

LEUKOCYTE INDICES IN DAIRY COWS UNDER HEAT STRESS

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Introduction. Heat stress is currently recognized as one of the major limiting factors in modern dairy production systems, particularly under conditions of ongoing climate change. Increasing ambient temperatures, the growing frequency of heat waves, and the combined effects of temperature and humidity significantly affect animal physiology, productivity, and welfare [1]. High-producing dairy cows, especially of the Holstein breed, are particularly sensitive to thermal load due to their high metabolic heat production and limited capacity for heat dissipation [5]. Prolonged exposure to elevated temperature–humidity index (THI) values is associated with disturbances in energy balance, reduced feed intake, and impaired productive performance. In addition, heat stress induces profound alterations in the immune system, including both cellular and humoral components, leading to increased susceptibility to diseases and reduced resilience [2,3]. These changes often occur at a subclinical level, which complicates their early detection under commercial farming conditions.

Although a wide range of laboratory methods is available to assess immune status, including the determination of immunoglobulins and circulating immune complexes, their routine application is limited by high cost, time consumption, and the need for specialized equipment. Therefore, there is a clear need for simple, rapid, and economically feasible indicators that can be used for large-scale monitoring of animal health.

Integral leucocyte indices (ILIs), calculated from standard haematological parameters, represent a promising alternative. These indices reflect the balance between different leucocyte populations and provide an integrated assessment of inflammatory response, immune reactivity, and adaptive capacity. Despite their widespread use in biomedical research, their application in dairy cattle, particularly under chronic heat stress conditions, remains insufficiently studied [3].

The aim of the study was to evaluate the informative value of integral leucocyte indices as accessible markers of immune status in dairy cows under conditions of chronic heat stress.

Materials and Methods. The study was conducted on a commercial dairy farm located in the Dnipropetrovsk region (Ukraine) under conditions typical for intensive milk production systems [1]. Holstein cows in the second and third lactation stages ($n = 20$) were selected and randomly assigned to two analogous groups (10 animals each): an experimental group exposed to chronic heat stress during the summer period and a control group maintained under thermoneutral conditions in autumn. Environmental conditions were monitored using a portable thermo-hygrometer, and the level of heat stress was assessed using the temperature–humidity index (THI) calculated according to standard approaches [1]. During the experimental period, THI values ranged from 77.9 to 78.6, indicating moderate to severe heat stress, whereas in the control period THI remained below 68, corresponding to thermal comfort. Blood samples were collected from the jugular vein in the morning before feeding. Haematological analysis, including total and differential leucocyte counts, was performed using an automated analyzer, followed by microscopic verification of leucocyte formula. Biochemical and immunological parameters included immunoglobulins (IgA, IgG, IgM), circulating immune complexes (CIC), serum lysozyme activity (SLA), and serum bactericidal activity (SBA). Integral leucocyte indices were calculated based on differential leucocyte counts, including nuclear shift index (NSI), neutrophil-to-lymphocyte ratio (NLR), leucocyte shift index (LSI), lymphocyte-to-eosinophil ratio (LER), and others adapted for cattle physiology [3]. These indices allow a comprehensive evaluation of immune response by integrating cellular interactions within the immune system.

Statistical analysis was performed using Statistica 12.0 software. Since the distribution of most variables deviated from normality, non-parametric methods were applied. Differences between

groups were assessed using the Mann–Whitney U test, and relationships between variables were evaluated using Spearman’s rank correlation coefficient. Statistical significance was set at $p < 0.05$.

Results. Chronic heat stress induced significant alterations in both humoral and cellular components of the immune system in dairy cows. One of the most pronounced changes was a decrease in serum lysozyme activity by 48% ($p < 0.05$), indicating suppression of innate immune defence mechanisms. At the same time, the concentration of circulating immune complexes increased by 36%, suggesting enhanced antigenic load and activation of immune responses.

Although the concentrations of major immunoglobulin classes (IgG and IgM) remained relatively stable, IgA showed a tendency to increase, reflecting activation of mucosal immunity under prolonged stress conditions. This pattern is consistent with adaptive responses aimed at maintaining barrier protection.

Significant changes were also observed in the leucocyte profile. The proportion of band neutrophils increased 2.2-fold ($p < 0.01$), indicating activation of granulopoiesis and a shift of the leucogram to the left. In contrast, lymphocyte and monocyte counts decreased by 23.8% and 42.1%, respectively ($p < 0.05$), suggesting suppression of adaptive immune responses and reduced phagocytic reserve.

These changes were clearly reflected in the values of integral leucocyte indices. The nuclear shift index increased more than twofold, confirming enhanced mobilisation of immature neutrophils. The neutrophil-to-lymphocyte ratio increased by approximately 65%, indicating a shift toward innate immunity dominance. Conversely, indices associated with lymphocyte activity, such as LER and lymphocyte-related indices, decreased significantly, reflecting reduced immune regulation and adaptive capacity.

Correlation analysis revealed strong relationships between ILIs and humoral immune parameters. In particular, a strong positive correlation was found between NLR and IgA levels ($r = 0.873$; $p < 0.05$), indicating a coordinated response between cellular and humoral immunity under heat stress. Similar relationships were observed for the leucocyte shift index.

Importantly, ILIs calculated from a single complete blood count were found to be approximately 4.5 times less expensive than the determination of separate immunological parameters, highlighting their practical value for routine use.

Conclusions. Chronic heat stress in dairy cows leads to a complex restructuring of the immune system, characterized by activation of neutrophil-associated innate defence mechanisms and suppression of adaptive immune responses. Integral leucocyte indices demonstrated high sensitivity to these changes and reflected both cellular and humoral components of immune response. Their strong correlation with immunological parameters confirms their diagnostic relevance. Due to their simplicity, cost-effectiveness, and rapid availability, ILIs can be considered a practical tool for primary monitoring of immune status in dairy cows under commercial farming conditions.

References

1. Mylostyvyi, R., & Izhboldina, O. (2025). An Integrated Approach Using Temperature–Humidity Index, Productivity, and Welfare Indicators for Herd-Level Heat Stress Assessment in Dairy Cows. *Animals*, 15(22), 3341. <https://doi.org/10.3390/ani15223341>
2. Mylostyvyi, R., Lacetera, N., Amadori, M., Sejian, V., Souza-Junior, J. B. F., & Hoffmann, G. (2023). The autumn low milk yield syndrome in Brown Swiss cows in continental climates: hypotheses and facts. *Veterinary Research Communications*, 48(1), 203–213. <https://doi.org/10.1007/s11259-023-10203-0>
3. Mylostyvyi, R., Sejian, V., Izhboldina, O., Sanzhara, R., Chernenko, Olena, Dochkin, D., & Hoffmann, G. (2026). Changes in leukocyte indices of Holstein cows under prolonged heat stress conditions. *Tropical Animal Health and Production*, 58(2). <https://doi.org/10.1007/s11250-026-04910-4>
4. Varkholiak, I. S., Gutyj, B. V., Gufriy, D. F., Sachuk, R. M., Mylostyvyi, R. V., Radzykhovskiy, M. L., Sedilo, H. M., & Izhboldina, O. O. (2021). The effect of the drug

“Bendamine” on the clinical and morphological parameters of dogs in heart failure. Ukrainian Journal of Veterinary and Agricultural Sciences, 4(3), 76–83. <https://doi.org/10.32718/ujvas4-3.13>

5. Vysokos, M. P., Milostiviy, R. V., & Tiupina, N. P. (2014). Comparative assessment of the effect of technologies and housing systems on the longevity of productive use of Holstein cows of foreign breeding. Scientific and Technical Bulletin of the Research Center for Biosafety and Environmental Control of Agricultural Resources, 2(1): 143–147. URL: <http://dspace.dsau.dp.ua/jspui/handle/123456789/3347>

DIETHYL PHTHALATE-INDUCED CHANGES IN XANTHINE OXIDOREDUCTASE ACTIVITY AS A MARKER OF OXIDATIVE STRESS IN THE CYTOSOLIC FRACTION OF RATS

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Introduction. Diethyl phthalate (DEP) is one of the widespread environmental xenobiotics capable of inducing oxidative stress development and disrupting cellular function [1]. One of the key enzymes involved in the generation of reactive oxygen species (ROS) is xanthine oxidoreductase (XOR), a purine metabolism enzyme that catalyzes the conversion of hypoxanthine to xanthine and xanthine to uric acid. This enzyme exists in two interconvertible forms: xanthine dehydrogenase (xanthine:NAD⁺ oxidoreductase, EC 1.17.1.4) and xanthine oxidase (xanthine:oxygen oxidoreductase, EC 1.17.3.2). The dehydrogenase form uses NAD⁺ as an electron acceptor and is characterized by minimal ROS production. In contrast, the oxidase form of XOR utilizes molecular oxygen, resulting in the formation of superoxide anion and hydrogen peroxide [2]. The conversion of the dehydrogenase form into the oxidase form is one of the key mechanisms underlying oxidative stress development under toxic conditions.

The relevance of this study is determined by the widespread occurrence of phthalates in the environment and the insufficient understanding of the mechanisms of their toxic effects on the prooxidant–antioxidant balance of the organism. Since activation of the oxidase form of XOR is associated with increased ROS production, the investigation of changes in the activity of different enzyme forms under prolonged DEP exposure is essential for understanding the mechanisms of oxidative stress development and associated structural and functional tissue alterations.

Aim of the study – to investigate the effect of diethyl phthalate on the activity of the dehydrogenase and oxidase forms of xanthine oxidoreductase in the cytosolic fraction of rat liver.

Materials and methods. The study was performed on outbred white rats weighing 180–200 g, kept under standard vivarium conditions. The animals were divided into three groups: Group I – intact animals; Group II – rats receiving daily oral administration of DEP at a dose of 2.5 mg/kg body weight; Group III – rats receiving DEP at a dose of 5 mg/kg body weight. The experiment lasted for 21 days. Euthanasia was performed on days 14 and 21 of the study.

The cytosolic fraction was obtained from rat liver by tissue homogenization in an appropriate buffer solution followed by differential centrifugation. After removal of cell debris and organelles, the supernatant was used as the cytosolic fraction for the determination of XOR activity. The activity of the dehydrogenase and oxidase forms of XOR was measured spectrophotometrically based on the rate of uric acid formation from xanthine. The activity of the dehydrogenase form was assessed in the