

The informative value of modified leukocyte indices for assessing the immune response of dairy cows to short-term heat stress

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ABSTRACT

Heat stress (HS) remains a significant challenge in dairy cattle production, affecting immune function and productivity. This study evaluated adapted leukocyte indices in Brown Swiss cows under short-term heat stress at the beginning and end of the summer, compared to thermoneutral controls. Acute heat stress resulted in a significant increase in segmented neutrophils (+17%, $P<0.01$) and a decrease in lymphocytes (–15%, $P<0.05$), with corresponding reductions in the Nuclear Shift Index (NSI, –40%, $P<0.01$) and Leukocyte Index (LI, –28%, $P<0.001$). In contrast, late summer exposure led to higher monocyte (+192%, $P<0.001$) and eosinophil counts (+159%, $P<0.05$), and marked declines in the Leukocyte Index of Intoxication (LII, –43%, $P<0.001$), Neutrophil-to-Monocyte Ratio (NMR) and Lymphocyte-to-Monocyte Ratio (LMR) (2–3 fold, $P<0.001$), and the Index of Immune Reactivity (IIR, –40%, $P<0.001$). Indices such as NSI, Neutrophil-to-Lymphocyte Ratio (NLR), LMR, IIR and LII were particularly sensitive in distinguishing acute adaptation from immune fatigue. These findings highlight the diagnostic value of adapted leukocyte indices for early detection and monitoring of immune adaptation to heat stress in dairy cows, supporting targeted interventions and improved herd resilience.

Key words: heat stress; dairy cattle; leukocyte indices; immune adaptation; thermal stress biomarkers

Introduction

Heat stress (HS) is one of the principal challenges in dairy cattle farming, exerting negative effects on both productivity and animal welfare. The combined impact of excessively high ambient temperatures and humidity provokes characteristic physiological responses in the organism, including an increase in respiratory and heart rates, as well as

elevated rectal temperature. These adaptive mechanisms are primarily aimed at maintaining homeostasis and dissipating excess body heat. However, due to additional energy expenditure on thermoregulation and reduced feed intake, milk yields decline and the reproductive performance of the herd deteriorates. Most critically, the development of systemic inflammation and suppression of immune function

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have emerged as key pathogenic mechanisms underpinning the adverse effects of HS in dairy cattle ([BAGATH et al., 2019](#)).

The intensity of physiological and immune responses to HS in dairy cows is determined by breed, genetic background, metabolic profile, and immunological reactivity ([JOO et al., 2021](#); [CHEN et al., 2023](#)). Hyperthermia leads to increased production of proinflammatory cytokines and activation of a systemic immune response, the magnitude of which varies depending on the duration of HS exposure and the individual immunobiological characteristics of the animal ([KOCH et al., 2023](#); [ALHUSSIEN et al., 2024](#)).

The use of environmental indices enables the quantitative assessment of thermal stress in cattle ([HOFFMANN et al., 2022](#)). The temperature-humidity index (THI) is the most widely accepted indicator for evaluating HS in dairy cows. A THI value of approximately 72 is generally considered the threshold at which milk yield begins to decline ([GUPTA et al., 2023](#)). However, even lower THI values have been shown to cause reductions in milk fat and protein, highlighting the high sensitivity of metabolic processes in dairy cows to HS ([MAGGIOLINO et al., 2022](#)). The use of THI as a background indicator in clinical studies of hyperthermia is widely regarded as a standard and well-established approach in this area of research ([KIM et al., 2023](#); [LEES et al., 2026](#)).

Among the available tests, physiological parameters are commonly assessed such as respiratory and heart rates, internal or surface body temperature, and biological markers including blood glucose, beta-hydroxybutyrate, hormones, or heat shock proteins ([CHEN et al., 2023](#); [RAKIB et al., 2024](#)). Each of these indicators provides insight into specific aspects of the organism's response to stress. Nevertheless, these methods often fall short when a holistic evaluation of immune response is required ([KIM et al., 2024](#)).

HS is also closely associated with the metabolic adaptations that accompany immune changes in dairy cows. Previous studies have demonstrated alterations in glucose utilisation, lipid mobilisation, β -hydroxybutyrate concentration, oxidative balance

and antioxidant capacity during periods of thermal load, indicating that the physiological response to HS is systemic rather than restricted to individual organs or functions ([TURK et al., 2015](#); [TURK et al., 2024](#)). In this context, leukocyte-derived indices may be considered not only as markers of immune reactivity, but also as practical indicators reflecting the broader adaptive status of the organism under HS conditions.

The white blood cell system is highly labile and, as such, leukocyte indices are widely utilised in biomedical research to assess pathological states ([LOMAKO and SHYLO, 2024](#)). Given the systemic nature of the disturbances caused by HS and the complex interplay of defence mechanisms within biological systems, the application of leukocyte indices has recently gained traction as a practical tool for evaluating animal welfare under conditions of thermal load ([MYLOSTYVYI et al., 2026](#)).

Differences in the leukocyte profiles of humans and laboratory animals, along with the limited data on the application of leukocyte indices under conditions of seasonal hyperthermia, somewhat constrain their relevance for use in ruminants. There is also insufficient evidence on how these indices reflect the distinct phases of the immune response, particularly during the initial adaptation to acute HS, and the possible development of immune fatigue or early signs of immune exhaustion following repeated exposures or during chronic thermal stress. This necessitates appropriate modification of the indices to account for the leukocyte profile of ruminants, and careful interpretation of results from the perspective of the systemic effects of HS on the organism and the immune system in particular.

This study hypothesised that short-term heat stress episodes of equal duration, but occurring at different periods of the summer, induce distinct immune responses in dairy cows, which can be detected using adapted leukocyte indices. The aims were: (i) to modify commonly used biomedical leukocyte indices for application in ruminants, and (ii) to evaluate their ability to differentiate between acute immune adaptation and later-stage immune dysregulation or possible immune exhaustion in cows exposed to heat stress at the beginning and end of the summer. Thus, the use of adapted leukocyte in-

dices was intended to offer a universal approach for assessing immune response and to validate them as practical biomarkers for monitoring cow health under conditions of HS.

Materials and methods

Animals and experimental design. This study was conducted on Brown Swiss dairy cows at a commercial dairy farm in central Ukraine (48°34'03.1" N, 34°54'47.0" E). All the animals were of the same breed, maintained under identical housing and management conditions, and were in their second lactation at the time of sampling (135±5.0 days in milk). The average daily milk yield was approximately 36±1.5 kg per cow. Two experimental groups were established on the basis of climatic conditions. Each group consisted of cows that had been exposed to a five-day period of moderate heat stress prior to blood sampling. The first experimental group (Group 1, mid-June) included 12 cows subjected to short-term heat stress during the second ten-day period of June. The second experimental group (Group 2, late August) included 12 cows exposed to moderate heat stress of equal duration during the final ten-day period of August. The control group consisted of 36 cows sampled under thermoneutral conditions, with blood collection carried out between the last ten days of February and the first ten days of May. All the animals were clinically healthy at the time of sampling.

Random assignment was used to allocate animals to the experimental and control groups, ensuring uniform parity, stage of lactation, and milk production, in order to minimise physiological variation. All procedures involving animals were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes and were approved by the Commission on Bioethics of Dnipro State Agrarian and Economic University (protocol No. 2/1, 26 February 2025). Every effort was made to ensure the welfare and humane treatment of the animals throughout the study.

The cows were housed in naturally ventilated barns (NVB), with sand bedding to ensure optimal hygiene and comfort. The animals were kept un-

tethered throughout the year and fed a total mixed ration (TMR), formulated in accordance with National Research Council (NRC) ([NRC, 2001](#)) recommendations. The diet was based primarily on maize silage and was supplemented with barley, rye, maize grain, lucerne silage, grass hay, and wheat straw. Protein sources included rapeseed meal, sunflower meal, soybean meal, and dried beet pulp. Mineral and vitamin supplements were provided to ensure dietary balance. All feed ingredients were thoroughly mixed using automated mixers equipped with electronic scales, and ration distribution was managed using computer software. Cows had ad libitum access to both feed and water, ensuring consistent nutritional intake across all experimental groups. The same TMR was provided to all cows regardless of group or season, in accordance with their lactational stage.

Assessment of climatic conditions. To assess environmental conditions and quantify heat stress exposure, the temperature–humidity index (THI) was calculated using the [KIBLER \(1964\)](#) formula, as described by [MYLOSTYVYI et al. \(2023\)](#). Data on ambient temperature and relative humidity were obtained from the nearest meteorological station, located approximately 20 km from the farm, and made publicly available by the Ukrainian Hydrometeorological Centre. Climatic parameters were recorded at three-hour intervals, and daily mean values were used to determine the degree of thermal load.

During the control period (February to early May), the mean daily THI ranged from approximately 35 to 67, with an average of 51.6±9.4, indicating thermoneutral conditions for dairy cows. For Group 1 (mid-June), a short-term heat wave resulted in mean THI values of 71.1±4.7 (range: 64.1 to 79.1). For Group 2 (late August), the mean THI was 71.4±3.9 (range: 63.9 to 77.6). According to internationally accepted thresholds ([MYLOSTYVYI and IZHBOLDINA, 2025](#)), THI values from 68.0 to 71.9 are classified as mild heat stress, and 72.0 to 79.9 as moderate heat stress. In both experimental groups, the mean THI was at the upper border of mild heat stress and occasionally entered the moderate range, reflecting mild to moderate heat stress exposure.

This design enabled a comparative assessment of leukocyte dynamics in cows exposed to mild-to-moderate short-term heat stress at different periods of the summer versus those maintained under thermoneutral conditions.

Determination of indicators. All blood samples were collected in the morning (between 7:00 and 8:00 a.m.) before feed distribution in the barn section where the animals were housed. Samples were obtained via jugular venipuncture using 2-ml EDTA Vacutainer® tubes (Aichele Medico AG, Basel, Switzerland). The analysis included the determination of total leukocyte count (WBC) and the leukocyte differential count. The total leukocyte count was measured using an automatic Sysmex XS-1000i haematology analyser (Sysmex Corporation, Japan) following the manufacturer's calibration and quality control procedures. The leukocyte differential count was determined by microscopic examination of blood smears stained using the Romanowsky-Giemsa method. A total of 200 cells were counted per smear, including band and segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Only smears with a high-quality monolayer and without artefacts were included in the analysis.

Leukocyte indices calculation and adaptation. To evaluate the immune-physiological response to HS, a set of integral leukocyte indices was calculated based on methods widely accepted in clinical practice ([LOMAKO and SHYLO, 2024](#)). These indices were specifically modified to improve their applicability for assessing immune and inflammatory shifts associated with HS in dairy cattle.

Most leukocyte indices were originally developed for humans, where neutrophils are the predominant leukocyte type. In contrast, dairy cattle exhibit a lymphocyte-dominant leukocyte profile, necessitating methodological adaptations for valid application. In this study, the indices were adjusted by excluding immature myeloid precursors (such as myelocytes, metamyelocytes, and plasma cells), which are typically absent in healthy cattle.

Additionally, correction factors were introduced in situations where leukocyte subpopulations approached zero to prevent computational errors,

while maintaining biological interpretability.

These modifications ensured that the calculated indices more accurately reflected immune dynamics in response to HS in cattle, where both neutrophilic and lymphocytic responses contribute to adaptation. Unlike infectious diseases, which are often characterised by a pronounced left shift in leukocyte maturation, HS primarily induces functional immunomodulation rather than overt inflammatory responses. Therefore, the adapted indices were designed to capture subtle alterations in leukocyte balance and immune function, rather than severe pathological changes. All indices were calculated according to the formulas listed below.

Leukocyte indices used. A comprehensive set of leukocyte indices was applied to assess various aspects of immune function and adaptation to HS in dairy cows. All indices were calculated according to the adapted formulas described below.

Indices reflecting neutrophil activation and left shift:

Nuclear Shift Index (NSI). Assesses the degree of left shift by evaluating the proportion of band neutrophils relative to segmented neutrophils. A correction factor (+1) is added to the numerator to account for animals with no band neutrophils:

$$NSI = \frac{\text{Band neutrophils} + 1}{\text{Segmented neutrophils}} \quad /1/$$

Leukocyte Index (LI). Reflects the balance between lymphocytic and neutrophilic components, indicative of general immune activity:

$$LI = \frac{\text{Lymphocytes}}{\text{Band neutrophils} + \text{Segmented neutrophils}} \quad /2/$$

Indices related to inflammatory and intoxication responses:

Leukocyte Index of Intoxication (LII). Estimates the level of systemic inflammatory response and endogenous intoxication, adapted by removing immature myeloid precursors:

$$LII = \frac{(2 \times \text{Band neutrophils} + \text{Segmented neutrophils})}{(\text{Lymphocytes} + \text{Monocytes}) \times (\text{Eosinophils} + 1)} \quad /3/$$

Lymphocyte-Granulocyte Index (LGI). Characterises the balance between adaptive (lymphocytes) and innate (granulocytes) immune responses:

$$LGI = \frac{10 \times \text{Lymphocytes}}{\text{Band neutrophils} + \text{Segmented neutrophils} + \text{Eosinophils}} \quad /4/$$

Indices of leukocyte ratio dynamics:

Neutrophil to Lymphocyte Ratio (NLR). Assesses the balance between innate and adaptive immunity, commonly used as a marker of systemic stress:

$$NLR = \frac{\text{Band neutrophils} + \text{Segmented neutrophils}}{\text{Lymphocytes}} \quad /5/$$

Lymphocyte to Eosinophil Ratio (LER). May reflect changes in immune regulation and allergic potential:

$$LER = \frac{\text{Lymphocytes}}{\text{Eosinophils} + 1} \quad /6/$$

Neutrophil to Monocyte Ratio (NMR). Provides insight into the proportion of short-lived phagocytes (neutrophils) relative to longer-lived monocytes:

$$NMR = \frac{\text{Band neutrophils} + \text{Segmented neutrophils}}{\text{Monocytes} + 1} \quad /7/$$

Lymphocyte to Monocyte Ratio (LMR). Indicates trends in adaptive immunity and stress adaptation:

$$LMR = \frac{\text{Lymphocytes}}{\text{Monocytes} + 1} \quad /8/$$

Indices of functional immune reactivity and adaptation:

Index of Allergisation (IA). Evaluates immune reactivity in relation to eosinophilic response, with adjustments for small eosinophil counts:

$$IA = \frac{\text{Lymphocytes} - (\text{Eosinophils} + 1)}{\text{Band neutrophils} + \text{Segmented neutrophils} + \text{Monocytes}} \quad /9/$$

Index of Adaptation by Garkavi (IAG). Reflects the organism's adaptive potential by analysing the ratio of lymphocytes to segmented neutrophils:

$$IAG = \frac{\text{Lymphocytes}}{\text{Segmented neutrophils}} \quad /10/$$

Index of Immune Reactivity (IIR). Estimates immune response patterns based on the interaction between lymphocytes, eosinophils, and monocytes:

$$IIR = \frac{\text{Lymphocytes} + \text{Eosinophils}}{\text{Monocytes} + 1} \quad /11/$$

Indices of leukocyte redistribution and neutrophil reactivity:

Leukocyte Shift Index (LSI). Characterises shifts in the granulocyte-to-lymphocyte ratio, which may indicate immune adaptation under stress:

$$LSI = \frac{\text{Eosinophils} + \text{Segmented neutrophils} + \text{Band neutrophils}}{\text{Lymphocytes} + \text{Monocytes}} \quad /12/$$

Neutrophil Reactive Response (NRR). Evaluates the reactive potential of neutrophils, with adjustments for zero counts in band neutrophils or eosinophils:

$$NRR = \frac{(\text{Band neutrophils} + 1) \times \text{Segmented neutrophils}}{(\text{Lymphocytes} + \text{Monocytes}) \times (\text{Eosinophils} + 1)} \quad /13/$$

Thus, the indices NSI, LER, NMR, LMR, IA, IIR, NRR, and partially LII were specifically modified by introducing correction factors (+1) to prevent division by zero when band neutrophils, eosinophils, or monocytes were absent. In addition, all indices that originally incorporated immature myeloid precursors (myelocytes, metamyelocytes, and plasma cells) were adapted by excluding these elements, as such cells are not present in the peripheral blood of clinically healthy cattle. The formulas for LI, LGI, NLR, IAG, and LSI were applied without further modification, except for the removal of immature cell forms where required. These targeted adjustments enable reliable assessment of immune status in dairy cows. The effectiveness of this adapted index set for detecting HS-related immune changes was also confirmed in Holstein cows in our recent study ([MYLOSTYVYI et al., 2026](#)), supporting its applicability across major dairy breeds.

Statistical analysis. Descriptive statistics were presented as mean values (Mean) and the standard

error of the mean (SE). Prior to statistical analysis, normality was assessed using the Shapiro-Wilk test. Since most indicators did not meet normality criteria ($P < 0.05$), non-parametric methods were applied. The Mann-Whitney U test was used to compare differences between groups, with statistical signifi-

cance set at $P < 0.05$. Effect sizes (r) were calculated for pairwise comparisons to provide additional insights into the magnitude of the observed differences. Data processing and analysis were conducted using Statistica 12 (StatSoft, Inc., Tulsa, OK, USA).

Table 1. Leukocyte profile and modified integral indices in dairy cows under short-term HS in different periods of the summer

Indicator1	Control (n=36)	Group 1 (n=12)	Group 2 (n=12)
White blood cells (cells/μL)	10.47 $\pm$ 2.82	10.61 $\pm$ 1.52	9.95 $\pm$ 1.85
Types of leukocytes (%)			
Band	1.02 $\pm$ 1.19	0.40 $\pm$ 0.83*	1.61 $\pm$ 1.85
Segmented	46.13 $\pm$ 9.74	54.13 $\pm$ 7.43*	42.07 $\pm$ 6.65
Monocytes	1.63 $\pm$ 1.10	0.60 $\pm$ 0.91*	4.76 $\pm$ 2.42*
Lymphocytes	49.15 $\pm$ 9.67	41.73 $\pm$ 9.00*	46.23 $\pm$ 8.38
Eosinophils	2.05 $\pm$ 1.57	3.27 $\pm$ 2.76	5.31 $\pm$ 3.73*
Basophils	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
Integral leukocyte indices			
NSI	0.043 $\pm$ 0.021	0.026 $\pm$ 0.015*	0.056 $\pm$ 0.023*
LI	1.099 $\pm$ 0.305	0.787 $\pm$ 0.143*	1.088 $\pm$ 0.266
LII	0.414 $\pm$ 0.156	0.483 $\pm$ 0.121	0.236 $\pm$ 0.083*
LGI	10.95 $\pm$ 2.61	7.32 $\pm$ 1.45*	10.04 $\pm$ 1.98
NLR	1.017 $\pm$ 0.268	1.358 $\pm$ 0.323*	0.998 $\pm$ 0.278
LER	19.76 $\pm$ 6.14	17.75 $\pm$ 6.34	15.73 $\pm$ 3.46*
NMR	20.51 $\pm$ 5.72	44.87 $\pm$ 5.83*	10.29 $\pm$ 2.85*
LMR	20.98 $\pm$ 6.97	34.60 $\pm$ 6.57*	11.19 $\pm$ 3.97*
IA	0.994 $\pm$ 0.300	0.719 $\pm$ 0.173*	0.874 $\pm$ 0.196
IAG	1.109 $\pm$ 0.312	0.792 $\pm$ 0.148*	1.151 $\pm$ 0.301
IIR	21.21 $\pm$ 5.70	36.20 $\pm$ 6.73*	12.67 $\pm$ 3.43*
LSI	1.036 $\pm$ 0.334	1.456 $\pm$ 0.262*	1.021 $\pm$ 0.369
NRR	0.788 $\pm$ 0.481	0.677 $\pm$ 0.102	0.522 $\pm$ 0.163

Note: Nuclear Shift Index (NSI); Leukocyte Index (LI); Leukocyte Index of Intoxication (LII); Lymphocyte-Granulocyte Index (LGI); Neutrophil-to-Lymphocyte Ratio (NLR); Lymphocyte-to-Eosinophil Ratio (LER); Neutrophil-to-Monocyte Ratio (NMR); Lymphocyte-to-Monocyte Ratio (LMR); Index of Allergisation (IA); Index of Adaptation by Garkavi (IAG); Index of Immune Reactivity (IIR); Leukocyte Shift Index (LSI); Neutrophil Reactive Response (NRR). * – significantly different from control group ($P < 0.05$).

Results

In accordance with the study hypothesis, the adapted leukocyte indices enabled the identification of specific features of the immune response in dairy cows exposed to short-term HS of varying intensity during different periods of the summer (Table 1). During acute HS in early summer (Group 1, mid-June), there was a significant increase in the proportion of segmented neutrophils, accompanied by a reduction in the relative number of lymphocytes. This reflects a classical acute immune reaction with a shift towards neutrophilic dominance. These changes were associated with a decrease in NSI and LI values by 40 percent and 28 percent respectively compared to the control group ($P < 0.01$), as well as a marked increase in NLR, NMR, LMR, IIR and LSI. This pattern indicates the activation of the innate immune response, with the relative suppression of the lymphocytic component and the enhanced functional reactivity of neutrophils.

In contrast to early summer, the cows exposed to HS in late summer (Group 2, late August) showed a transition from an acute immune response to features of immune exhaustion. There was a significant elevation of monocyte and eosinophil counts, a reduction in LII by 43 percent compared to the control ($P < 0.001$), a pronounced decline in NMR and LMR by two to threefold ($P < 0.001$), and a 40 percent decrease in IIR ($P < 0.001$). The profile of these indices at this stage suggests a disruption of the balance among major leukocyte lineages and a possible reduction in the adaptive capacity of the immune system in response to repeated HS.

The modified indices proved to be highly sensitive to the different phases of the immune response. Acute adaptive changes predominated in Group 1, while markers suggestive of immune fatigue and imbalance were evident in Group 2. The results confirm the high informative value of the adapted leukocyte indices for assessing the immune response of dairy cows to short-term HS. The most sensitive indicators of immune status included NSI, NLR, LMR, IIR and LII. These indices enabled differentiation between acute adaptive responses and the phenomena of immune fatigue that arise under prolonged or repeated exposure to adverse temperatures. Changes in the dynamics of these indices re-

flect alterations in the immune response to HS of identical duration, depending on the period of the summer. Such observations may be important for evaluating the adaptive potential of dairy cows, and for identifying the health risks associated with repeated or prolonged heat exposure.

Discussion

The results of this study confirm that the adapted leukocyte indices may serve as valuable biomarkers of the immune response in dairy cows under short-term HS. The observed changes in leukocyte dynamics are consistent with previous research demonstrating alterations in immune function under HS conditions. HS induces complex physiological and metabolic adaptations in animals, and there is reason to believe that immune modulation plays an important role in maintaining homeostasis ([KIM et al., 2022](#)). Our findings demonstrate that modified leukocyte indices are capable of providing relevant information about immune status, offering a practical tool for characterising immunological shifts and their impact on animal health under real farm conditions.

During the summer, distinct immune responses of leukocyte subpopulations were observed in dairy cows depending on the timing of the HS exposure. Acute HS at the beginning of the season (Group 1) was associated with the features of classical immune activation, characterised by a transient shift towards neutrophil predominance, accompanied by relative suppression of the lymphocytic component. This response was reflected in a significant decrease in NSI and LI, along with increases in NLR, NMR, LMR, IIR, and LSI, indicating enhanced activation of innate immunity and the increased functional reactivity of neutrophils. These findings are consistent with previous studies reporting similar immune responses to HS in dairy cattle ([SAFA et al., 2018](#); [KIM et al., 2022](#)).

In contrast, exposure to HS at the end of the summer (Group 2) manifested in alterations of the leukocyte profile, with signs suggestive of immune fatigue and possible early immune exhaustion. In particular, there was a significant increase in the number of monocytes and eosinophils, a marked decrease in LII, NMR, and LMR, as well as a pro-

nounced reduction in IIR. This may suggest a disruption in the balance among the main leukocyte lineages and a potential decline in the adaptive capacity of the immune system following repeated HS against a background of a prolonged period of high summer temperatures. Such changes are consistent with previous reports describing immune exhaustion in response to cumulative HS in dairy cattle ([DAHL et al., 2020](#); [SCERRI et al., 2023](#); [WIJFFELS et al., 2023](#)).

These immunological changes should be interpreted within the broader context of systemic metabolic adaptation to HS. It has been shown that thermal load in dairy cows affects glucose metabolism, enhances lipid mobilisation, alters β -hydroxybutyrate concentrations, and is accompanied by oxidative imbalance and reduced antioxidant protection ([TURK et al., 2015](#); [TURK et al., 2024](#)). Such metabolic shifts are closely linked to immune regulation and may contribute to the redistribution of leukocyte subpopulations observed in the present study. Therefore, the modified leukocyte indices used here may reflect not only immune reactivity per se, but also the integrated adaptive responses of the organism to HS.

HS induces significant metabolic changes that can affect both energy balance and immune function. According to previous studies, cows exposed to HS show elevated blood lactate and pyruvate levels, indicating increased anaerobic glycolysis as a compensatory mechanism aimed at maintaining energy homeostasis ([YUE et al., 2020](#); [AJANOVIC et al., 2023](#)). Changes in glucose and fatty acid levels have also been reported, confirming impaired energy metabolism, which may also contribute to immune suppression during HS ([ALBERGHINA et al., 2024](#)). These metabolic changes are often accompanied by alterations in other haematological parameters. For example, increased levels of reactive oxygen metabolites and reduced antioxidant defence markers highlight the role of oxidative stress in immune modulation ([SKLYAROV et al., 2020](#); [KIM et al., 2024](#)). Therefore, the integration of leukocyte indices with metabolic biomarkers may contribute to a more comprehensive assessment of adaptation mechanisms in dairy cows exposed to HS.

Breed-related differences in thermotolerance have been previously reported. For example, Brown Swiss cows are generally considered more resistant to HS than Holstein cows ([MYLOSTYVYI et al., 2023](#); [CECILIANI et al., 2024](#)). These differences may be associated with genetic factors, such as the presence of single nucleotide polymorphisms (SNPs) related to thermotolerance, which are linked to immune modulation and heat dissipation mechanisms ([CUELLAR et al., 2023](#); [NÚÑEZ SOTO et al., 2023](#)). However, since the present study was limited to Brown Swiss cows, the results and conclusions regarding the dynamics of the immune response and the diagnostic value of leukocyte indices are specific to this breed. Potential breed-specific variations in leukocyte profiles and adaptation to HS must be acknowledged as a limitation of this work. Further comparative studies involving other dairy breeds, such as Holstein, are needed for the broader applicability of leukocyte biomarkers in the context of breed-related variability in immune response to HS ([CASTELLANI et al., 2025](#)).

The use of the modified leukocyte indices proposed in this study as biomarkers of HS may provide valuable information for improving dairy cattle management as part of preventive strategies. Incorporating these indices into routine diagnostic investigations could facilitate the early detection of changes in immune status in response to increasing thermal load. This would allow for timely intervention measures, such as advanced cooling systems and dietary adjustments to maintain immune homeostasis ([SCERRI et al., 2023](#)). Additionally, genetic selection for thermotolerance is a promising direction for mitigating the negative effects of HS, especially in breeding programmes focused on immune resilience traits ([JURKOVICH et al., 2023](#)). Therefore, the potential for using leukocyte indices to select more resilient animals under the increasing number of heat waves appears to be justified. The relatively small sample size in the heat-stressed groups ($n=12$) should be considered as a limitation of the study, and may restrict the generalisability of the observed patterns.

Future research should focus on the applicability of leukocyte indices as biomarkers depending on the intensity and duration of HS in dairy cows,

including individual and breed-specific differences. Studying the interactions between leukocyte indices and other physiological and metabolic indicators of adaptation under HS conditions may also be promising. Furthermore, the development of predictive models integrating leukocyte indices, metabolic parameters, and environmental data could facilitate timely decision-making in precision livestock management, supporting animal health and productivity under challenging thermal conditions.

Conclusions

The adapted leukocyte indices proved to be effective biomarkers for assessing the immune response of dairy cows to short-term heat stress. Acute heat stress at the beginning of the summer elicited an adaptive reaction with a predominance of neutrophils, as evidenced by the increased integral leukocyte indices (NLR, NMR, LMR, IIR, LSI) and decreased NSI and LI. Repeated exposure to heat stress of equal intensity and duration at the end of the summer suggested the development of immune suppression, manifested by increased monocyte and eosinophil counts, decreased integral leukocyte indices (LII, NMR, LMR, IIR), and an imbalance among the main leukocyte lineages. These results underscore the value of the modified leukocyte indices for monitoring immunological adaptation to heat stress. Further research is needed to confirm their differential diagnostic significance for heat stress of varying duration and intensity, as well as their applicability to other breeds under different production systems.

Ethics approval

This study was conducted in accordance with the guidelines approved by the Bioethics Commission of Dnipro State Agrarian and Economic University (Approval No. 2/1, 26 February 2025).

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Authorship contribution statement

Roman Mylostyvyi conceived and designed the study, collected and analysed the data, interpreted the results, and wrote the manuscript. The author critically reviewed and approved the final version of the manuscript.

Declaration of competing interest

There is no conflict of interest.

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SAŽETAK

Toplinski stres (HS) i dalje je znatan izazov u proizvodnji mliječnih krava, koji utječe na imunosnu funkciju i produktivnost. U ovom su istraživanju procijenjeni modificirani leukocitni pokazatelji u krava pasmine švicarsko smeđe govedo. Uspoređeni su podaci za krave pod utjecajem toplinskog stresa na početku i na kraju ljeta s podacima za krave u termoneutralnim uvjetima (kontrolna skupina). Akutni toplinski stres rezultirao je znatnim povećanjem segmentiranih neutrofila (+17%, $P<0,01$) i smanjenjem limfocita (−15%, $P<0,05$), uz odgovarajuća smanjenja indeksa skretanja leukocita (NSI, −40%, $P<0,01$) i leukocitnog indeksa (LI, −28%, $P<0,001$). Nasuprot tome, izloženost kasnom ljetu dovela je do većeg broja monocita (+192%, $P<0,001$) i eozinofila (+159%, $P<0,05$), kao i do izraženog pada leukocitnog pokazatelja intoksikacije (LII, −43%, $P<0,001$), omjera neutrofila i monocita (NMR) i omjera limfocita i monocita (LMR) (2 do 3 puta, $P<0,001$) te pokazatelja imunosne reaktivnosti (IIR, −40%, $P<0,001$). Pokazatelji kao što su NSI, omjer neutrofila i limfocita (NLR), LMR, IIR i LII bili su osobito osjetljivi u razlikovanju akutne adaptacije od imunosnog zamora. Ovi nalazi naglašavaju dijagnostičku vrijednost modificiranih leukocitnih pokazatelja za rano otkrivanje i praćenje imunosne prilagodbe na toplinski stres u mliječnih krava. Navedeno pruža potporu za ciljane intervencije i bolju otpornost na razini stada.

Ključne riječi: toplinski stres; mliječna goveda; leukocitni pokazatelji; imunosna prilagodba; biomarkeri toplinskog stresa
