocytes were counted under oil immersion (1000× magnification) to determine the percentage distribution of each leukocyte type.

2.6. Experiment Procedure

Participants were divided into control and intervention groups. Baseline leukocyte levels were measured prior to intervention. The intervention group received *tempe semangit* cookies at a predetermined dosage for a specified duration, while the control group did not receive the intervention. Blood samples were collected aseptically and analyzed promptly to maintain sample stability.

2.7. Data Analysis

Statistical analysis was performed using SPSS Statistics (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro-Wilk test. For within-group comparisons, normally distributed data were analyzed using the paired t-test, whereas non-normally distributed data were analyzed using the Wilcoxon signed-rank test. For between-group comparisons, the independent t-test was used for normally distributed data, while the Mann-Whitney U test was applied for non-normally distributed data. Statistical significance was set at $p < 0.05$.

3. Results and Discussion

The present study aimed to evaluate differences in total leukocyte count and differential leukocyte count before and after the consumption of *tempe semangit* cookies in adolescents. The intervention dose was 40 g/day, with each cookie weighing

approximately 4 g, consumed between 9:00 a.m. and 4:00 p.m. This dosage was selected based on practical considerations, representing a realistic daily portion for adolescents without replacing main meals. At this intake level, the estimated daily consumption of *tempe semangit* flour was 9.2 g/day.

3.1. Pre- and Post-Intervention Total Leukocyte Count After Tempe Semangit Cookies Consumption

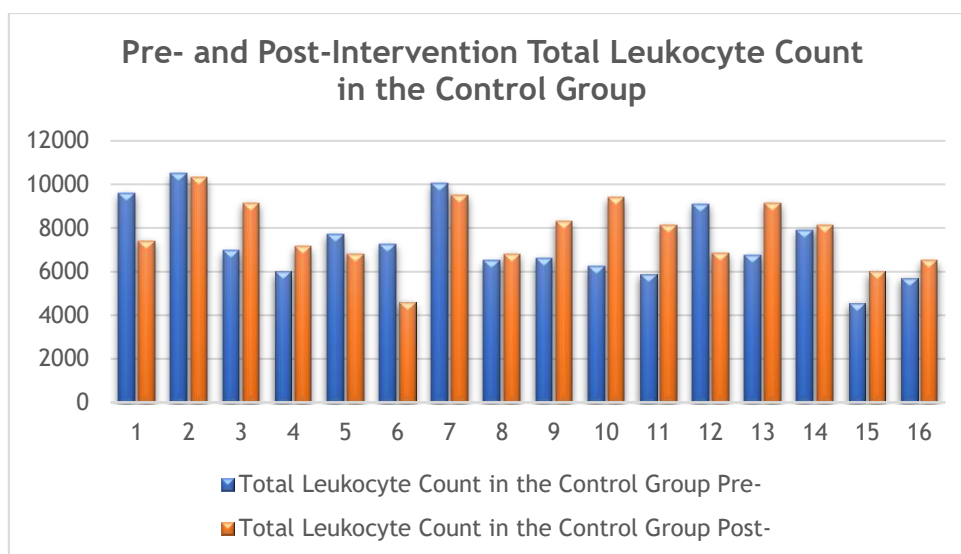


Figure 1. Pre- and Post-Intervention Total Leukocyte Count in the Control Group

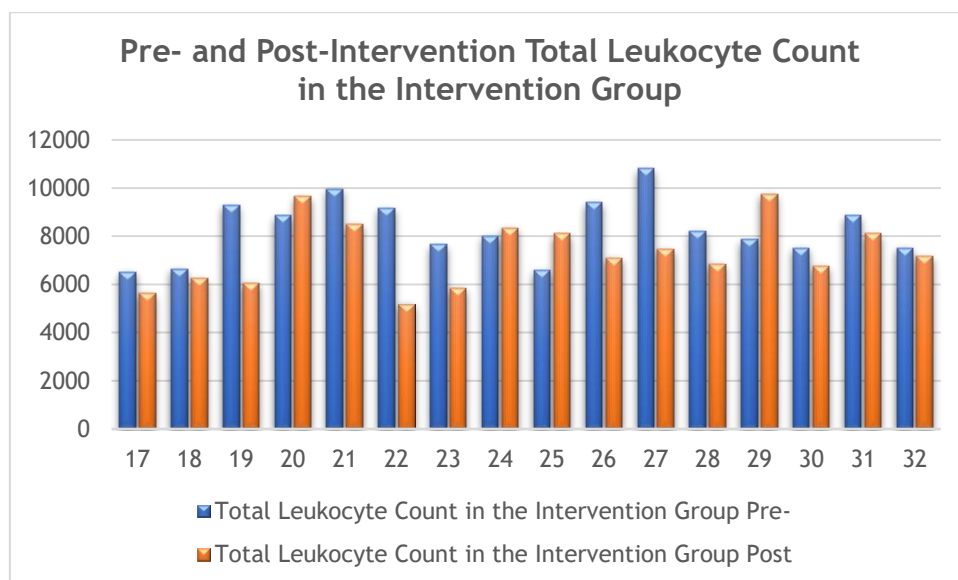


Figure 2. Pre- and Post-Intervention Total Leukocyte Count in the Intervention Group

The total leukocyte count in the control group demonstrated heterogeneous fluctuations between pre- and post-observation measurements, with no consistent directional trend. In contrast, the intervention group exhibited a more pronounced tendency toward reduced leukocyte counts following the consumption of *tempe semangit* cookies. Although some variability remained, the majority of samples showed decreased values post-intervention, indicating a general downward trend compared to the control group.

Statistical analysis showed that there was no significant difference in total leukocyte count between pre- and post-observation measurements in the control group ($p = 0.349$). In contrast, the intervention group demonstrated a statistically significant difference ($p = 0.031$), with a tendency toward decreased leukocyte counts following the intervention.

The present study demonstrates that the daily consumption of *tempe semangit* cookies induces a selective immunomodulatory effect in adolescents, as evidenced by the significant reduction in total leukocyte count. The absence of significant changes in the control group supports the notion that, under normal physiological conditions, the immune system operates in a state of homeostasis, maintaining leukocyte levels within a tightly regulated range (Calder *et al.*, 2020).

The observed decrease in leukocyte count in the intervention group should not be interpreted as immunosuppression, but rather as a regulatory adaptation reflecting improved immune balance. Within the framework of nutritional immunology, dietary interventions may act as immunomodulators by attenuating low-grade inflammation and oxidative stress, thereby optimizing immune responsiveness rather than amplifying it (Gombart *et al.*, 2020).

This immunodulatory effect may be associated with the bioactive compounds present in *tempe semangit*, a fermented soybean product. Fermentation has been reported to enhance the bioavailability of functional components, including bioactive peptides and isoflavones, which possess antioxidant and anti-inflammatory properties. These compounds may contribute to the regulation of inflammatory pathways and oxidative stress, thereby supporting immune homeostasis (Liu *et al.*, 2024)(Teoh *et al.*, 2024). Furthermore, dietary patterns play an important role in modulating immune and inflammatory responses, highlighting the potential of functional foods as part of a strategy

to maintain immune balance (Liu *et al.*, 2024). Recent metabolomic evidence also highlights the potential of *tempe* as a source of bioactive compounds with immunoregulatory properties (Astawan *et al.*, 2025).

Collectively, these findings indicate that *tempe semangit* cookies function as a dietary immunomodulator with a regulatory, rather than stimulatory, effect on the immune system. The results underscore the potential application of fermented soybean products as functional foods in supporting immune health, particularly among adolescents. Future investigations incorporating longer intervention periods and molecular biomarkers are warranted to further elucidate the underlying mechanisms.

3.2. Pre- and Post-Intervention Differential Leukocyte Count After *Tempe Semangit* Cookies Consumption

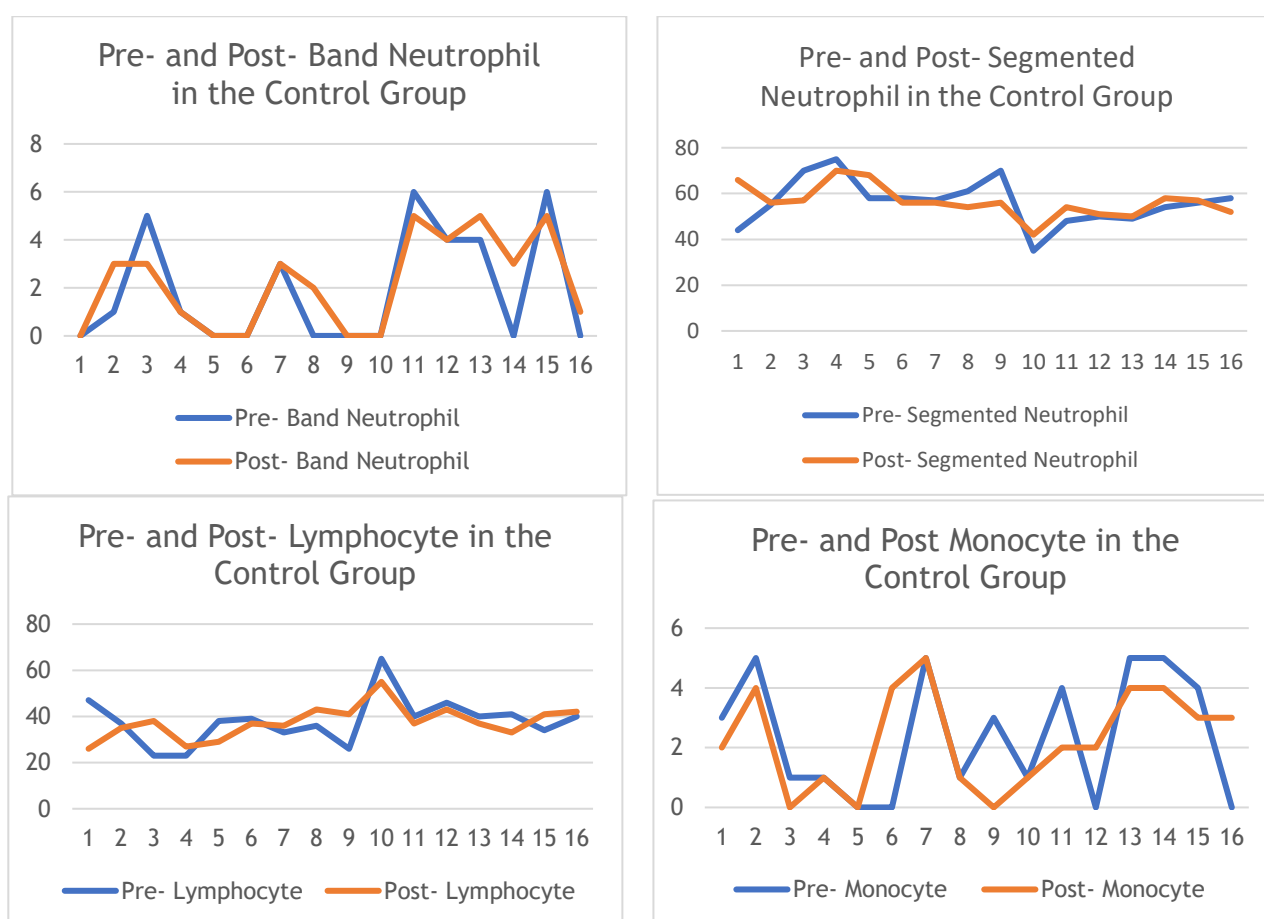


Figure 3. Pre- and Post-Intervention Differential Leukocyte Count After *Tempe Semangit* Cookies Consumption in the Control Group

The differential leukocyte profile in the control group (Figure 3) showed no substantial changes between pre- and post-observation. Band neutrophils remained low with minimal variation, while segmented neutrophils fluctuated slightly without a consistent trend. Lymphocyte and monocyte proportions also exhibited minor, non-directional changes across samples. Overall, these findings indicate a stable leukocyte distribution, reflecting maintained immune homeostasis in the control group.

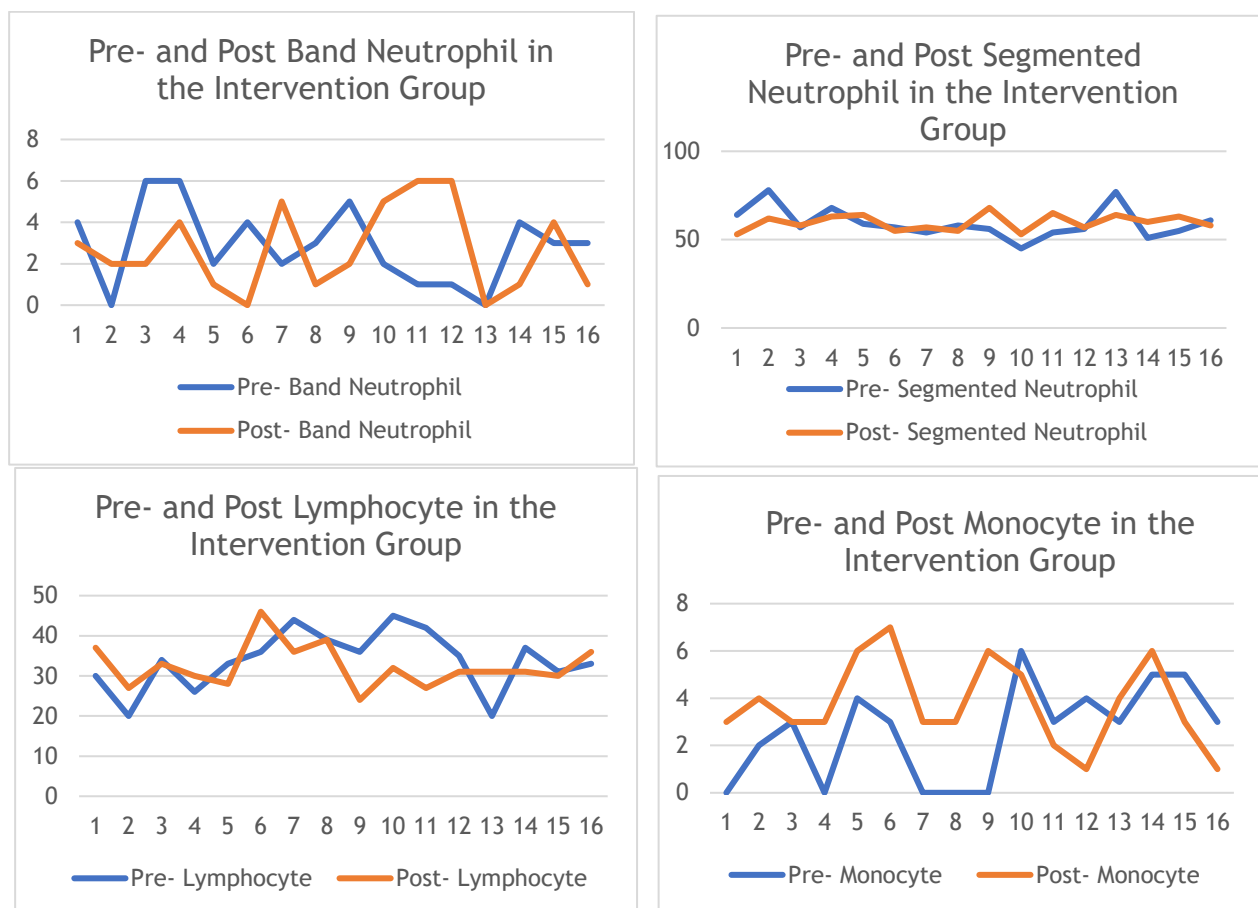


Figure 4. Pre- and Post-Intervention Differential Leukocyte Count After *Tempe Semangit* Cookies Consumption in the Intervention Group

The differential leukocyte profile in the intervention group (Figure 4) demonstrated modest variations following the intervention. Band and segmented neutrophils showed slight fluctuations without a consistent directional pattern. Lymphocyte and monocyte proportions exhibited some individual variability, with a tendency toward redistribution in several samples. Overall, these changes were not substantial, indicating that the short-

term intervention did not markedly alter leukocyte distribution, although minor shifts in immune cell proportions were observed.

The analysis of differential leukocyte counts, including band neutrophils, segmented neutrophils, lymphocytes, and monocytes, demonstrated no statistically significant differences between pre- and post-intervention measurements in both the control and intervention groups ($p > 0.05$). This finding indicates that the 7-day dietary intervention was not sufficient to induce measurable alterations in leukocyte distribution.

From a biological perspective, changes in differential leukocyte profiles require coordinated processes of hematopoiesis, cellular differentiation, and maturation in the bone marrow, which are regulated by cytokines and growth factors. These processes typically occur over a longer time frame, thereby limiting the likelihood of detecting short-term changes. Similar findings have been reported in previous nutritional intervention studies, where short-duration interventions resulted in minimal changes in leukocyte subsets despite observable effects on systemic immune markers.

In addition, the stability of leukocyte distribution observed in this study suggests that the immune system remained within a physiological baseline state. Under normal conditions, leukocyte dynamics are tightly controlled and tend to respond significantly only to strong stimuli such as infection or inflammation (Calder *et al.*, 2020). Therefore, the absence of significant changes in differential leukocyte counts may reflect that the intervention exerted a mild regulatory effect without triggering a pronounced immune response.

3.3. Between-Group Comparison of Control and Intervention Groups (Pre- and Post-Intervention)

Table 1. Independent t-test for between group comparison of control and intervention groups

			N	Sig.
Pre-Intervention Count	Total	Leukocyte	32	0,077
Post-Intervention Count	Total	Leukocyte	32	0,368
Pre-Intervention		Segmented	32	0,338

Neutrophils			
Post-Intervention Neutrophils	Segmented	32	0,129
Pre-Intervention Lymphocytes		32	0,191
Post-Intervention Lymphocytes		32	0,029
Post-Intervention Monocytes		32	0,02

Table 2. Mann-Whitney test between group comparison of control and intervention groups

	N	Sig.
Pre-Intervention Monocytes	32	0,985
Pre-Intervention Band Neutrophil	32	0,149
Post-Intervention Neutrophil	Band 32	0,468

However, between-group analysis revealed statistically significant differences in lymphocyte ($p = 0.029$) and monocyte ($p = 0.020$) levels at post-intervention. This finding suggests that the effect of the intervention is more evident in comparisons between groups rather than within-group changes. Lymphocytes, as key components of adaptive immunity, play a central role in antibody production and immune regulation, whereas monocytes, as part of the innate immune system, are involved in phagocytosis and differentiation into macrophages.

These differences may be attributed to the bioactive compounds in tempe semangit, which act as immunomodulators. Soybean-derived bioactive compounds have been reported to regulate immune responses by modulating inflammatory mediators, cytokine production, and immune cell function, thereby contributing to the maintenance of immune homeostasis (Tezuka and Imai, 2024). Therefore, the consumption of tempe semangit may help regulate immune responses, particularly innate immunity.

The absence of significant changes in band and segmented neutrophils further indicates that the intervention did not trigger an acute inflammatory response. Neutrophils typically increase during bacterial infection; thus, their stability suggests that the participants

remained in a normal physiological condition and that the intervention did not induce inflammatory stress (Calder *et al.*, 2020).

Overall, the findings of this study indicate that a 7-day intervention with *tempe semangit* cookies exerts a selective immunomodulatory effect, influencing specific parameters such as total leukocyte count, lymphocytes, and monocytes, without significantly altering the overall leukocyte differential profile. This suggests that the effect of the intervention is primarily regulatory rather than stimulatory and may be influenced by the relatively short duration of exposure and the baseline physiological condition of the participants.

4. Conclusions

The present findings demonstrate that short-term consumption of *tempe semangit* cookies is associated with specific alterations in immune-related parameters among adolescents. The observed effects were primarily reflected in total leukocyte count, along with variations in lymphocyte and monocyte levels, while the overall distribution of leukocyte subsets remained largely unchanged.

These patterns suggest that *tempe semangit* exerts a modulatory influence on the immune system, contributing to the maintenance of immunological equilibrium rather than eliciting an exaggerated inflammatory response. The unchanged neutrophil profile further supports the absence of acute immune activation during the intervention period.

From a nutritional perspective, *tempe semangit* cookies may represent a viable functional food with potential benefits for immune regulation. Nevertheless, the relatively brief duration of intervention limits the extent of observable effects. Future studies involving extended intervention periods, increased sample sizes, and more comprehensive immunological assessments are recommended to provide deeper insights into the mechanisms involved.

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Author Contributions: MH; conceptualize the study, conducted the research, and performed data analysis.

MFF, YEN and IAF: supervised the study, reviewed the manuscript, and provided scientific input. All authors read and approved the final manuscript.

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