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THE EFFECT OF PROLONGED COLD EXPOSURE ON LEUKOCYTE INDICES OF DAIRY COWS

The effect of prolonged winter cold exposure on leukocyte composition and integral leukocyte indices (ILIs) in Brown Swiss dairy cows was investigated. Cows in the cold stress group were exposed to low ambient temperatures for 10 days (mean –9.6 ± 2.1 °C, minimum –14 °C). Prolonged cold exposure was associated with a decrease in the total leukocyte count, an increase in the proportion of monocytes, and no significant changes in other leukocyte subpopulations. Concurrently, decreases in the neutrophil-to-monocyte ratio (NMR), lymphocyte-to-monocyte ratio (LMR), and index of immune reactivity (IIR) indicated a shift in immune balance towards the monocytic component and reduced immune reactivity. Changes in leukocyte indices reflected a restructuring of immune homeostasis and activation of nonspecific defence mechanisms under low-temperature exposure. The results demonstrate the applicability of leukocyte indices for assessing adaptive changes in dairy cows under prolonged cold exposure.

Key words: *blood leukocytes, cold stress, prolonged cold exposure, leukocyte indices, immune adaptation, dairy cows.*

Low temperature is one of the key factors affecting physiological adaptation in dairy cows [2, 20]. In livestock science, cold stress (CS) is commonly used to describe environmental low-temperature conditions that impose additional thermoregulatory and metabolic load on animals. Although the effects of heat stress on metabolism and defence mechanisms in cows have been widely studied [11, 19], the role of CS in shaping the immune response remains insufficiently elucidated, particularly with regard to immune remodelling during prolonged exposure to low temperatures [8, 14]. In contemporary livestock science literature, the term "cold stress" is widely used to describe a complex of low-temperature environmental factors that impose additional thermoregulatory and metabolic load on the animal organism.

Periods of abrupt cooling lead to increased energy expenditure on thermoregulation, reduced feed intake, decreased productivity, and activation of stress-related immune pathways [10, 13]. A critical feature of the response to CS is the alteration of both quantitative and qualitative composition of blood leukocytes, reflecting a shift in the balance between innate and adaptive immunity [1, 9]. In contrast to HS, which is characterised primarily by neutrophil activation and systemic inflammation [4, 6], CS is associated with suppression of adaptive immune responses, activation of the monocytic component, and the development of immunosuppression [10, 16].

Modern approaches to the assessment of thermal load involve the use of environmental indices, such as the temperature-humidity index (THI) and wind chill temperature (WCT), which allow for quantitative evaluation of risks to animals [6]. It has been established that CS in cows may develop at relatively moderate sub-zero temperatures when combined with wind and high humidity [2].

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The leukocyte system is highly sensitive to stressors and rapidly reflects changes in the distribution of immune cells in response to extreme conditions [15]. In practice, the use of integral leukocyte indices (ILIs) enables a quantitative assessment of the balance between the principal arms of the immune system [16, 22]. However, classical indices adapted for humans or laboratory animals do not always accurately reflect the specificity of the immune response in cattle, due to the lymphocytic blood profile and immunobiological peculiarities of ruminants [15, 21].

The aim of this study was to assess alterations in blood leukocyte composition and integral leukocyte indices in dairy cows subjected to prolonged winter cold exposure.

MATERIALS AND METHODS

This study was conducted on Brown Swiss dairy cows at a commercial farm in central Ukraine. For blood analysis, two analogue groups of 15 cows each were formed by random sampling: a control group (CON), sampled under thermoneutral conditions (May 2025), and an experimental group (winter cold stress, CS), represented by animals kept under low ambient temperatures during the winter period (January 2026). All animals were matched for lactation stage and productivity (second lactation, 135 ± 5.0 days in milk, 36 ± ± 1.5 kg/day), were clinically healthy, and were housed in naturally ventilated barns on sand bedding. Brown Swiss cows are widely used under continental climatic conditions and are regarded as a relatively hardy dairy breed with good adaptive capacity to environmental stressors. Cows received the same balanced total mixed ration (TMR) throughout the year in accordance with NRC (2001) [18], with feed and water available *ad libitum*, which reduced the potential influence of seasonal feeding variation. The study was approved by the Bioethics Committee of Dnipro State Agrarian and Economic University (protocol No. 2/1 of 26 February, 2025).

The degree of thermal load was determined using the temperature–humidity index (THI) [17] and wind chill temperature (WCT) [2], based on data from the nearest meteorological station and continuous monitoring of the microclimate within the livestock facility throughout the experiment. Cold stress conditions were characterised by a 10-day period of low ambient temperatures (mean −9.6 ± 2.1 °C, minimum −14 °C), with animals housed in naturally ventilated barns under typical winter conditions.

Blood samples were collected from the jugular vein in the early morning hours (07:00—08:00), prior to feed distribution, into EDTA tubes (Aichele Medico AG, Switzerland). The total leukocyte count (WBC) was determined using an automated haematology analyser Sysmex XS-1000i (Sysmex Corp., Japan) in accordance with the manufacturer's protocol. Differential leukocyte counts were performed on blood smears stained using the Romanowsky–Giemsa method, with 200 cells counted per smear (band and segmented neutrophils, lymphocytes, monocytes, eosinophils, basophils). The results were independently verified by two specialists in a blinded manner. To assess the immune response, a range of integral leukocyte indices (ILIs), commonly used in biomedical research [15, 16], were calculated using leukocyte fractions identified in cattle blood smears. Where necessary, a value of +1 was added to avoid division by zero.

The following ILIs were used in the research:

index of neutrophil-to-monocyte ratio (N/M) characterizes the components of the micro- and macrophage systems;

index of lymphocyte-to-monocyte ratio (L/M) reflects the ratio of the affector and effector components of immunological process;

index of neutrophil-to-lymphocyte ratio ((BLs + + SLs)/L) reflects the ratio of non-specific and specific defense cells;

lymphocyte-granulocyte index (10L/(MCs + + MMCs + BLs + SLs + E + B)) allows differentiation between infectious and auto-intoxication;

nuclear shift index ((MCs + MMCs + BLs)/SLs) shows the ratio of the content of all young forms of neutrophils to their mature forms;

lymphocyte-to-eosinophil ratio (L/E) reflects the ratio of immediate and delayed hypersensitivity processes;

leukocyte shift index ((E + B + SLs + BLs)/(L + + M)), its increase indicates an active inflammatory process and impaired immunoreactivity;

leukocyte index (L/N) reflects the relationship between the humoral and cellular components of the immune system; leukocyte intoxication index by Kalf-Kalif ((4MCs + 3MMCs + 2BLs + SLs) × × (PCs + 1))/((L + M) × (E + 1)) characterizes the level of endogenous intoxication and activation of tissue breakdown;

neutrophil reactive response index (BLs × SLs/((L + M) × E)) is an index of endogenous intoxication;

as well as allergization index ((L + 1 – (E + 1))/(BLs + SLs + M)), Garkavi adaptation index (L/SLs), and index of immune reactivity ((L + E)/M).

The abbreviations in the above formulae refer to cell types such as: BLs, band and SLs, segmented leukocytes; L, lymphocytes; M, monocytes; N, neutrophils; E, eosinophils; B, basophils; MCs, myelocytes; MMCs, metamyelocytes (immature cells), PCs, plasma cells (expressed as percentage).

The data were processed using Statistica 12 software (StatSoft, USA). The normality of distribution was assessed using the Shapiro–Wilk test. Since most blood parameters did not meet the criteria of normal distribution ($p < 0.05$), the non-parametric Mann–Whitney U test was used for between-group comparisons. Statistical significance was accepted at $p < 0.05$. Blood parameters are presented as the median (Me) and interquartile range (25^{th}—75^{th} percentiles, Q1—Q3) due to high individual variability. For environmental parameters, the mean value (Mean) and standard error of the mean (SE) are also given.

RESULTS AND DISCUSSION

It is well established that leukocyte parameters in animals are sensitive indicators of physiological state and may respond to multiple endogenous and environmental factors, including thermal conditions. Therefore, prior to the direct analysis of changes in the blood system, particular attention was given to a comprehensive assessment of environmental parameters defining the level of thermal load (Table 1). The control group (CON) included cows sampled under thermoneutral conditions in spring, whereas the cold stress group (CS) comprised animals exposed to low ambient temperatures during the winter period.

As shown in Table 1, in the CON group, THI and WCT were within thermoneutral ranges for dairy cows, indicating the absence of environmental stress. In the CS group, a prolonged period of low temperatures combined with high relative humidity was recorded, resulting in a marked decrease in both THI and WCT. Such conditions created pronounced cold load, which, according to modern classifications, corresponds to a moderate ($8 < $ $< \text{THI} \leq 25$ or $-25 < \text{WCT} \leq -10$) level of stress [10]. It should be emphasised that at the time of blood sampling in the CS group, both indices remained within ranges characteristic of CS, indicating the persistence of adverse environmental factors throughout the experimental period. This experimental design allowed the comparison of changes in haematological parameters under contrasting thermal conditions.

The following section presents the results of the analysis of changes in the blood leukocyte formula and ILIs (Table 2), reflecting the specific features of the immune response of cows to CS.

Analysis of the dynamics of the leukocyte formula in dairy cows under CS demonstrated a range

Table 1. **Environmental parameters under experimental conditions**

Indicator	Control	Cold stress
Mean T, °C (week)	11.3 ± 3.3	–9.6 ± 2.1
Maximum T, °C (week)	18.0	–6.0
Minimum T, °C (week)	4.0	–14.0
Relative humidity, % (week)	73.2 ± 17.4	84.1 ± 3.7
Mean THI (week)	51.4 ± 4.9	18.5 ± 3.0
Maximum THI (week)	60.1	23.8
Minimum THI (week)	40.6	12.7
WCT, °C (week)	7.2 ± 4.0	–15.1 ± 3.2
Duration of adverse period, days	0 (THI < 68 entire period)	10 (THI < 38 entire period)
T, °C on the day of blood sampling	11.0	–8.0
THI on the day of blood sampling	52.6	20.7

Note. Environmental parameters are presented as mean ± SE, maximum, or minimum values for the 7-day period preceding blood sampling in each group. Air temperature, relative humidity, and temperature-humidity index (THI) were calculated using meteorological data [17]. WCT is wind chill temperature calculated from air temperature and wind speed [2]. Temperature and THI values on the day of blood sampling (at the time of collection) are shown separately.

of characteristic changes reflecting the specificity of the immune response to prolonged thermal load at low temperatures.

Under CS, in contrast to the CON group, a significant decrease in WBC (leukopenia) was recorded, confirming suppressed leukopoiesis and enhanced migration or destruction of leukocytes in circulation due to cold exposure [12, 13]. Similar effects have been reported in laboratory animals [16] and are considered markers of stress-induced immunosuppression.

A marked increase in monocytes (by 50%, $p < 0.05$) may indicate activation of the macrophage component and enhancement of phagocytic and reparative processes, a typical mechanism of long-term adaptation to adverse environmental conditions [5, 12]. No significant changes were observed for other leukocyte subpopulations, indicating the absence of an acute inflammatory response [13].

ILIs deserve particular attention. Under CS, there was a statistically significant decrease in NMR (by 29%, $p < 0.05$), LMR (by 40%, $p < 0.05$), and IIR (by 40%, $p < 0.05$) compared with CON, reflecting a shift in the balance of cellular populations towards the monocytic component, a reduction in adaptive immune reserve, and reduced immune reactivity typical of adaptation to chronic cold load [10]. The remaining indices did not differ significantly ($p > 0.05$).

These changes in the leukocyte profile are consistent with literature data on the immune response of cattle and other mammals to cold exposure, particularly with the findings of Lomako *et al.* [16], where leukopenia, activation of the macrophage link, and a decrease in immune reactivity indices were also described in rats under cold load. According to current understanding, a decrease in NMR and LMR reflects a transition from acute inflammatory response to chronic adaptation with activation of macrophage defence, while a reduction in IIR is indicative of immunosuppression [3, 7, 22].

Thus, the results obtained in dairy cows indicate that prolonged CS is associated with a characteristic immune response pattern, including leukopenia, monocyte activation, and decreased NMR, LMR, and IIR, reflecting a chronic restructuring of immune balance in favour of nonspecific defence mechanisms [3, 16, 22].—

It should be noted that the present study was conducted under real production conditions rather than as a strictly controlled experiment. Therefore, the comparison between spring and winter periods may include not only temperature-related effects

Table 2. **Blood leukocyte parameters and leukocyte indices in dairy cows under control (CON, $n = 15$) and cold stress (CS, $n = 15$) conditions (median and interquartile range, 25th—75th percentiles)**

Indicator	Control	Cold stress	*p*–value
WBC (cells/μL)	11.3 (9.3—13.0)	8.11 (6.59—9.96)	0.0036
Band neutrophils, %	1.0 (0.0—3.0)	0.0 (0.0—1.0)	0.2159
Segmented neutrophils, %	49.0 (37.0—58.0)	46.0 (43.0—51.0)	0.6629
Monocytes, %	2.0 (1.0—2.0)	3.0 (2.0—4.0)	0.0001
Lymphocytes, %	46.3 (36.0—57.0)	47.0 (43.0—52.0)	0.8032
Eosinophils, %	2.93 (1.0—4.0)	1.0 (1.0—3.0)	0.2788
Integral leukocyte indices			
Nuclear Shift Index (NSI)	0.033 (0.027—0.081)	0.026 (0.021—0.045)	0.1645
Leukocyte Index (LI)	0.877 (0.600—1.425)	1.022 (0.862—1.209)	0.8681
Leukocyte Intoxication Index (LII, Kalf-Kalif)	0.355 (0.161—0.619)	0.401 (0.235—0.552)	0.6481
Lymphocyte–Granulocyte Index (LGI)	8.49 (5.71—13.90)	9.58 (7.96—11.81)	0.9503
Neutrophil-to-Lymphocyte Ratio (NLR)	1.13 (0.70—1.66)	0.97 (0.82—1.15)	0.8682
Lymphocyte-to-Eosinophil Ratio (LER)	14.75 (7.00—28.50)	16.33 (12.25—26.0)	0.4678
Neutrophil-to-Monocyte Ratio (NMR)	17.33 (14.33—30.00)	12.25 (8.60—15.33)	0.0042
Lymphocyte-to-Monocyte Ratio (LMR)	18.0 (15.0—28.0)	10.8 (8.25—15.33)	0.0005
Allergization Index (IA)	0.796 (0.524—1.309)	0.865 (0.711—1.063)	0.8519
Garkavi Adaptation Index (IAG)	0.918 (0.620—1.621)	1.022 (0.862—1.209)	0.7557
Index of Immune Reactivity (IIR)	19.33 (15.5—29.0)	11.50 (9.00—15.66)	0.0003
Leukocyte Shift Index (LSI)	1.12 (0.69—1.70)	0.96 (0.78—1.17)	0.9173
Neutrophil Reactive Response Index (NRR)	0.797 (0.226—1.779)	0.424 (0.347—1.041)	0.5337

but also other season-dependent physiological factors. The spring period was selected as a thermoneutral baseline reflecting the physiological state of animals without pronounced thermal stress and to minimise the influence of additional seasonal factors, including known declines in milk yield observed in autumn in Brown Swiss cows, which could confound the interpretation of temperature-related effects [18]. At the same time, seasonal variations, including potential endocrine changes, cannot be completely excluded and should be considered when interpreting the results. These aspects represent a limitation of the study but also reflect the practical relevance of the obtained data under commercial farming conditions.

CONCLUSIONS

It was established that prolonged exposure to low temperatures in dairy cows led to changes in the leukocyte system, manifested by leukopenia, an increased proportion of monocytes, and decreased NMR, LMR, and IIR values. These changes indicate a shift in immune homeostasis towards nonspecific defence mechanisms and reduced immune reactivity under prolonged low-temperature exposure.

The obtained results indicate that leukocyte indices can be used to assess adaptive changes in dairy cows under low-temperature exposure and may be useful for monitoring animal status under prolonged cold exposure conditions.

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ВПЛИВ ТРИВАЛОГО ХОЛОДОВОГО НАВАНТАЖЕННЯ
НА ЛЕЙКОЦИТАРНІ ІНДЕКСИ МОЛОЧНИХ КОРІВ

Досліджено вплив тривалого холодового навантаження, пов’язаного з природним впливом низьких температур у зимовий період, на лейкоцитарний склад крові та лейкоцитарні індекси у молочних корів швіцької породи. Холодове навантаження тривало 10 діб і характеризувалося середньою температурою –9,6 ± 2,1 °C з мінімальними значеннями до –14 °C. За умов холодового навантаження встановлено зниження загальної кількості лейкоцитів, підвищення частки моноцитів та відсутність суттєвих змін інших субпопуляцій лейкоцитів. Одночасно виявлено зниження співвідношення нейтрофілів до моноцитів (НМР), лімфоцитів до моноцитів (ЛМР) та індексу імунореактивності (ІІР), що свідчило про зміщення імунного балансу в напрямку моноцитарного компонента та зниження імунної реактивності. Зміни лейкоцитарних індексів відображали перебудову імунного гомеостазу та активацію неспецифічних механізмів захисту за умов низькотемпературного впливу. Результати демонструють інформативність лейкоцитарних індексів для оцінки адаптаційних змін у молочних корів за тривалого холодового навантаження.

Ключові слова: лейкоцити крові, холодовий стрес, тривале холодове навантаження, лейкоцитарні індекси, імунна адаптація, молочні корови.