Research Article

Multi Feed Supplement Prevents the Impact of Heat Stress on Dairy Cattle at Low Altitudes in Indonesia

Ujang Hidayat Tanuwiria[1], Bambang Kholiq Mutaqin[1], Amriana Hifizah[2], Andi Mushawwir[1]

[1]Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, Padjadjaran University, Jl. Ir. Soekarno KM. 21 Bandung-Sumendang, 45363, Jawa Barat, Indonesia; [2]Department of Animal Science, UIN Alauddin Makassar. Jl. H.M. Yasin Limpo No. 36, Gowa 92118, South Sulawesi, Indonesia.

Abstract | This study was conducted over 12 weeks in dairy cattle housing at multiple lowland locations in West Java. A total of 32 Friesian Holstein (FH) dairy cows, spanning their first to fifth lactations particularly their second and third were used to analyze leukocyte differentiation profiles and stress biomarkers and to determine which treatment had the greatest positive effect on these parameters. A completely randomized design with four replicates was employed. The treatments included FS0 (control): 60% forage + 40% concentrate; FS1: 60% forage + 40% concentrate (97% concentrate + 3% bypass protein); FS2: 60% forage + 40% concentrate (95% concentrate + 3% bypass protein + 2% Ca-PUFA); and FS3: 60% forage + 40% concentrate (93% concentrate + 3% bypass protein + 2% Ca-PUFA + 2% organic minerals). The results showed that feed supplementation significantly affected (P<0.05) the leukocyte differentiation profile and stress biomarkers in lactating dairy cows in the lowlands of West Java. Cows fed FS3—60% forage + 40% concentrate (93% concentrate + 3% bypass protein + 2% Ca-PUFA + 2% organic minerals)—showed lower mean levels of leukocyte differentiation (Neutrophils 2.59x 10^2/µL) and stress biomarkers (ATPase Na+/K+ Transporting Subunit Alpha 1 0.75 ng/dL; Interleukin-6 1.73 ng/dL)), as well as increased milk fat content (5.64 g/100 g), compared to the control group (P<0.05). Based on these findings, it was concluded that diets supplemented with three feed additives can reduce heat stress, as indicated by leukocyte activity, differentiation, and stress marker enzymes in the blood.

Keywords | Dairy cows, Feed supplement, Stress, Metabolism, Production, Milk

Received | June 03, 2026; **Accepted** | July 15, 2026; **Published** | July 28, 2026
***Correspondence** | Ujang Hidayat Tanuwiria, Department of Animal Nutrition and Feed Technology, Faculty of Anim. Sci, Padjadjaran University, Jatinangor-Sumedang, West Java 45363, Indonesia; **Email:** ujang.hidayat@unpad.ac.id
Citation | Tanuwiria UH, Mutaqin BK, Hifizah A, Mushawwir A (2026). Multi feed supplement prevents the impact of heat stress on dairy cattle at low altitudes in Indonesia. Adv. Anim. Vet. Sci., 14(8):1605-1613.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.8.1605.1613
ISSN (Online) | 2307-8316

INTRODUCTION

Friesian Holstein (FH) dairy cows are homeothermic animals that require an optimal ambient temperature to live comfortably and produce milk (Ahmed-Farid *et al*., 2021). The comfort zone for European dairy cows ranges from 13-18°C (Razzaghi *et al*., 2022). Meanwhile, Indonesia, especially in lowland areas, has an environmental temperature range of 28-35 °C and air humidity of 85% (Tanuwiria *et al*., 2022). These climatic differences in husbandry can affect livestock's physiological condition and productivity, thus requiring monitoring to ensure their survival.

Maintaining dairy cows above their comfort zone can cause heat stress, which damages their physiology and blood hematology (Tanuwiria *et al*., 2022) and alters total leukocyte counts, leukocyte differential counts, and immune and inflammatory responses (Tanuwiria *et al*., 2025). Leukocyte levels, including neutrophils,

lymphocytes, monocytes, eosinophils, and basophils in the blood, serve as indicators for evaluating heat stress experienced by dairy cows. Additionally, heat stress can disrupt physiological and metabolic functions, leading to excessive organ activity, increased inflammation (Wang *et al*., 2022), and cell damage and death. This affects blood metabolites such as alkaline phosphatase, creatinine, creatine kinase, gamma-Glutamyl Transpeptidase (γ-GT), and Lactate dehydrogenase (LDH), which then enter the bloodstream (Hong *et al*., 2019). Higher plasma levels of these compounds indicate greater heat stress in cattle (Jiang *et al*., 2025).

The negative impacts of heat stress on dairy cattle when maintained above their comfort zone can be mitigated through nutritional strategies, such as feed supplementation. These include bypass protein, Ca-PUFA, and organic minerals. These supplements help by stimulating lipid metabolism, boosting immunity, preventing inflammation, and supporting thermoregulation, thereby reducing the adverse effects on the animal's physiological condition. The nutritional modulation of dairy cow diets significantly impacts milk yield and the complex fatty acid profile of milk (Dhakal *et al*., 2024). This is vital for enhancing farm profitability and improving the nutritional quality of milk for consumers (Ahmed-Farid *et al*., 2021). Specifically, strategies such as supplementing with calcium salts of polyunsaturated fatty acids (PUFAs), protected proteins, and organic minerals have shown potential to influence ruminal biohydrogenation and nutrient partitioning, thereby directly affecting milk fat production and overall yield (Herrera *et al*., 2024). The goal of incorporating these dietary elements is to reduce the breakdown of beneficial nutrients in the rumen, increasing their availability for milk synthesis in the mammary glands (Lee *et al*., 2020; Mosoni *et al*., 2023). This approach employs advanced nutritional science to target metabolic pathways in dairy cows, enabling more efficient conversion of feed into high-quality milk components (Agle *et al*., 2020; Mosley *et al*., 2017). This study reviews current research on the effects of Ca-PUFA, protected proteins, and organic minerals on milk production and fatty acid composition, focusing on their mechanistic roles in modulating ruminal fermentation and systemic metabolism.

MATERIALS AND METHODS

Materials

The experimental animals used in this study were 32 Friesian Holstein (FH) dairy cows in the third lactation period, which were randomly distributed into predetermined treatment groups. The study was conducted over 16 weeks in an open housing system. The experimental animals were housed in individual pens measuring 1.5 x 2 m, each pen equipped with a treatment label. Feed was provided in limited quantities in accordance with standard requirements, and drinking water was provided ad libitum. EBI 25–TH Wireless Data Logger has been used in this research to record temperature and humidity in real time. The entire research procedure is visualized in Figure 1.

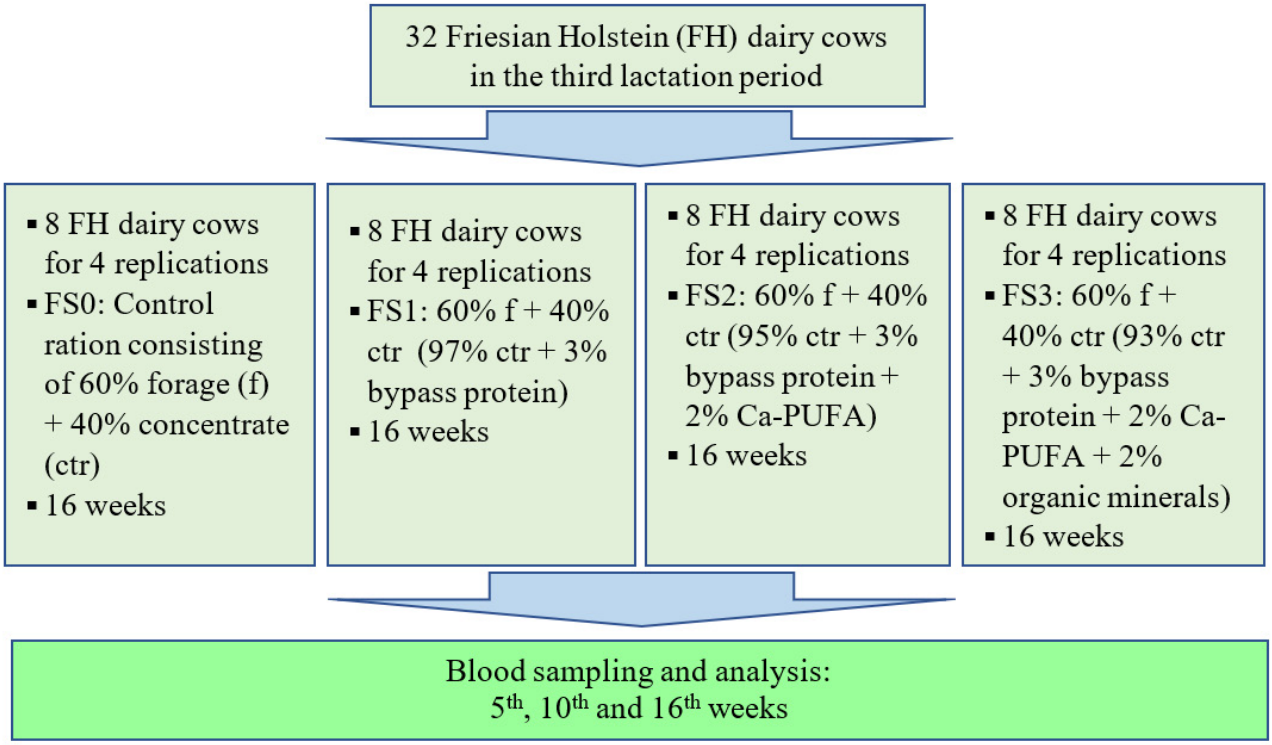

Figure 1: Experimental design flowchart.

Treatment and housing microclimate

This study was conducted using a completely randomized design (CRD). It consisted of four treatments, each with four replicates. The four treatments were as follows, FS0: Control ration consisting of 60% forage (f) + 40% concentrate (ctr) ; FS1: 60% f + 40% ctr (97% ctr + 3% bypass protein); FS2: 60% f + 40% ctr (95% ctr + 3% bypass protein + 2% Ca-PUFA); FS3: 60% f + 40% ctr (93% ctr + 3% bypass protein + 2% Ca-PUFA + 2% organic minerals).

Temperature and humidity recordings were performed during the study. The average daytime temperature and humidity were 31˚C and 89%, respectively. At night, they were lower, at 26˚C and 78%, respectively.

Feed supplement preparation

The feed supplements in this study included bypass protein, Ca-PUFA, and organic minerals. Bypass protein was produced by mixing tannin with fishmeal. The process involves tannin binding to the amino and/or carboxyl groups of the protein (Tanuwiria *et al*., 2025).

Tannin-Protected Fish Meal Prepation, Fish meal was homogeneously sprayed with a tannin extract solution at 3% w/w of the fish meal and then dried in an oven at 600^0C for three days. The nutrient composition of the tannin-protected fish meal was 17.09% water, 32.29% ash, 28.56% crude protein, 12.58% crude fat, 0.77% crude fiber, and 25.80% BETN.

The Ca-PUFA complex was produced via saponification using peanut oil and calcium hydroxide ($Ca(OH)_2$). As noted by Tanuwiria *et al.* (2025), the process involves hydrolyzing oil with a base to produce glycerol and fatty acid salts, in which the COOH groups of fatty acids bind to base cations. The Ca-PUFA was administered at 120 grams per fish daily. The PUFA from the peanut oil used consisted of 35% linoleic acid.

Organic minerals are produced by mixing zinc (Zn), copper (Cu), selenium (Se), and chromium (Cr) into corn and soybean media, which are then fermented with the help of *Saccharomyces cerevisiae* (SC) and/or *Aspergillus oryzae* (AO). The principle of producing organic Zn, organic Cu, organic Se, and organic Cr is to incorporate Zn, Cu, Se, and Cr into the proteins of the fungi *Saccharomyces cerevisiae* (SC) and/or *Aspergillus oryzae* (AO). The procedure for producing Zn-organic, Cu-organic, Se-organic, and Cr-organic compounds is as follows: the base substrate is a mixture of corn flour and soybean flour. The substrate is mixed with a standard solution (0.5% NH4NO3, 0.05% KCl, 0.05% MgSO4.7H2O, 0.001% FeSO4.7H2O, 0.0001% CuSO4.5H2O) and a solution of Zn, Cu, Cr, and Se at the specified dosage. The substrate is then sterilized at 121˚C, 15 psi for 15 minutes. Next, it was inoculated with *A. oryzae* and *S. cerevisiae* cultures at a 2% (2 g per 100 g of substrate) inoculum. Incubation was carried out for four days at room temperature. The resulting product was dried in an oven at 60˚C and ground. Bound minerals were quantified using standard procedures (Tanuwiria *et al*., 2025). The administration of organic Zn and organic Cu was 120 grams/animal/day, while organic Cr and organic Se were 10 g/animal/day.

Feed administration

Feed was provided twice daily at 07:00 and 15:00. The feed consisted of 6 kg of concentrate supplemented with *feed supplements* in the form of bypass protein, Ca-PUFA, and organic minerals according to the treatment, as well as forage equivalent to 10% of the dairy cow's body weight.

Sample collection and analysis

Sampling was conducted twice, at the midpoint and end of the study, namely in the 5^{th}, 10^{th} and 16^{th} weeks. Blood samples were obtained from *the coccygeal vein* using 3 mL tubes containing the anticoagulant Ethylenediaminetetraacetic Acid (EDTA).

Blood samples were analyzed using a hematology analyzer to determine erythrocyte, leukocyte, and differential counts. Stress biomarker parameters were obtained from blood plasma through centrifugation for 5 minutes at 5000 rpm. The concentration was measured with a spectrophotometer by mixing reagents and buffer solutions according to the Biolabo Kit analysis procedure, using a wavelength appropriate for the parameter (500–550 nm).

Data analysis

The data were analyzed using a completely randomized design variance analysis. Differences between treatments were determined with Duncan's multiple range test. All analyses were performed with IBM SPSS 25 at a 5% significance level or 95% confidence level.

RESULTS AND DISCUSSION

Thermoregulation and hematological

The study's Table 1 illustrates how adding the feed supplement to the diet influences thermoregulation and leukocyte differentiation, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

The analysis of variance shows that feed supplements significantly affect leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, and basophils ($P<0.05$). Duncan's multiple range test was used to compare treatment means. According to Table 1, leukocyte levels decrease from FS0 to FS3, with values of 9.38 $x10^3/\mu L$, 8.89 $x10^3/\mu L$,

Table 1: Average thermoregulatory and hematological levels in lactating dairy cows with feed supplement administration of various treatments.

Parameter	Treatment			
	FS0	FS1	FS2	FS3
Thermoregulation				
Heart rate (x minute^{-1})	78.15^{a}	75.25^{ab}	74.50^{b}	73.76^{b}
Respiratory rate (x minute^{-1})	32.64^{a}	30.83^{ab}	29.28^{b}	27.35^{b}
Rectal temperature (^{0}C)	39.05^{a}	38.15^{a}	38.05^{a}	38.05^{a}
ATPase Na^{+}/K^{+} Transporting Subunit Alpha 1 (ng/dL)	3.04±0.02	2.83±0.05	2.26±0.06	1.75±0.03
Thyroxine (ng/dL)	1.04±0.03^{a}	1.62±0.07^{b}	1.93±0.04^{b}	2.32±0.03^{c}
Haematological				
Red blood cells (x10^{6}/µL)	5.87^{a}	6.12^{b}	6.15^{b}	6.10^{b}
Haemoglobin (g%)	7.15^{a}	9.20^{b}	10.15^{b}	10.10^{b}
Haematocrit	24.55^{a}	27.41^{b}	28.75^{b}	30.34^{c}
Leukocytes (×10^{3}/µL)	9.38± 0.21^{c}	8.89± 0.24^{b}	8.63± 0.28^{b}	8.68± 0.61^{a}
Neutrophils (x 10^{2}/µL)	7.01±0.07^{d}	2.56± 0.18^{c}	2.28± 0.24^{b}	2.59±0.25^{a}
Lymphocytes (x 10^{2} /µL)	3.65± 0.11^{c}	5.36± 0.07^{b}	5.37± 0.04^{b}	5.14± 0.90^{a}
Monocytes (x 10^{2}/µL)	0.23±0.04^{d}	0.18± 0.04^{c}	0.42± 0.06^{b}	0.33±0.07^{a}
Eosinophils (x 10^{2}/µL)	0.27±0.01^{c}	0.32±0.01^{b}	0.23±0.01^{b}	0.29±0.02^{a}
Basophils (x 10^{3}/µL)	0.10± 0.00^{c}	0.01± 0.00^{b}	0.02± 0.00^{ab}	0.04± 0.01^{a}

^{a,b,c,d}DiDifferent superscripts on the same row indicate significant differences (P<0.05).

8.63 x10^{3}/µL, and 8.68 x10^{3}/µL, respectively. These levels remain within the normal range of 5.83 to 12.23 x10^{3}/µL, as reported by Tanuwiria *et al.* (2022). The rise in leukocyte levels may be linked to increased neutrophil counts (especially in the FS0 group) or to changes in lymphocyte counts, since both cell types are predominant in the blood circulation.

Neutrophils can be a good and accurate indicator of ruminant stress. Neutrophils function to phagocytose and kill organisms (Jain *et al.*, 2025). The average neutrophil count in experimental lactating dairy cows ranged from 2.28 to 7.01 x10^{3}/µL (Table 1). The results of this study were generally within the normal range reported by Jiang *et al.* (2025), which was 1.7-6.0 x10^{3}/µL, except for the FS0 group, which showed a slight increase.

Lymphocytes play an important and comprehensive role in the body's defense system. The average lymphocyte count in this study ranged from 3.65 to 5.37 x10^{3}/µL (Table 1). The average lymphocyte count was higher in the FS0 treatment group (4.65 × 10^{3}/µL) than in the control group. This value is still within the range reported by Hong *et al.* (2019) and Guo *et al.* (2025), which is 1.8-8.1 x10^{3}/µL.

Monocytes protect the body from invading organisms through phagocytosis (Goetz *et al.*, 2022). The results of this study show that the average monocyte levels in lactating dairy cows range from 0.18 to 0.42 x10^{3}/µL (Table 1). However, the average monocyte count was higher than the values reported by Rahayu *et al.* (2023) and Razzaghi *et al.* (2022), who reported a normal monocyte count of 0.3 x10^{3}/µL in Friesian Holstein (FH) dairy cows. The high average monocyte count in this study is thought to be a response to heat stress. This is supported by D'Oliveira *et al.* (2021) and Guinard-Flament *et al.* (2024), who emphasize that animal stress can be assessed using monocyte counts and that stress factors can increase them.

One way to evaluate stress resistance is to administer multi-feed supplements and assess livestock's response to feed additives that boost immunity. Eosinophils, basophils, and monocytes are appropriate parameters for this purpose. Eosinophils are produced in large numbers in patients with parasitic infections and migrate to tissues (Bach *et al.*, 2005; Gobikrushanth *et al.*, 2023; Chena *et al.*, 2024). The average eosinophil count in this study ranged from 0.23 to 0.32 x10^{3}/µL (Table 1). This value is slightly lower than that reported by Cavallini *et al.* (2025), who stated that the normal eosinophil count in Friesian-Holstein (FH) dairy cows ranges from 0.6 x10^{3}/µL. However, the results of this study align with those of Michalowski (2005), who reported that the normal eosinophil count in cows ranges from 0-1.5 x10^{3}/µL.

Basophils play a role in the development of hypersensitivity reactions and in the secretion of vasoactive mediators (Cuervo *et al.*, 2025). This study showed that the average basophil count in lactating dairy cows ranged from 0.01 to 0.10 x10^{3}/µL (Table 1). These results are in line with the view of Franzolin *et al.* (2010) that the normal basophil count in cows is 0.01-0.03 x10^{3}/µL. Basophils are the

myeloid cells with the lowest circulating concentrations and are commonly found in inflammatory and allergic conditions (Denton *et al*., 2022; Abouelezz *et al*., 2022).

Based on this study's Findings, leukocyte counts and differentiation in experimental lactating dairy cows receiving feed supplements tended to be lower than in the control group. The FS3 treatment, which included 60% forage and 40% concentrate (comprising 93% concentrate, 3% bypass protein, 2% Ca-PUFA, and 2% organic minerals), resulted in the lowest average leukocyte differentiation profile ($P<0.05$) among all treatments. According to studies by Barłowska *et al*. (2023) and Puastuti *et al*. (2021), both genetic and environmental factors impact leukocyte levels and differentiation, with environmental influences including infection and feed.

Leukocyte formation and differentiation depend on amino acids for protein synthesis. Bypass protein supplementation provides many amino acids crucial for supporting the immune response during heat stress in dairy cows, as these amino acids improve immune function and gluconeogenesis (Tanuwiria *et al*., 2022; Razzaghi *et al*., 2022). Moreover, amino acids boost protein metabolism, help alleviate heat stress, and increase blood concentrations, especially leukocyte counts, to within normal levels (Mosoni *et al*., 2023; Michalowski, 2005).

Research by Wijayanti *et al*. (2022) and Theodorou *et al*. (1994) confirms that PUFA effectively mitigates heat stress, enhancing FH cattle performance and immune health during hot conditions. Additionally, PUFAs can elevate leukocyte levels and support their differentiation within normal ranges. They also influence immune and inflammatory responses, playing a crucial role in cellular metabolism and leukocyte function (Mosley *et al*., 2017; Barloska *et al*., 2023).

Administering organic minerals can enhance enzyme and hormone activity and support metabolic processes in the body (Abouelezz *et al*., 2022; Ahmed-Farid *et al*., 2021). Essential organic minerals such as chromium (Cr), copper (Cu), selenium (Se), and zinc (Zn) are vital for dairy cow health. Cr supplementation can increase milk production, boost immune function, and enhance overall health, especially in stressed cows and during early lactation (Chena *et al*., 2024). Copper (Cu) is involved in cellular energy metabolism, nerve impulse transmission, and the health of the cardiovascular and immune systems (Denton *et al*., 2022). Its biological roles include ceruloplasmin, superoxide dismutase (SOD), lysine oxidase, and cytochrome oxidase (Jiang *et al*., 2025). Moreover, Cu improves immunity and fertility (D'Oliveira *et al*., 2021). Selenium (Se) functions as an antioxidant within selenium metabolism to maintain homeostasis (Dhakal *et al*., 2024), reducing cell damage caused by heat stress. Se also modulates immune responses in livestock by activating neutrophil phagocytosis, increasing antibody production, and promoting lymphocyte proliferation (Tanuwiria *et al*., 2022; Jiang *et al*., 2025). Zinc (Zn) has anti-inflammatory properties and participates in many biological functions (Muslim *et al*., 2014; Mosoni *et al*., 2023).

The decrease in ATP1A1 levels or expression in dairy cows that are not experiencing heat stress reflects the reduced cellular need to maintain ionic homeostasis previously disrupted during heat stress. Under thermoneutral conditions, body temperature, electrolyte balance, and acid-base status return to stability, resulting in decreased Na^+ and K^+ transport activity across cell membranes (Mosoni *et al*., 2023). Consequently, the energy requirement for operating the Na^+/K^+-ATPase pump is reduced, and ATP1A1 expression is re-regulated toward basal levels. This mechanism represents a physiological adaptation aimed at increasing the efficiency of cellular energy use when environmental stress has subsided and reducing unnecessary metabolic load.

Stress biomarkers for inflammation

The effects of feed supplement administration in rations on stress biomarkers, including alkaline phosphatase, creatinine, creatine kinase, Gamma Glutamyl Transpeptidase (γ-GT), and Lactate Dehydrogenase (LDH), are shown in Table 2, based on the study.

The variance analysis showed that administering feed supplements had a significant effect ($P<0.05$) alkaline phosphatase, creatinine, creatine kinase, gamma glutamyl transpeptidase (γ-GT), and lactate dehydrogenase (LDH). Furthermore, Duncan's multiple range test was conducted to assess differences in means among treatments. As shown in Table 2, the alkaline phosphatase (ALP) parameter differed significantly ($P<0.05$) across treatments. Based on the results of the study, lactating dairy cows given the FS0 treatment had higher averages than FS1 to FS3, which were 43.33 U/L, 37.58 U/L, 37.31 U/L, and 34.52 U/L, respectively. Previous research by Lee *et al*. (2020) reported that ALP levels in dairy cows decreased with increasing parity.

Table 2 presents the effect of feed supplement administration on creatinine levels in lactating dairy cows. The study shows that cows in FS0 had significantly higher creatinine levels (123.41 µmol/L, $P<0.05$) than those in FS1 to FS3, which recorded 109.46 µmol/L, 99.38 µmol/L, and 96.52 µmol/L, respectively. These values fall within the typical range reported by Ahmed-Farid *et al*. (2021), 97.5-111 µmol/L. Elevated blood creatinine levels can indicate kidney issues such as glomerular filtration problems, acute tubular necrosis, dehydration, or renal failure (Gobikrushanth *et al*., 2023; Herrera *et al*., 2024).

Table 2: Levels of stress biomarkers in lactating dairy cows with feed supplement administration.

Parameter	Treatment			
	FS0	FS1	FS2	FS3
Malondialdehyde (MDA) (ng/dL)	3.72±0.06[a]	2.93±0.17[a]	1.05±0.08[b]	0.95±0.07[b]
Total Antioxidant (ng/dL)	0.13±0.04[a]	0.22±0.05[b]	0.46±0.03[c]	0.93±0.03[d]
Interleukin-6 (ng/dL)	2.76±0.07[a]	2.62±0.04[a]	2.04±0.04[b]	1.73±0.02[c]
Creatinine (µmol/L)	123.41±3.22[d]	109.46±4.03[c]	99.38±3.33[b]	96.52±3.11[a]
Creatine Kinase (U/L)	4.33±0.06[d]	2.22±0.02[c]	1.85± 0.11[b]	0.36±0.02[a]
Lactate dehydrogenase (LDH) (U/L)	18.53±1.11[c]	11.05±0.22[b]	8.84±0.44[ab]	7.01±0.42[a]
Gamma-Glutamyl Transpeptidase (U/L)	16.63±1.04[d]	14.34±1.06[c]	11.63±1.01 [b]	8.44±0.11[a]
Alkaline Phosphatase (U/L)	43.33±1.46[c]	37.58±1.42[b]	37.31±1.42[b]	34.52±1.24[a]

[a,b,c,d] Different superscripts on the same row indices significant differences (P<0.05)

Table 2 shows the effect of feed supplement administration on creatine kinase in lactating dairy cows. Each treatment differs significantly (P<0.05). Based on the results of the study, the average creatine kinase in lactating dairy cows given the FS0 treatment was higher (4.33 U/L) than in those given FS1 (2.22 U/L), FS2 (1.85 U/L), or FS3 (0.36 U/L). According to Michalowski (2005) and Chena *et al.* (2024), heat stress causes muscle metabolism to provide energy by breaking down creatine phosphate into creatinine via the enzyme creatine kinase. This indicates that high plasma levels of creatinine and creatine indicate that the cattle are experiencing heat stress.

Gamma-glutamyl transpeptidase (γ-GT) is an enzyme prevalent in cardiac cells. Homeostatic mechanisms related to cardiac function (Gobikrushanth *et al.*, 2023; Denton *et al.*, 2022), especially those governing energy supply (Razzaghi *et al.*, 2022), can lead to increased cardiac cell death (necrosis). Past research indicates that necrosis promotes metabolite leakage into the bloodstream (Puastuti *et al.*, 2021; Rahayu *et al.*, 2023; Mosoni *et al.*, 2023). As shown in Table 2, γ-GT levels were significantly higher (P<0.05) in lactating dairy cows fed only FS0 (control diet) than in those fed FS1, FS2, and FS3. The findings reveal notable differences (P<0.05) in γ-GT levels across treatments. Levels declined from FS0 to FS3, measuring 16.63 U/L, 14.34 U/L, 11.63 U/L, and 8.44 U/L, respectively. Overall, the FS3 group exhibited the lowest average γ-GT level across treatments.

Table 2 shows the effect of feed supplement administration on LDH levels. According to the table, LDH levels in FS0 were significantly higher (P<0.05) at 18.53 U/L than in FS1-FS3, which received the feed supplement treatment. Their LDH levels were 11.05 U/L, 8.84 U/L, and 7.01 U/L, respectively. Elevated blood LDH levels can indicate damage to cells, muscle membranes, and tissues (Franzolin *et al.*, 2010; Bach *et al.*, 2005) and also serve as an early blood marker for heat stress.

This study showed that the levels of certain metabolic compounds in the blood such as Alkaline Phosphatase (ALP), creatinine, creatine kinase, Gamma Glutamyl Transpeptidase (γ-GT), and Lactate Dehydrogenase (LDH) which serve as stress indicators in experimental lactating dairy cows receiving feed supplements, tended to be lower on average than in the control group. The FS3 treatment, composed of 60% forage and 40% concentrate (including 93% concentrate, 3% bypass protein, 2% Ca-PUFA, and 2% organic minerals), showed the lowest stress levels (P<0.05) among all treatments.

Milk production and fat profile

Table 3 shows milk production and fat profile from dairy cows raised in lowland areas with feed supplement administration. Current research results indicate that administering FS2 and FS3 significantly increases average milk production (P<0.05) compared to the experimental group of cows without feed supplements and those receiving FS1. The content of long-chain polyunsaturated fatty acids also appears to be higher in cows that received rations supplemented with FS2 and FS3. In contrast, the saturated fatty acid content was lower.

Previous research indicates that concentrates rich in crude protein and Ca-FA can markedly increase intake of dry matter, protein, fat, and energy, thereby boosting milk production (Puastuti *et al.*, 2021). Additional energy, particularly from fatty acids, can increase milk fat and protein yields (Mosley *et al.*, 2017).

Adding Ca-PUFA to dairy cow diets has been shown to alter macromineral and metabolic marker levels in the bloodstream, potentially reducing postpartum health issues and improving reproductive efficiency (Gobikrushanth *et al.*, 2023). Additionally, increased nutrient uptake by the mammary gland from Ca-PUFA supplementation could explain the rise in milk fat levels by altering nutrient utilization (Guinard-Flament *et al.*, 2024). Calcium is

Table 3: Milk production and fat profile of dairy cows in lowland areas with feed supplement addition.

Milk production and fat profile	Feed supplement			
	FS0	FS1	FS2	FS3
Average daily milk production (L)	13.5±1.31[a]	13.7±0.67[a]	14.87±1.65[b]	16.85±2.03[c]
Fatty acids (%)				
C4:0	3.87±0.61[a]	2.92±0.04[a]	2.36±0.16[c]	2.05±0.03[d]
C6:0	2.64±0.01[a]	2.56±0.11[a]	2.15±0.11[ab]	2.08±0.04[b]
C8:0	1.62±0.06[a]	1.64±0.07[a]	1.37±0.01[ab]	1.28±0.03[b]
C10:	4.28±0.84[a]	3.94±0.64[a]	3.32±0.21[ab]	3.11±0.02[b]
C12:0	4.44±1.04[a]	4.39±0.51[a]	3.85±0.11[ab]	3.42±0.03[b]
C14:0	11.12±1.53[a]	11.02±1.03[a]	10.44±1.43[a]	10.07±1.95[b]
C16:0	28.71±3.034[a]	28.74±3.05[a]	28.28±1.53[ab]	26.13±2.42[b]
C18:0	12.21±2.64[a]	12.19±1.51[a]	12.16±1.07[ab]	11.17±1.05[b]
18:*1* cis-9	24.34±3.02[a]	24.38±2.55[a]	24:56±2.35[a]	25.55±3.17[b]
18:2 cis-9, cis-12	3.57±0.31[a]	3.57±0.333[a]	3.92±0.17[a]	4.48±0.09[b]
total n-6	3.83±0.53[a]	3.88±0.42[a]	4.08±0.44[a]	5.17±0.81[b]
total n-3	0.36±0.07[a]	0.55±0.02[a]	1.16±0.02[b]	1.38±0.06[b]
SFA	70.25±3.70[a]	69.77±2.64[a]	67.25±3.05[b]	66.17±3.45[b]
MUFA	27.08±1.48[a]	27.26±1.64[a]	28.57±1.63[b]	29.17±1.57[c]
PUFA	4.08±0.72[a]	4.41±0.66[a]	4.53±0.15[a]	5.87±0.85[b]
Total fat (g.100g $^{-1}$)	5.15±0.92[a]	5.26±0.47	5.41±1.63[a]	5.64±0.44[b]

[a,b,c,d] Different superscripts on the same row indicate significant differences (P<0.05). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.

Organic minerals play a crucial role in shaping the fatty acid profile of dairy cow milk, influencing milk fat synthesis and desaturation (Razzaghi *et al*., 2022). For instance, certain organic trace minerals, such as zinc and selenium, serve as cofactors for enzymes involved in fatty acid metabolism, potentially affecting the concentration of beneficial unsaturated fatty acids in milk (Barłowska *et al*., 2023). This suggests that targeted supplementation with organic minerals could be an effective strategy for dairy farmers seeking to enhance the nutritional value and functional qualities of milk (Puastuti *et al*., 2021; Cavallini *et al*., 2025).

CONCLUSION

This study concluded that providing feed supplements effectively reduced the risk of heat stress in dairy cattle in Indonesia's lowlands. Physiological indicators like thermoregulation and hematology remained normal or improved, and free radical activity decreased even under heat stress. The most effective treatment was FS3, which involved lactating dairy cows fed a diet comprising 60% forage and 40% concentrate, including 93% concentrate, 3% bypass protein, 2% Ca-PUFA, and 2% organic minerals.

ACKNOWLEDGMENT

The author wishes to thank everyone who supported and helped with this research, especially the Ruminant Nutrition and Animal Feed Chemistry laboratory assistants and the Animal Physiology and Biochemistry laboratory at Padjadjaran University.

NOVELTY STATEMENT

This study's key innovation is the use of a multi-component feed supplement that combines calcium-protecting polyunsaturated fatty acids (Ca-PUFA), organic oils, and bypass proteins as a comprehensive nutritional strategy. This combination is believed to act synergistically to alleviate heat stress in dairy cattle raised in lowland tropical regions, particularly in Indonesia, a novel application not previously reported. The approach broadens current knowledge by examining how these feed components interact to influence thermophysiological responses focusing on specific thermoregulatory markers such as ATPase Na+/K+ Transporting Subunit Alpha 1 and interleukin-6, which indicate free radical activity and productive performance. Overall, it offers a sustainable approach to enhancing the resilience and productivity of dairy cattle under chronic heat stress.

AUTHORS CONTRIBUTION

The authors listed in this article have contributed equally to its preparation and writing. UHT, AM: Planned and designed the research, supervised implementation, guided sample analysis, analyzed and interpreted data, and wrote,

edited, and finalized the article. BKM, AH: Conducted research in the barn, analyzed samples, performed statistical analysis, and authored the article.

Ethical approval

This experiment was designed and conducted with the understanding that no experimental techniques violated animal experimentation ethics rules, as approved by the Research Supervisory Agency, DP Indonesia, under number 183/Ret/rt.23/2025.

Generate AI and AI-assisted technology statement

The authors declare that they did not utilize any technological assistance in the form of AI software or similar tools in drafting and writing this article or in the data analysis contained herein.

Conflict of interest

The authors have declared no conflict of interest. All scientific information and research data published in this article are stated to have no conflict of interest whatsoever with any party, whether in the form of patents, financing, or rights related to them.

REFERENCES

Abouelezz K, Jiang Z, Gou Z, Wang Y, Jiang S (2022). Effects of metabolic energy intervention on lipid content and liver transcriptome in finisher yellow-feathered chickens. Ital. J. Anim. Sci., 21: 1362–1370. https://doi.org/10.1080/1828051X.2022.2116607

Agle M, Hristov AN, Zaman S, Schneider CR, Ndegwa PM, Vaddella V (2020). The effects of ruminally degraded protein on rumen fermentation and ammonia losses from manure in dairy cows. J. Dairy Sci., 93(4): 1625–1637. https://doi.org/10.3168/jds.2009-2579

Ahmed-Farid OA, Salah AS, Nassan MA, El-Tarabany MS (2021). Effects of chronic thermal stress on performance, energy metabolism, antioxidant activity, brain serotonin, and blood biochemical indices of broiler chickens. Animals, 11: 2554. https://doi.org/10.3390/ani11092554

Bach À, Calsamiglia S, Stern MD (2005). Nitrogen Metabolism in the Rumen. J. Dairy Sci., 88. https://doi.org/10.3168/jds.S0022-0302(05)73133-7

Barłowska J, Polak G, Janczarek I, Tkaczyk E (2023). The Influence of selected factors on the nutritional value of the milk of cold-blooded mares: The example of the sokólski breed. Animals, 13(7): 1152. https://doi.org/10.3390/ani13071152

Cavallini D, Lamanna M, Colleluori R, Silvestrelli S, Ghiaccio F, Buonaiuto G, Formigoni A (2025). The use of rumen-protected amino acids and fibrous by-products can increase the sustainability of milk production. Front. Vet. Sci., 12. https://doi.org/10.3389/fvets.2025.1588425

Chena Q, Yun-feng C, Zhao-xi Z, Fu-cheng J, Xiang-yu M, Jin-jin L, Da-yong C, Jian-lei J (2024). Effect of alfalfa supplementary change dietary non-fibrous carbohydrate (NFC) to neutral detergent fiber (NDF) ratio on rumen fermentation and microbial function in Gansu alpine fine wool sheep (*Ovis aries*). Animals, 35(1): 2262539. https://doi.org/10.1080/10495398.2023.2262539

Cuervo W, Gómez-López C, Di Lorenzo N (2025). Methane synthesis as a source of energy loss impacting microbial protein synthesis in beef cattle: A review. Methane, 4(2): 10. https://doi.org/10.3390/methane4020010.

D'Oliveira MC, Souza MIL, Corrêa FRAC, Morais MG, Ítavo CCBF, Franco GL (2021). Effects of road transportation or droving on the weight and metabolism of young bulls. Trop. Anim. Health Prod., 46(8): 1447–1453. https://doi.org/10.1007/s11250-014-0663-9

Denton BL, Diese LE, Firkins JL, Hackmann TJ (2022). Accumulation of reserve carbohydrate by rumen protozoa and bacteria in competition for glucose. Appl. Environ. Microbiol., 81(5): 1832-1838. https://doi.org/10.1128/AEM.03736-14

Dhakal R, Neves ALA, Sapkota R, Khanal P, Ellegaard-Jensen L, Winding A, Hansen HH (2024). Temporal dynamics of volatile fatty acids profile, methane production, and prokaryotic community in an *in vitro* rumen fermentation system fed with maize silage. Front. Microbiol., 15. https://doi.org/10.3389/fmicb.2024.1271599

Franzolin R, Rosales FP, Soares WVB (2010). Effects of dietary energy and nitrogen supplements on rumen fermentation and protozoan population in buffalo and zebu cattle. Rev. Brasil. Zoot., 39(3): 549– 555. https://doi.org/10.1590/S1516-35982010000300014

Gobikrushanth M, Macmillan K, Behrouzi A, López-Helguera I, Hoff B, Colazo MG (2023). Circulating Ca and its relationship with serum minerals, metabolic and nutritional profiles, health disorders, and productive and reproductive outcomes in dairy cows. Livest. Sci., 233: 103946. https://doi.org/10.1016/j.livsci.2020.103946

Goetz HM, Winder CB, Costa JHC, Creutzinger KC, Uyama T, Kelton DF, Dunn J, Renaud DL (2022). Characterizing the literature surrounding transportation of young dairy calves: A scoping review. J. Dairy Sci., 105(2): 1555–1572. https://doi.org/10.3168/jds.2021-21211

Guinard-Flament J, Lemosquet S, Delamaire É, Bris GL, Lamberton P, Hurtaud C (2024). Alteration of the nutrient uptake by the udder over an extended milking interval in dairy cows. J. Dairy Sci., 94(11): 5458. https://doi.org/10.3168/jds.2011-4268

Guo W, Liu Y, Na M, Zhang Y, Na R (2025). Effects of rumen-degradable starch levels on *in vitro* rumen fermentation and microbial protein synthesis in alfalfa silage. Fermentation, 11(2): 106. https://doi.org/10.3390/fermentation11020106

Herrera D, Harris WG, Nair VD, Josan MS, Staples CR (2024). Effect of dietary modifications of calcium and magnesium on reducing the solubility of phosphorus in feces from lactating dairy cows. J. Dairy Sci., 93(6): 2598. https://doi.org/10.3168/jds.2009-2766

Hong H, Lee E, Lee IH, Lee SR (2019). Effects of transport stress on physiological responses and milk production in lactating dairy cows. Asian-Australas. J. Anim. Sci., 32(3): 442–451. https://doi.org/10.5713/ajas.18.0108

Jiang Y, Zhang J, Wang K, Feng H, You Y, Dai P, Wang Z, Hu R, Peng Q, Zou H, Xiao J, Wang L, Bai X (2025). The effects of dietary energy level on the growth performance of yaks (*Bos grunniens*) were studied using omics techniques. Front. Microbiol., 16. https://doi.org/10.3389/

fmicb.2025.1621581

Lee S, Ha J, Cheng KJ (2020). Influence of an anaerobic fungal culture administration on *in vivo* ruminal fermentation and nutrient digestion. Anim. Feed Sci. Technol., 88(3–4): 201–217. https://doi.org/10.1016/S0377-8401(00)00216-9

Michalowski T (2005). Rumen protozoa in the growing domestic ruminant. Biol. Growing Anim., 2: 54-74. https://doi.org/10.1016/S1877-1823(09)70036-0

Mosley SA, Mosley EE, Hatch B, Szasz JI, Corato A, Zacharias N, Howes D, McGuire MA (2017). Effect of varying levels of fatty acids from palm oil on feed intake and milk production in holstein cows. J. Dairy Sci., 90(2): 987. https://doi.org/10.3168/jds.S0022-0302(07)71583-7

Mosoni P, Martin C, Forano E, Morgavi DP (2023). Long-term defaunation increases the abundance of cellulolytic ruminococci and methanogens but does not affect the bacterial and methanogen diversity in the rumen of sheep. J. Anim. Sci., 89(3): 783–791. https://doi.org/10.2527/jas.2010-2947

Muslim G, Sihombing JE, Fauziah S, Abrar A, Fariani A (2014). Activity of various rumen fluid proportions in overcoming tannin with *in vitro* techniques. Sriwijaya J. Anim. Husb., 3(1): 25-36. https://doi.org/10.33230/JPS.3.1.2014.1727

Puastuti W, Magrianti T, Hanifah VW, Sianturi RG, Romjali E, Talib C (2021). Introduction of 16% crude protein concentrate and Ca-FA feed to increase milk production for dairy cows on smallholder farms in Bogor Regency. IOP Conf. Ser. Earth Environ. Sci., 788(1): 12044. https://doi.org/10.1088/1755-1315/788/1/012044

Rahayu RI, Subrata A, Achmadi J (2023). *In vitro* ruminal fermentation of ammoniated rice straw-based diets with banana flour and molasses supplementation. J. Anim. Husband. Indones., 20(3): 166-174. https://doi.org/10.25077/jpi.20.3.166-174.2018

Razzaghi A, Vakili A, Khorrami B, Ghaffari M, Rico DE (2022). Effect of dietary supplementation or cessation of magnesium-based alkalizers on milk fat output in dairy cows under milk fat depression conditions. J. Dairy Sci., 105(3): 2275. https://doi.org/10.3168/jds.2021-20457

Tanuwiria UH, Susilawati I, Tasripin DS, Salman LB, Mushawwir A (2022). Behavioral, physiological, and blood biochemistry of Friesian Holstein dairy cattle at different altitudes in West Java, Indonesia. Biodiversity, 23: 533-539. https://doi.org/10.13057/biodiv/d230157

Tanuwiria UH, Zain M, Syamsu JA, Yunilas Y, Mushawwir A, Yanza YR (2025). The influence of different proportions of rumen-degradable protein and non-fiber carbohydrate in the feed ration on *in vitro* rumen fermentation, digestibility, gas production kinetics, and enteric methane emission. J. Adv. Vet. Anim. Res., 12(3): 784–784. https://doi.org/10.5455/javar.2025.l941

Theodorou MK, Williams BA, Dhanoa MS, McAllen AB, France J (1994). A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Tech., 48(3-4): 185-197. https://doi.org/10.1016/0377-8401(94)90171-6

Wang X, Xu T, Zhang X, Zhao N, Hu L, Liu H, Zhang Q, Geng Y, Kang S, Xu S (2022). The response of ruminal microbiota and metabolites to different dietary protein levels in tibetan sheep on the qinghai-tibetan plateau. Front. Vet. Sci., 9(10): 922817. https://doi.org/10.3389/fvets.2022.922817

Wijayanti E, Wahyono F, Surono F (2022). Nutrient digestibility and fermentative capacity of complete feed with different levels of bagasse *in vitro*. J. Anim. Agric., 1(1): 167-179.