



Potential of Palm Oil, Sacha Inchi Oil, and Turmeric Combination as Feed Supplementation on Leukocyte Profile in Laying Hens

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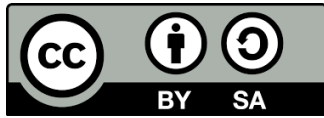
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Abstract

Feed quality plays an important role in maintaining the productivity and physiological health of laying hens. This study aimed to evaluate the effects of dietary supplementation with palm oil, sachu inchi oil, and turmeric on the leukocyte profile of laying hens. The study employed an experimental method using a completely randomized factorial design with two factors: the combination of oil types and turmeric supplementation. The parameters evaluated included total leukocyte count, differential leukocyte counts (monocytes, lymphocytes, heterophils, eosinophils, and basophils), and the heterophil-to-lymphocyte (H/L) ratio. Data were analyzed using analysis of variance (ANOVA), followed by Duncan's multiple range test. The results showed that the treatment combinations affected the leukocyte profile and H/L ratio, although all measured values remained within normal physiological ranges. Supplementation with 1.5% palm oil and 1.5% sachu inchi oil without turmeric resulted in the lowest H/L ratio and maintained a balanced leukocyte profile. In conclusion, dietary supplementation with 1.5% palm oil and 1.5% sachu inchi oil without turmeric showed the greatest potential for maintaining physiological homeostasis and a balanced leukocyte profile in laying hens.



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INTRODUCTION

The poultry industry, particularly the laying-hen sector, plays an important role in supplying affordable, high-quality animal protein. In Indonesia, the demand for eggs continues to increase with population growth and greater public awareness of nutritional needs (Kementerian Pertanian, 2023). This trend highlights the importance of maintaining optimal physiological health in laying hens because their health status is closely associated with their ability to sustain normal egg production (Al-Khalaifah & Al-Nasser, 2026; Bonnefous et al., 2022; Gao et al., 2025; Kim et al., 2024; Sharma & Kim, 2024). Feed is one of the major factors affecting the physiological condition of laying hens and may account for up to 60% of production costs. Therefore, the appropriate selection of feed ingredients, including dietary fat sources and functional feed additives, is important for supporting physiological and immune responses (Natha et al., 2025).

Palm oil is commonly used as a dietary energy source and has relatively high oxidative stability because of its fatty acid composition. In contrast, sachu inchi oil (*Plukenetia volubilis* L.) contains high levels of unsaturated fatty acids, particularly omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), which have the potential to support immune function and improve the nutritional quality of animal products (Maya & Sriwidodo, 2022; Yang et al., 2025). However, its high PUFA content also makes sachu inchi oil more susceptible to lipid oxidation, particularly during prolonged storage or exposure to unfavorable environmental conditions, potentially increasing the oxidative challenge associated with the diet (Leskovec et

al., 2024). Therefore, combining an oxidatively stable dietary fat source with appropriate antioxidant supplementation may improve the utilization of sachai oil in poultry diets.

Turmeric (*Curcuma longa* L.) is a natural feed additive containing curcuminoids, particularly curcumin, and other bioactive compounds with antioxidant properties (Aderemi & Alabi, 2023; Ardelean et al., 2026; Jalali & Ghasemzadeh Rahbardar, 2026; Jyotirmayee et al., 2024). Dietary supplementation with turmeric may help mitigate oxidative processes associated with diets rich in unsaturated fatty acids and thereby support physiological homeostasis (Sahara et al., 2022). Although the nutritional and biological properties of palm oil, sachai oil, and turmeric have been investigated individually, evidence regarding their combined supplementation and effects on the leukocyte profile of laying hens remains limited. This represents a relevant research gap because leukocyte profiles may serve as hematological indicators of physiological and immune responses in poultry. Differential leukocyte counts, including heterophils, lymphocytes, monocytes, eosinophils, and basophils, together with the heterophil-to-lymphocyte (H/L) ratio, can provide information on changes in the physiological and stress responses of laying hens.

Based on this research gap, the study investigated whether dietary supplementation with combinations of palm oil, sachai oil, and turmeric affected total leukocyte counts, differential leukocyte counts, and the H/L ratio of laying hens. The study employed an experimental feeding trial involving different combinations of dietary fat sources and turmeric supplementation. The novelty of this study lies in evaluating the combined supplementation of palm oil, sachai oil, and turmeric in relation to the leukocyte profile of laying hens rather than examining each dietary component separately. The findings were expected to provide scientific evidence regarding the potential use of these feed components as a complementary dietary strategy for maintaining physiological homeostasis and supporting immune function in laying hens.

This study aimed to evaluate the effects of dietary supplementation with palm oil, sachai oil, and turmeric on the leukocyte profile of laying hens. Theoretically, this research contributes to the literature on poultry nutrition, particularly regarding the use of dietary lipid sources and functional feed additives in relation to hematological and immune responses. Practically, the findings may provide a scientific basis for developing dietary formulations that support the physiological and immune health of laying hens. The principal contribution of this research lies in integrating different dietary lipid sources with a natural antioxidant-containing feed additive within a single experimental design, an approach that remains relatively underexplored in studies of laying-hen nutrition.

METHOD

Experimental Design

The study employed a completely randomized design (CRD) with a 3×2 factorial arrangement. The first factor was dietary oil supplementation at three levels: 3% crude palm oil (M0), 1.5% crude palm oil combined with 1.5% sachai oil (M1), and 3% sachai oil (M2). The second factor was turmeric supplementation at two levels: 0% (K0) and 1% (K1).

The factorial arrangement produced six dietary treatments (M0K0, M0K1, M1K0, M1K1, M2K0, and M2K1). Each treatment was replicated three times, resulting in 18 experimental units, with one laying hen assigned to each experimental unit.

Animals, Housing, and Feeding

A total of 18 laying hens (Isa Brown strain), aged 16 week were used in this study. Birds were randomly assigned to six treatment groups and housed individually in battery cages under similar environmental conditions at the Integrated Field Laboratory, Universitas Islam Malang, Malang, East Java, Indonesia. The experiment was conducted for 6 weeks.

The basal diet was formulated by the researchers to meet standard nutrient requirements and was designed to be isoenergetic and isoprotein. The composition and nutrient content of the basal diet are presented in Table 1. Feed and drinking water were provided ad libitum throughout the experimental period.

Palm oil (crude palm oil, CPO), sacha inchi oil (*Plukenetia volubilis*), and turmeric were used as feed supplements. Sacha inchi oil and palm oil were obtained from commercial suppliers via an online marketplace, while turmeric was used in the form of commercial turmeric powder (maceration-based product). All materials were sourced from local suppliers in Malang, Indonesia.

Sample Collection and Laboratory Analysis

At the end of the experimental period, approximately 2 mL of blood samples were collected from the brachial vein (wing vein) using a 3 mL syringe and transferred into heparinized vacutainer tubes. Samples were stored in an icebox with ice packs and transported to the laboratory for further analysis. Blood smears were prepared and stained using 2% Giemsa or May–Grünwald–Giemsa (MDT) staining methods. Leukocyte differentiation was observed under a standard laboratory light microscope at 1000× magnification using immersion oil. Differential leukocyte counts (heterophils, lymphocytes, monocytes, eosinophils, and basophils) were determined by counting 100 leukocytes per sample (Samudra et al., 2024). The heterophil/lymphocyte (H/L) ratio was calculated as an indicator of physiological stress.

Statistical Analysis

Data on total leukocyte counts, differential leukocyte percentages, and H/L ratio were analyzed using analysis of variance (ANOVA) based on a factorial completely randomized design. The statistical model included the effects of oil type, turmeric supplementation, and their interaction. When significant differences were observed ($P < 0.05$), Duncan's multiple range test was applied for mean separation.

Table 1. Feed Ingridients Formulation

No	Feed Ingredient (%)	M0K0	M0K1	M1K0	M1K1	M2K0	M2K1
1	Yellow corn	50.00	50.00	50.00	50.00	50.00	50.00
2	Rice bran	11.50	11.50	11.50	11.50	11.50	11.50
3	Soybean meal	12.00	12.00	12.00	12.00	12.00	12.00
4	Corn gluten meal	5.00	5.00	5.00	5.00	5.00	5.00
5	Fish meal	7.00	7.00	7.00	7.00	7.00	7.00
6	Meat bone meal (MBM)	5.00	5.00	5.00	5.00	5.00	5.00
7	Palm oil (CPO)	3.00	3.00	1.50	1.50	0.00	0.00
8	Sacha inchi oil	0.00	0.00	1.50	1.50	3.00	3.00
9	Dicalcium phosphate	1.50	1.50	1.50	1.50	1.50	1.50

No	Feed Ingredient (%)	M0K0	M0K1	M1K0	M1K1	M2K0	M2K1
10	Calcium carbonate	3.00	3.00	3.00	3.00	3.00	3.00
11	Sodium chloride	1.00	1.00	1.00	1.00	1.00	1.00
12	Premix	1.00	1.00	1.00	1.00	1.00	1.00
13	Turmeric powder	0.00	1.00	0.00	1.00	0.00	1.00
Nutrient Content							
No.	Nutrient	M0K0	M0K1	M1K0	M1K1	M2K0	M2K1
1	Moisture (%)	11.29	11.29	11.29	11.29	11.29	11.29
2	Dry matter (%)	88.71	88.71	88.71	88.71	88.71	88.71
3	Metabolizable energy (kcal/kg)	3003.60	2996.10	3003.60	3003.60	3011.10	3011.10
4	Crude protein (%)	20.26	20.26	20.26	20.26	20.26	20.26
5	Crude fat (%)	3.29	3.29	3.29	3.29	3.29	3.29
6	Crude fiber (%)	3.21	3.21	3.21	3.21	3.21	3.21
7	Calcium (%)	2.51	2.51	2.51	2.51	2.51	2.51
8	Phosphorus (%)	0.92	0.92	0.92	0.92	0.92	0.92

RESULTS AND DISCUSSION

The effects of dietary oil type and turmeric supplementation on leukocyte profile, including total leukocyte count, differential leukocytes, and H/L ratio, are presented in Table 2. Overall, all parameters remained within the normal physiological range, indicating that the dietary treatments did not induce pathological conditions in laying hens.

Total Leukocyte Count

Total leukocyte counts across all treatments remained within the normal physiological range, indicating that the dietary interventions did not induce pathological conditions in laying hens. Turmeric supplementation significantly increased leukocyte levels compared to non-supplemented groups ($P < 0.05$), suggesting an enhancement of immune activity. This effect is likely associated with the immunomodulatory and antioxidant properties of curcumin, which can stimulate immune cell function and protect against oxidative stress (Sahara et al., 2022).

The type of dietary oil showed a moderate effect on leukocyte levels. Diets containing palm oil tended to produce higher leukocyte counts compared to those containing sachai inchi oil. This may be attributed to the higher saturated fatty acid content in palm oil, which is associated with increased inflammatory responses (Hamid et al., 2025). In contrast, sachai inchi oil, rich in omega-3 fatty acids, may exert anti-inflammatory effects by modulating immune cell membrane composition and reducing excessive immune activation (Samrit et al., 2024).

The interaction between oil type and turmeric supplementation indicated that turmeric enhanced leukocyte responses across treatments, although the effect was more pronounced in palm oil-based diets. These findings suggest that turmeric plays a dominant role in stimulating immune responses, while dietary fat composition modulates the intensity of the response.

Differential Leukocyte Profile

Monocytes

Monocyte values remained within the normal physiological range, indicating the absence of chronic inflammation. Oil type significantly influenced monocyte levels ($P < 0.05$), with higher values observed in palm oil treatments compared to combined oil treatments. This suggests that saturated fatty acids may stimulate innate immune activity, whereas omega-3 fatty acids from sachai inchi oil may suppress excessive inflammatory responses (Alagawany

et al., 2019). Turmeric supplementation did not significantly affect monocyte levels, indicating that its role is more likely related to immune regulation rather than direct stimulation of monocyte production. The lowest monocyte values observed in the combined oil treatment reflect a more balanced immune response due to the interaction between saturated and unsaturated fatty acids.

Table 2. Two-Way Table of Total Leukocytes and Their Differentiation

		0% Turmeric	1% Turmeric	Mean
Total leukocyte	3% Palm oil	17.00 ± 0.60a	21.33 ± 0.58c	19.17 ± 2.48A
	1.5% palm oil + 1.5% sacha inchi oil	17.33 ± 0.58a	19.00 ± 0.00ab	18.17 ± 0.98B
	3% sacha inchi oil	17.00 ± 0.00a	18.00 ± 1.00a	17.50 ± 0.84B
	Mean	17.11 ± 0.60A	19.44 ± 1.59B	
heterophils	3% Palm oil	30.33 ± 0.12d	29.00 ± 0.40c	29.67 ± 0.78C
	1.5% palm oil + 1.5% sacha inchi oil	26.40 ± 0.53a	27.53 ± 0.31ab	26.97 ± 0.73A
	3% sacha inchi oil	27.60 ± 0.20ab	28.20 ± 1.40bc	27.90 ± 0.95B
	Mean	28.11 ± 1.77	28.24 ± 0.98	
Lymphocytes	3% Palm oil	31.67 ± 0.58c	32.33 ± 0.12b	32.00 ± 0.52B
	1.5% palm oil + 1.5% sacha inchi oil	33.80 ± 0.35a	32.87 ± 0.23b	33.33 ± 0.58A
	3% sacha inchi oil	32.93 ± 0.23b	33.00 ± 0.40b	32.97 ± 0.29A
	Mean	32.80 ± 0.99	32.73 ± 0.39	
heterophil/ lymphocyte (H/L)	3% Palm oil	0.96 ± 0.02d	0.90 ± 0.01c	0.93 ± 0.04C
	1.5% palm oil + 1.5% sacha inchi oil	0.78 ± 0.02a	0.84 ± 0.01b	0.81 ± 0.03A
	3% sacha inchi oil	0.84 ± 0.01b	0.85 ± 0.05bc	0.85 ± 0.04B
	Mean	0.86 ± 0.08	0.86 ± 0.04	
Monocytes	3% Palm oil	11.87 ± 0.31d	11.67 ± 0.61cd	11.77 ± 0.45C
	1.5% palm oil + 1.5% sacha inchi oil	10.07 ± 0.12a	11.27 ± 0.12bc	10.67 ± 0.67A
	3% sacha inchi oil	11.40 ± 0.00cd	10.73 ± 0.31b	11.07 ± 0.41B
	Mean	11.11 ± 0.83	11.11 ± 0.53	
basophils	3% Palm oil	0.47 ± 0.12	0.33 ± 0.23	0.40 ± 0.18
	1.5% palm oil + 1.5% sacha inchi oil	0.33 ± 0.12	0.20 ± 0.00	0.27 ± 0.10
	3% sacha inchi oil	0.47 ± 0.12	0.27 ± 0.12	0.37 ± 0.15
	Mean	0.42 ± 0.12B	0.27 ± 0.14A	
Eosinophils	3% Palm oil	25.67 ± 0.70	26.67 ± 0.90	26.17 ± 0.91C
	1.5% palm oil + 1.5% sacha inchi oil	29.40 ± 0.72	28.13 ± 0.23	28.77 ± 0.84A
	3% sacha inchi oil	27.60 ± 0.00	27.80 ± 1.20	27.70 ± 0.77B
	Mean	27.56 ± 1.69	27.53 ± 1.01	

Notes: Values are presented as mean ± standard deviation. Lowercase superscript letters within treatment-combination cells indicate significant differences among the six treatment combinations based on Duncan's multiple range test ($P < 0.05$) when the oil × turmeric interaction was significant. Uppercase superscript letters in the marginal means indicate significant differences in the corresponding main effects of oil type or turmeric supplementation ($P < 0.05$). For parameters without a significant interaction, treatment-combination cells are presented without superscripts, and significant main effects are indicated in the marginal means. M0 = 3% palm oil; M1 = 1.5% palm oil + 1.5% sacha inchi oil; M2 = 3% sacha inchi oil; K0 = 0% turmeric; K1 = 1% turmeric.

Lymphocytes

Lymphocyte levels remained stable across treatments, indicating that adaptive immune function was well maintained. Diets containing sachai inchi oil, particularly in combination with palm oil, tended to produce higher lymphocyte values, suggesting a supportive role of omega-3 fatty acids in lymphocyte proliferation and immune regulation (Wang et al., 2024). Turmeric supplementation did not significantly affect lymphocyte levels, supporting the notion that curcumin primarily acts as an immunomodulator rather than a direct stimulator of lymphocyte proliferation. The relatively higher lymphocyte values observed in balanced oil treatments indicate improved immune homeostasis.

Heterophils

Heterophil values varied among treatments but remained within normal ranges. Lower heterophil levels were observed in diets combining palm oil and sachai inchi oil, suggesting reduced activation of innate immune responses. This may reflect the anti-inflammatory properties of omega-3 fatty acids, which can modulate inflammatory pathways and limit excessive immune activation (Alagawany et al., 2019). Turmeric supplementation did not significantly influence heterophil levels, indicating that its antioxidant effects may contribute to maintaining physiological balance without directly altering innate immune cell populations.

Basophils

Basophil levels were low and within normal limits across all treatments. Turmeric supplementation significantly reduced basophil levels ($P < 0.05$), which may be attributed to the antihistamine and anti-inflammatory properties of curcumin. This suggests that turmeric can suppress mild inflammatory responses without disrupting overall immune stability. Oil type did not significantly affect basophil levels, indicating that variations in dietary fat composition have minimal influence on this leukocyte type.

Eosinophils

Eosinophil values varied among treatments, with oil type exerting a more pronounced effect than turmeric supplementation. Balanced oil combinations tended to produce higher eosinophil values, which may reflect physiological immune adaptation rather than pathological responses (Agboola et al., 2017). Although eosinophil levels were relatively higher than typical reference values, no clinical signs of disease were observed, suggesting that these variations represent normal immune responses to dietary and environmental factors.

Heterophil/Lymphocyte (H/L) Ratio

The H/L ratio, an indicator of physiological stress, was significantly influenced by oil type ($P < 0.05$). The lowest H/L ratio was observed in the combined oil treatment, indicating better immune balance and lower stress levels. In contrast, higher ratios in palm oil treatments suggest increased activation of innate immune responses. Turmeric supplementation did not significantly affect the H/L ratio, indicating that its primary role may be related to antioxidant protection rather than modulation of stress-related immune parameters (Korver, 2012). The overall stability of H/L values across treatments confirms that the birds remained under normal physiological conditions.

Implication and Optimal Treatment

Among all treatments, the combination of 1.5% palm oil and 1.5% sachai inchi oil without turmeric (M1K0) demonstrated the most optimal leukocyte profile. This treatment maintained leukocyte counts within normal ranges, supported balanced differential leukocyte

values, and resulted in the lowest H/L ratio, indicating improved physiological stability and immune balance. These findings highlight the importance of balancing saturated and unsaturated fatty acids in poultry diets. While turmeric enhances immune activity, the combination of different lipid sources appears to play a more critical role in maintaining immune homeostasis. This study provides valuable insight into the development of functional feed strategies integrating lipid composition and natural antioxidants to improve poultry health and productivity.

CONCLUSION

The combination of palm oil, sachai inchi oil, and turmeric supplementation significantly affected the leukocyte profile and heterophil-to-lymphocyte (H/L) ratio of laying hens, indicating changes in their physiological and immune responses. Supplementation with 1.5% palm oil and 1.5% sachai inchi oil without turmeric resulted in the lowest H/L ratio while maintaining leukocyte values within normal physiological ranges, suggesting the potential role of balanced dietary fatty acids in supporting physiological homeostasis. However, given the relatively short experimental period, further studies are recommended to evaluate the effects of longer feeding periods and to include additional parameters, such as oxidative stress biomarkers, fatty acid profiles, immune function indicators, and egg quality characteristics.

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CONFLICT OF INTEREST

The author states that this research was conducted without any commercial or financial relationship that can be interpreted as a potential conflict of interest.

AUTHOR CONTRIBUTION

Isnin Ramadhani Nafiu contributed to the research design, data collection and processing, system development, analysis of results, and manuscript preparation.

GENERATIVE ARTIFICIAL INTELLIGENCE USAGE STATEMENT

In the preparation of this article, the author utilized generative artificial intelligence (AI) as a tool to assist with language refinement, structuring the text, and correcting grammar. The use of AI was limited and remained under the author's supervision. All data, analyses, interpretations, and scientific content within this article have been reviewed and verified by the author. The author bears full responsibility for the accuracy, originality, and final content of the manuscript.

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