Effects of Protein from Goat Milk Yogurt Enriched with Mung Bean Extract on White Blood Cell Profiles in Rats

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Abstract. Protein intake is crucial for white blood cell (WBC) formation and immune regulation. Goat milk yogurt enriched with mung bean extract, providing additional amino acids, may influence WBC levels, including monocytes, lymphocytes, neutrophils, eosinophils, and basophils. This study examined the impact of different yogurt doses on these WBC levels in male Wistar rats. A total of 24 rats were randomly assigned to four groups: P0 (control, no yogurt), P1 (3.6 mL/kg body weight), P2 (7.2 mL/kg body weight), and P3 (10.8 mL/kg body weight). Rats were treated for 21 days, and blood samples were collected on day 22 for analysis. The results showed a significant increase in neutrophil, monocyte, and basophil counts in the yogurt-treated groups compared to the control. No significant differences were observed in lymphocyte and eosinophil counts. The findings suggest that goat milk yogurt enriched with mung bean extract may enhance immunity.

Keywords: Goat milk yogurt, mung bean extract, white blood cells.

1 Introduction

Protein intake is critical for cellular function, enzymatic activity, and the orchestration of immune responses. At the cellular level, the quality and quantity of dietary protein influence the formation, maturation, and effector functions of white blood cells (WBCs), notably neutrophils and lymphocytes, which together underpin innate phagocytic defenses and adaptive antibody-mediated immunity, alongside key contributions from monocytes, eosinophils, and basophils in inflammatory regulation [1]. Beyond absolute intake, source and digestibility matter: animal proteins are typically complete and highly digestible, while strategic combinations of animal- and plant-derived proteins can complement amino-acid profiles and bioactive payloads to support immune function [2]. Emerging human trials further demonstrate that protein source modulates physiological mediators of health, including vascular and mineral-metabolism signals, highlighting the broader systemic ramifications of protein choice [3], [4]. Observations from aging and population studies link prudent protein patterning with successful health

trajectories, reinforcing the relevance of protein quality to immune resilience across the lifespan [5].

Goat milk–based foods are attracting attention as alternatives to bovine dairy because of favorable digestibility, lower allergenic potential, and a rich complement of bioactive peptides and essential amino acids. Fermentation into yogurt delivers both high-quality protein and probiotic microorganisms that can modulate the gut microbiota—an axis integral to systemic immunity [6]. Technological development has extended these concepts to prototypes and products in which dairy matrices are fortified with legume-derived ingredients to bolster nutritional and functional attributes [7], [8], [9]. Notably, goat milk yogurt enriched with mung bean extract has been characterized for its physicochemical, microbiological, and sensory properties, supporting the feasibility of such hybrid formulations for health-oriented applications [10].

Mung bean (*Vigna radiata*) is a legume notable for its protein density, essential amino acids, and a diverse portfolio of phytochemicals (e.g., flavonoids, phenolic acids) with antioxidant and anti-inflammatory potential. Germination and fermentation can further enhance polyphenol content and bioactivity, with reported benefits spanning glycemic control, redox balance, and immune modulation [11], [12]. In vitro and in vivo reports show antiviral, antistress, and chemopreventive activities of mung bean preparations, while probiotic-enriched sprouts yield potentially bioaccessible phenolics with antioxidant properties [13], [14], [15]. Broader botanical and processing perspectives—such as effects of fucoidan/alginate on germination, ultrasound/GABA pretreatments, and fungal bioprocessing—underscore how tailored bioprocessing can potentiate legume functionality, including antioxidant and antidiabetic activities [16], [17], [18]. Animal and cellular studies further document anti-inflammatory and hepatoprotective actions of untreated, germinated, or fermented mung bean extracts, and improvements in lipid profiles and liver protection with mung bean–based diets [19], [20]. Together, these data position mung bean as a promising immunonutritional adjunct when integrated into dairy matrices [21].

When animal and plant proteins are combined within a fermented dairy vehicle, complementary amino-acid profiles may converge with probiotic-mediated gut–immune crosstalk to influence WBC development and function. Proof-of-concept efforts in food systems demonstrate that incorporating legume components into yogurt can enhance antioxidant capacity, modify texture and microbiology, and maintain consumer-acceptable sensory profiles—prerequisites for real-world impact [7], [8], [9]. Goat milk yogurt enriched with mung bean extract exemplifies such a strategy and aligns with evidence that protein source and processing shape physiological endpoints relevant to immune competence [6], [22].

Preclinical models are well suited to disentangle dose–response relationships between novel food formulations and immune readouts. Wistar rats offer translational insights into immunological effects of dietary manipulations, with prior work showing that protein quality and bioactive legume components can alter oxidative status, stress physiology, and metabolic control—factors intimately tied to leukocyte dynamics [23], [24]. Although not immune models per se, aquaculture and zoological studies provide convergent evidence that dietary bioprocessed proteins—including plant sources and lactobacilli-inoculated feeds—can modulate performance and immunological indices, reinforcing diet–immunity linkages across species [25], [26]. Considerations of allergenicity and protein taxonomy further emphasize the

value of matrix and source when anticipating immune outcomes [27]. From a mechanistic vantage point, cellular maintenance pathways impacted by nutrition—such as lysosomal integrity—also intersect with immune cell homeostasis, suggesting broader diet-regulated levers that may influence WBC profiles [28].

Against this backdrop, the present study investigates whether varying doses of goat milk yogurt enriched with mung bean extract modulate circulating WBC subpopulations—neutrophils, lymphocytes, monocytes, eosinophils, and basophils—in male Wistar rats. By leveraging a hybrid animal–plant protein matrix with probiotic fermentation, this work addresses a clear gap in the experimental literature regarding combined protein sources within a single functional food and their immunological consequences [8], [9], [10]. The anticipated contribution is twofold: first, to delineate dose-dependent effects of the formulation on discrete leukocyte lineages; and second, to inform the rational design of functional foods that harness complementary protein sources and bioactives to enhance immune resilience—a thesis consistent with epidemiologic and clinical signals linking protein patterning to health outcomes [4], [29], [30].

2 Methods

2.1 Chemicals and reagents

All the ingredients used in this study were procured from local markets in North Sumatra, Indonesia, to ensure the use of regionally sourced materials. These included goat milk (*Capra aegagrus hircus*), mung bean (*Vigna radiata*), yogurt starter, and sugar. The goat milk was obtained from nearby dairy farms and served as the main base for yogurt production. The milk was fresh and pasteurized to guarantee its safety and consistency throughout the production process. The yogurt starter used was Biokul® plain yogurt (PT. Diamond Cold Storage, Indonesia), which contains *Lactobacillus acidophilus* and *Bifidobacterium species*. Reagents and materials for the white blood cell (WBC) analysis were obtained from certified laboratory suppliers, ensuring the reliability and accuracy of the blood tests. The blood samples were processed and analyzed to determine differential WBC counts, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, using standard hematological methods.

2.2 Animal experiments design

Study design. This study employed a randomized controlled design with four experimental groups: the control group (P0), which received a volume-matched sterile water gavage, and three experimental groups (P1, P2, P3), where rats received different doses of yogurt enriched with mung bean extract (3.6 mL/kg body weight for P1, 7.2 mL/kg body weight for P2, and 10.8 mL/kg body weight for P3). The study followed the ARRIVE Essential 10 checklist for reporting animal experiments [31] and was approved by the Animal Research Ethics Committees FMIPA USU (approval no. 0640/KEPH-FMIPA/2024).

Sample size. The sample size for this study was determined based on the method outlined by Charan et al. [32], which recommends a minimum number of animals per group for a one-way ANOVA design. Given that the study involved four experimental groups (control and three experimental doses), the sample size calculation for each group was based on the formula: $n = 10/(k+1)$ for the minimum and $n = 20/(k+1)$ for the maximum, where k represents the number of

groups. For this study, with k=4, the calculated sample size per group was 2 animals for the minimum and 4 animals for the maximum. However, to account for a 20% dropout rate, the sample size per group was increased to 6 animals, resulting in a total of 24 rats for the study (4 groups × 6 animals per group = 24 rats). This ensures adequate statistical power and compensates for potential exclusions during the experiment.

Inclusion and exclusion criteria. The inclusion criteria for this study were male Wistar rats (*Rattus norvegicus*) aged 8-10 weeks, with a body weight between 100-180 g at baseline, in good health, and showing no signs of injury or disease. The exclusion criteria included animals exhibiting signs of distress during the study. A total of 6 animals were assigned to each experimental group, and this number represents the final sample size analyzed for each group, as no animals were excluded throughout the course of the experiment.

Randomisation and blinding. Randomisation was used to allocate rats to one of the four experimental groups (control and three experimental doses) using a computer-generated sequence, ensuring unbiased distribution. To minimise potential confounders, treatments were administered at the same time daily, and animals were housed under identical conditions, with cage locations and treatment orders balanced. Blinding was not implemented, as the researchers were aware of group allocations during treatment administration, outcome assessments, and data analysis. The absence of blinding should be considered when interpreting the results.

Experimental prosedures. Rats from all experimental groups, including the control, were subjected to a daily oral gavage of either sterile water (control group) or goat milk yogurt enriched with mung bean extract for 21 days. The yogurt was formulated using a blend of 85% goat milk and 15% mung bean extract, with 10% sugar and 7.5% starter culture added to facilitate fermentation. The final yogurt formulation had a protein content of 3.2% [10]. The rats received specific doses of this yogurt (3.6 mL/kg body weight for P1, 7.2 mL/kg body weight for P2, and 10.8 mL/kg body weight for P3). The rats were weighed daily to adjust the volume of yogurt or water administered according to their body weight. The treatment was administered once a day between 08:00 and 10:00 AM to maintain consistency. The animals were housed in a temperature-controlled environment with a 12:12 light–dark cycle, and they were acclimated to the environment for 7 days before the treatment began to minimize stress and ensure they were comfortable in the experimental conditions. The rationale for these procedures was to assess the impact of different doses of yogurt enriched with mung bean extract on the white blood cell profiles, with the control group providing baseline data for comparison.

2.3 Outcome measures

The primary outcome measure was the white blood cell (WBC) profile of male Wistar rats, which was assessed to evaluate the immunomodulatory effects of goat milk yogurt enriched with mung bean extract. The WBC profile was measured by collecting blood samples on day 22 of the study, after 21 days of daily gavage administration. The blood samples were analyzed for the total number and differential count of five types of WBCs: neutrophils, lymphocytes, monocytes, eosinophils, and basophils. The analysis was conducted using a veterinary hematology analyzer, which provided precise counts of each WBC subtype. The primary outcome measure used to determine the sample size was the differential WBC count, as it was hypothesized that the intervention would influence the levels of these immune cells. The differential counts of WBCs were the key data for statistical analysis, aiming to compare the

effects of the different doses of yogurt enriched with mung bean extract across the experimental groups.

2.4 Statistical analysis

Data were analyzed using one-way ANOVA to compare the means between the control and experimental groups. The analysis was performed using SPSS software, with a significance level set at alpha = 0.05. If significant differences were found, post-hoc pairwise comparisons were conducted using the Duncan's multiple range test. The data are presented as mean ± standard deviation (SD).

3 Results and Discussion

This study aimed to examine the effects of goat milk yogurt enriched with mung bean extract on the white blood cell (WBC) profiles of male Wistar rats. Protein, a vital macronutrient, is essential for the formation and function of leukocytes, which are critical components of the immune system. Protein deficiency can result in abnormalities in cell structure and function [33]. Leukocytes are one of the cell types that function as agents of the body's immune system. Protein is also necessary for the formation of all types of leukocytes, including neutrophils, basophils, eosinophils, monocytes, and lymphocytes [34]. Table 1 demonstrated that, while eosinophils and lymphocytes did not show significant differences across treatments, neutrophils, basophils, and monocytes exhibited significant changes, highlighting a dose-dependent response to the yogurt intervention.

Table 1. White Blood Cell Counts with Mean ± SD by Treatment Group[1].

White blood cell type	P0 (Control)	P1 (3.6 mL/kg)	P2 (7.2 mL/kg)	P3 (10.8 mL/kg)
Neutrophils	15.67 ± 2.08^a	23.00 ± 3.83^{ab}	34.25 ± 7.63^b	34.75 ± 14.22^b
Lymphocytes	64.00 ± 5.20^a	66.00 ± 0.00^a	69.33 ± 3.05^a	73.33 ± 7.02^a
Monocytes	2.50 ± 0.71^a	6.33 ± 2.31^{ab}	10.00 ± 1.73^b	9.67 ± 2.08^b
Eosinophils	0.00 ± 0.00^a	0.00 ± 0.00^a	0.50 ± 0.54^a	0.17 ± 0.41^a
Basophils	3.00 ± 0.00^a	5.00 ± 0.00^{ab}	6.00 ± 2.64^b	6.00 ± 1.00^b

Neutrophils are a critical component of the body's innate immune system, serving as the first line of defense against infections. These cells are highly specialized in phagocytosis, a process by which they engulf and digest pathogens, such as bacteria and fungi, to prevent the spread of infection. Neutrophils are essential for maintaining tissue homeostasis during inflammation, and their rapid recruitment to infection sites is a hallmark of the early immune response [34]. The significant (p<0.05) increase in neutrophil counts observed in the yogurt-treated groups, particularly in Group P3 (10.8 mL/kg), suggests that the yogurt intervention significantly enhances the function of these immune cells. This increase in neutrophils implies that the intervention could be improving the body's ability to mount an effective and rapid response to bacterial infections, thereby strengthening the body's immune defense mechanisms.

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[1] The letters that differ on the same row indicate significant differences at the 0.05 alpha level.

The findings are consistent with previous studies, which have demonstrated that dietary interventions, especially those incorporating plant-based extracts, can activate neutrophil activity and boost immune responses [35]. These plant extracts, like those from mung beans, are known to contain bioactive compounds, including flavonoids and phenolic acids, that have antioxidant and anti-inflammatory properties [19]. These compounds likely play a role in the modulation of immune responses, stimulating immune cell proliferation and enhancing phagocytic capacity. The increased neutrophil count observed in this study suggests that mung bean extract, in combination with goat milk yogurt, may enhance immune responses by not only increasing the number of neutrophils but also potentially improving their functional capacity to clear pathogens more efficiently.

Moreover, the increase in neutrophil count observed in the higher dose group (P3) indicates a dose-dependent effect, where a higher concentration of the intervention likely provides a stronger stimulus for neutrophil activation. This aligns with the literature suggesting that probiotics and functional foods, like the yogurt used in this study, can activate immune responses through their direct effects on immune cells or via the modulation of gut microbiota, which in turn influences systemic immunity [34], [35]. These findings support the hypothesis that dietary interventions, particularly those involving fermented products such as yogurt, can have significant effects on immune function by enhancing the activity and efficacy of neutrophils, providing an important boost to the body's first line of defense against infections. The substantial increase in neutrophil counts observed in the high-dose yogurt group suggests that the intervention may play an essential role in improving the body's immune capacity, particularly in its response to bacterial pathogens. These findings underline the potential of using goat milk yogurt enriched with mung bean extract as a functional food to support and enhance immune function, particularly by strengthening the neutrophil-driven early immune response. Further investigations into the specific mechanisms of action of mung bean extract and its interaction with the gut microbiota could provide deeper insights into how this intervention can be utilized to boost immunity in broader contexts.

Monocytes play a pivotal role in the body's innate immune response, acting as versatile immune cells that respond to various pathogens and tissue damage. They help clear pathogens and debris through phagocytosis and are key in initiating and regulating inflammation. The significant increase in monocyte counts observed in the higher-dose groups, especially in Group P3 (p<0.05), suggests that the intervention with goat milk yogurt enriched with mung bean extract may stimulate an immune activation, potentially in response to microbial invaders or endogenous signals indicating infection or injury. This finding aligns with previous research showing that dietary interventions, particularly those involving plant-based extracts, can stimulate immune responses by modulating various immune cells, including monocytes [36].

Monocytes are precursors to macrophages, which are specialized in pathogen clearance, antigen presentation, and modulation of inflammation [34], [37]. When activated, monocytes differentiate into macrophages, which play a critical role in both acute and chronic inflammation. The increase in monocyte count in this study could, therefore, reflect a priming of the immune system, potentially leading to the differentiation of monocytes into macrophages, which enhance the body's ability to fight infections and regulate immune responses. This process is vital in controlling both the initiation and resolution of inflammation, as macrophages can either amplify or suppress immune responses depending on their activation state [38].

Moreover, mung bean extract, which is rich in bioactive compounds such as flavonoids and phenolic acids, has known anti-inflammatory and immunomodulatory properties [19]. These

compounds may influence the differentiation and activity of monocytes, as well as their ability to clear pathogens or inflammatory mediators, highlighting the bioactive potential of mung bean as an immunomodulatory agent. The increase in monocytes in response to the yogurt intervention may therefore be linked to these bioactive compounds, which could act as immune enhancers through their ability to modulate cytokine production and immune signaling pathways.

According to Widmaier et al. [36], monocytes are often mobilized in response to pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs), both of which trigger an immune response to protect the body. The significant increase in monocytes observed in this study may indicate that the yogurt with mung bean extract is enhancing the body's recognition and response to pathogens or tissue injury. In this context, the yogurt could act as an immune stimulant, contributing to better pathogen clearance and more effective immune responses. This aligns with studies showing that dietary interventions, especially those involving fermented foods, can enhance the immune system's ability to recognize and respond to foreign threats [35].

Basophils are a type of granulocyte that play a significant role in allergic reactions and inflammatory responses. These cells are involved in the release of histamine and other mediators during allergic reactions, which can lead to symptoms such as itching, swelling, and bronchoconstriction. The observed significant increase in basophil counts, particularly in Group P3, suggests that the yogurt intervention may have triggered an allergic or inflammatory response in the rats. This increase aligns with the role of basophils in responding to allergens, as they are known to release histamine and other pro-inflammatory cytokines when activated by immune stimuli [34].

The increase in basophils in this study may be attributed to the bioactive compounds present in mung bean extract, which have been shown to induce allergic reactions in some individuals [16]. Mung beans are rich in flavonoids, phenolic compounds, and lectins, all of which can interact with the immune system and potentially trigger allergic responses, especially in sensitized individuals. These compounds may stimulate basophils and other immune cells, resulting in increased basophil activity and the release of histamine. As basophils are key players in IgE-mediated allergic responses, their activation could indicate that the yogurt with mung bean extract may act as an allergen in this context.

Furthermore, basophils are not only involved in allergic reactions but also play a role in chronic inflammation and immune defense [34], [36]. The increase in basophils observed in the study suggests that the intervention could be modulating both acute and chronic aspects of the immune response. Basophils, along with other immune cells, may be involved in the regulation of inflammatory pathways and immune homeostasis. The presence of bioactive compounds in mung beans, which have anti-inflammatory and immunomodulatory properties, could be contributing to this heightened immune activation, particularly by stimulating basophils to release cytokines and other mediators that amplify the immune response [19], [35]. It is also worth considering that probiotic effects from the yogurt fermentation process might enhance gut-associated immunity, which is increasingly recognized as a crucial factor in systemic immune responses [37]. Fermented foods, including yogurt, contain beneficial bacteria that can influence the gut microbiota and in turn affect the gut-associated lymphoid tissue (GALT). This could lead to an amplification of systemic immune activation, including the recruitment and activation of basophils, which may have contributed to the observed increase in basophil counts in this study.

Overall, the observed increase in basophils supports the hypothesis that the yogurt intervention, particularly with the inclusion of mung bean extract, stimulates both immune activation and allergic responses. The presence of bioactive compounds in mung beans may be playing a central role in modulating the immune system by activating basophils and enhancing inflammatory pathways. However, the allergic response observed suggests that further research is needed to carefully evaluate the immunological impacts of such dietary interventions, particularly in individuals with sensitivities to plant-derived compounds. Future studies could explore the potential for allergenicity and the dose-response relationship of mung bean extract in influencing immune cell dynamics, particularly in relation to basophils and allergic responses.

While eosinophils and lymphocytes did not show significant changes, their roles in immune defense are also important. Eosinophils are involved in parasitic infections and allergic responses, but their role in response to dietary changes might not be as pronounced in this study. Lymphocytes, crucial for adaptive immunity, did not show significant changes, which suggests that a stronger or more prolonged stimulus may be needed to alter their levels. This indicates the complexity of immune responses to dietary interventions, as different immune cells may respond differently based on the type of stimulus [36], [39].

The results of this study support the hypothesis that mung bean extract, when incorporated into goat milk yogurt, can influence immune function by modulating leukocyte populations, particularly neutrophils, basophils, and monocytes. Mung beans contain flavonoids and phenolic compounds that have antioxidant and anti-inflammatory properties, which could play a role in modulating the immune system [19], [40]. The fermentation process involved in yogurt production may further enhance the bioavailability of these compounds, improving their absorption and effectiveness in immune modulation [15]. The findings from this study demonstrate that goat milk yogurt enriched with mung bean extract has the potential to modulate the immune system, particularly by increasing neutrophils, basophils, and monocytes. These results provide insight into the potential of functional foods to enhance immune function. However, the lack of significant changes in eosinophils and lymphocytes suggests that further research is needed to explore the specific mechanisms underlying these immune-modulating effects and to investigate whether these findings translate to long-term health benefits. Future studies should also examine the role of the bioactive compounds in mung beans and the yogurt fermentation process in influencing immune responses, as well as the potential implications for dietary strategies aimed at promoting immune health.

4 Conclusion

This study demonstrates that goat milk yogurt enriched with mung bean extract can modulate white blood cell profiles in male Wistar rats, specifically increasing neutrophil, monocyte, and basophil counts. These findings suggest that the combination of goat milk and mung bean extract may enhance immune function by stimulating key components of the immune system. However, no significant effects were observed on lymphocytes and eosinophils, indicating that the immune-modulating effects of the yogurt may be selective. Overall, the results support the potential of goat milk yogurt enriched with mung bean extract as a functional food with immunomodulatory benefits, warranting further investigation to explore its broader applications in immune health.

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