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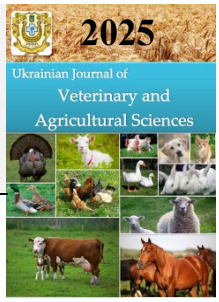

Impact of acute heat stress on hematological and biochemical profiles in Brown Swiss cows

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Abstract

Heat stress (HS) is a critical environmental factor that disrupts dairy cows' physiological and metabolic balance, leading to impaired productivity, immune suppression, and oxidative stress. The Brown Swiss breed is known for its relatively higher thermotolerance, yet its hematological and biochemical responses to acute HS remain poorly understood. This study aimed to evaluate the impact of acute heat stress on the hematological and biochemical parameters of Brown Swiss dairy cows, identifying key physiological adaptations and potential biomarkers for stress assessment. The experiment involved 16 Brown Swiss cows in their second lactation, divided into a heat-stressed group (HYP, n = 8) and a control group (CON, n = 8). Heat stress conditions were characterized by a temperature-humidity index (THI) of 77.6 for five consecutive days. Hematological and biochemical analyses were conducted using an automated haematology analyzer and biochemical assays to assess oxygen transport capacity, metabolic adaptations, and immune responses. The results demonstrated significant changes in key blood parameters due to acute HS. Haemoglobin concentration decreased by 8.8% ($P < 0.05$), while platelet count and leukocyte levels were reduced by 30.2 % and 25.1 %, respectively ($P < 0.05$), indicating hematopoietic and immune alterations. Biochemical findings showed a 21.8 % increase in albumin concentration ($P < 0.05$), along with a 77.5 % rise in blood urea nitrogen ($P < 0.05$), suggesting enhanced protein catabolism. Additionally, total lipoprotein levels increased by 56.3 % ($P < 0.05$), and β-carotene concentration rose by 87.1 % ($P < 0.05$), reflecting metabolic shifts and oxidative stress adaptation. Thus, acute HS induces significant hematological and biochemical alterations in Brown Swiss cows, affecting oxygen transport, immune function, and metabolic regulation. The findings highlight the physiological trade-offs necessary for thermoregulation, emphasizing the need for targeted nutritional and environmental strategies to enhance heat stress resilience in dairy cattle. Further research is warranted to explore long-term adaptations and develop practical mitigation approaches.

Keywords: heat stress; dairy cows; blood parameters; Brown Swiss; metabolic regulation.

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1. Introduction

Heat stress (HS) is a significant environmental challenge that disrupts the physiological homeostasis of dairy cows, leading to metabolic alterations, immune suppression, and reduced productivity. Rising global temperatures and prolonged periods of extreme heat exacerbate these effects, making the study of HS impact on cattle health and productivity increasingly relevant (Valencia et al., 2024). The physiological responses of dairy cows to HS involve complex interactions between thermoregulatory, endocrine, and hematological mechanisms, necessitating a comprehensive approach to understanding their adaptive capacity under heat stress conditions (Aleena et al., 2020; Duda et al., 2020).

HS has been shown to impair dairy cows' oxygen availability and metabolic efficiency. A study by Zeng et al. (2023) highlighted a significant decline in blood oxygen concentration, hemoglobin levels, and red blood cell counts under HS conditions. These physiological changes compromise oxygen transport to tissues, affecting metabolic processes essential for lactation performance. Moreover, HS stimulates the production of reactive oxygen species (ROS), leading to oxidative stress that further disrupts metabolism and reduces milk yield and quality. Such findings align with previous reports indicating that oxidative stress is key to HS-induced physiological dysfunction in dairy cattle.

Hematological parameters serve as critical indicators of cows' physiological state under HS. Research by Kim et al. (2023) demonstrated that exposure to HS significantly affects hematological markers, including increased white blood cell counts and elevated levels of heat shock proteins (HSP70). These changes reflect an adaptive response to mitigate cellular damage and maintain homeostasis. Similarly, Davidson et al. (2021) found that calves born to heat-

stressed heifers exhibited lower immunoglobulin G (IgG) concentrations, suggesting compromised passive immune transfer. These findings highlight the systemic effects of HS on immune function and stress adaptation, which extend beyond the immediate thermal exposure period (Mylostyvyi et al., 2021a; Wang et al., 2024).

The metabolic consequences of HS in dairy cows involve shifts in nutrient utilization. Gao et al. (2017) demonstrated that HS reduces milk yield by 17 % and milk protein concentration by 4.1 %, independent of feed intake reduction. Heat-stressed cows exhibited increased nitrogen excretion and reduced plasma-free amino acid concentrations, indicating metabolic shifts that prioritize thermoregulation over lactation efficiency. Similarly, Abbas et al. (2020) explored HS effects on glucose metabolism, emphasizing increased insulin activity and altered glucose transport mechanisms. These metabolic disruptions impair production efficiency and health challenges, underscoring the need for effective mitigation strategies.

Breed differences play a crucial role in HS resilience, as demonstrated by Mylostyvyi et al. (2021), who found that Brown Swiss cows exhibit greater thermotolerance compared to Holstein cows. Their study indicated that Brown Swiss cows maintain lower rectal temperatures and more stable hematological parameters under heat stress conditions, suggesting breed-specific adaptive mechanisms. The ability to sustain homeostasis under HS conditions is vital for ensuring dairy productivity, and understanding these differences can aid in developing targeted breeding and management strategies (Vasilenko et al., 2018; Hoffmann et al., 2021).

Beyond metabolic and hematological alterations, HS affects immune responses in dairy cows. Research by Chen et al. (2023) highlighted that heat-sensitive cows exhibit significantly higher serum cortisol levels and interleukin-6 (IL-6) concentrations compared to heat-tolerant cows. These findings suggest that heightened stress responses in heat-sensitive breeds contribute to increased inflammation and oxidative stress, further compromising their health and productivity. Nabi et al. (2022) reported that HS induces leukocyte profile alterations, with lymphopenia and neutrophilia characteristic hematological responses to thermal stress.

The implications of HS on dairy cow productivity extend to long-term physiological consequences. Blond et al. (2024) demonstrated that sustained exposure to HS alters metabolic, endocrine, and inflammatory parameters, significantly increasing rectal and body surface temperatures. The researchers found increased extracellular heat shock protein 70 (eHsp70), tumor necrosis factor α (TNFα), cortisol, and insulin levels, while reductions were noted in glucose, calcium, and total protein concentrations. These findings highlight the systemic nature of HS effects and the necessity of adaptive strategies to mitigate its impact on dairy cattle.

The objective of this study is to investigate the hematological and biochemical responses of Brown Swiss dairy cows to acute heat stress. This research aims to assess the physiological adaptations and metabolic changes induced by elevated ambient temperatures by analyzing key blood parameters. The findings will contribute to a deeper understanding of breed-specific thermotolerance and provide insights into potential biomarkers for monitoring heat stress in dairy cattle.

2. Materials and methods

Animals and Experimental Design

The study involved Brown Swiss dairy cows in their second lactation. A total of 16 cows were selected and divided into two groups: the experimental group (HYP, n = 8), which experienced heat stress during summer (June), and the control group (CON, n = 8), kept under thermally comfortable conditions in autumn (October). Lactation parameters were similar between the groups, with an average lactation duration of 106.7 ± 19.12 days in the HYP group and 136.75 ± 19.1 days in the CON group. The average daily milk yield was 36.4 ± 6.49 kg in the HYP group and 38.1 ± 6.32 kg in the CON group. Milk production data, including daily milk yield (kg) and fat and protein content (%), were recorded using the DairyComp 305 herd management system throughout the experimental period.

Climatic Condition Assessment

Heat stress levels were assessed using the temperature-humidity index (THI), calculated according to Kibler (1964). Data on air temperature (°C) and relative humidity (%) were obtained from the nearest meteorological station, approximately 20 km from the farm. Measurements were recorded eight times daily at three-hour intervals to calculate the mean values. During the five-day heat stress period in the Brown Swiss HYP group, the THI increased to 77–82 units (mean value: 77.6), whereas in the control group, the THI remained below 68 throughout the study period.

Blood Sampling and Sample Preparation

Blood samples (10 ml) were collected from the jugular vein into sterile tubes without anticoagulants. The samples were immediately cooled and centrifuged at 3000×g for 20 minutes. The resulting serum was transferred into 1.5-ml microtubes (Eppendorf AG, Germany) for further analysis.

Biochemical Analysis

Biochemical analyses were conducted at the Biosafety Center of Dnipro State Agrarian and Economic University. The following parameters were measured: total protein (biuret method), albumin (bromocresol green method), urea (urease method with Warburg optical test), creatinine (Jaffe reaction), glucose (glucose oxidase method), total bilirubin (oxidation with vanadate), total calcium (arsenazo III method), inorganic phosphorus (ammonium molybdate reaction), and carotene (spectrophotometry using the Bessey method modified by Levchenko et al., 1998). Enzymatic activity was assessed for alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Warburg kinetic test), alkaline phosphatase (ALP) (reaction with nitrophenyl phosphate). Biochemical parameters and enzyme activity were measured using the Miura-200 automatic biochemical analyzer (Italy) with commercial reagent kits from Felicit-Diagnostics (Ukraine), Cormay (Poland), and Spinreact (Spain). The nephelometric method determined the protein fraction content in serum (Vlizlo et al., 2012).

Vitamin Analysis

Vitamins A and E were analysed using reverse-phase high-performance liquid chromatography (RP-HPLC). The analysis was performed using an Agilent Technologies 1260 Infinity liquid chromatograph with a C18 column and spec-

trophotometric detection. The detection wavelengths were 328 nm for vitamin A and 286 nm for vitamin E. The mobile phase flow rate was 0.750 ml/min, and the column thermostat was maintained at +30.0 °C. Sigma (Germany) reagents were used for analysis.

Haematological Analysis

Blood samples for haematological analysis were collected into 2-ml EDTA Vacutainer® tubes (Aichele Medico AG, Basel, Switzerland). Complete blood counts (CBC) were performed using a Sysmex XS-1000i automated hematology analyzer (Sysmex Corporation, Japan). The CBC included total white blood cell (WBC) count, red blood cell (RBC) count, haemoglobin (HGB) concentration, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT) count, mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT). The erythrocyte sedimentation rate (ESR) was measured using the Westergren method.

Statistical Analysis

Data were presented as mean values (Mean) and standard error of the mean (SE). As most variables did not meet normality criteria, non-parametric statistical methods were used for analysis. Statistical processing was performed using STATISTICA 12 (StatSoft, Inc., USA). Differences between groups were evaluated using the Mann–Whitney U test, with statistical significance at $P < 0.05$.

3. Results and discussion

3.1 Results

Hematological Parameters

Table 1 presents the hematological parameters of cows exposed to acute heat stress. Under these conditions, the haemoglobin level in the HYP group was 8.8 % lower than in the control group ($P < 0.05$). At the same time, a slight increase in haematocrit by 3.0 % was observed in HYP cows compared to CON, though this difference was not statistically significant.

Table 1
Hematological parameters (Mean ± SE)

Indicator	Experimental groups, n = 8		P-value
	HYP	CON	
Haemoglobin, g/L	96.50 ± 4.53	105.87 ± 2.69	0.0016
Haematocrit, %	29.74 ± 1.05	28.88 ± 0.69	0.0918
Erythrocytes, 10^{12}/L	6.25 ± 0.13	6.00 ± 0.22	0.0284
MCV, fL	48.81 ± 1.39	48.59 ± 1.19	1.0000
MCH, pg	15.91 ± 0.38	17.91 ± 0.42	0.0013
MCHC, g/L	322.2 ± 5.15	367.4 ± 11.5	0.0014
Color index, units	0.871 ± 0.018	0.869 ± 0.023	0.8117
ESR, mm/h	1.00 ± 0.001	1.37 ± 0.52	0.0955
Platelets, 10^9/L	257.1 ± 48.78	368.3 ± 45.99	0.0031
Leukocytes, 10^9/L	8.13 ± 1.01	10.85 ± 1.73	0.0172

The erythrocyte count in the HYP group was 4.2 % higher compared to CON ($P < 0.05$), suggesting a compensatory response to heat stress. In contrast, the mean corpuscular volume (MCV) remained unchanged between the groups, indicating stable erythrocyte morphology.

The MCH and MCHC were lower in the HYP group by 11.2 % and 12.3 %, respectively ($P < 0.05$), suggesting reduced oxygen transport efficiency. The color index remained stable across groups, indicating no substantial shifts in the hemoglobin-to-erythrocyte ratio.

A 30.2 % reduction in platelet count was observed in the HYP group ($P < 0.05$), which may indicate heat stress-induced alterations in haemostasis. Leukocyte levels were 25.1 % lower in HYP ($P < 0.05$), suggesting immunosuppressive effects of acute heat stress, potentially increasing susceptibility to infections.

Biochemical Blood Parameters

The biochemical analysis revealed significant alterations in cows subjected to acute heat stress compared to thermoneutral conditions (Table 2).

Table 2
Biochemical blood parameters (Mean ± SE)

Indicator	Experimental groups, n = 8		p-value
	HYP	CON	
Total protein (g/L)	82.0 ± 5.59	77.4 ± 4.63	0.1307
Albumin (g/L)	41.7 ± 2.43	34.3 ± 1.49	0.0013
Globulins (g/L)	40.3 ± 6.68	43.1 ± 5.54	0.6015
α-globulins (%)	12.3 ± 3.32	15.3 ± 6.56	0.1747
β-globulins (%)	14.9 ± 3.10	18.3 ± 4.44	0.1747
γ-globulins (%)	32.2 ± 10.29	27.3 ± 7.79	0.3531
Albumin/Globulin ratio	1.07 ± 0.29	0.80 ± 0.09	0.0057
Urea (mmol/L)	8.2 ± 0.77	4.6 ± 0.62	0.0014
Blood urea nitrogen (mg/dL)	15.7 ± 1.50	8.8 ± 1.19	0.0014
Creatinine (μmol/L)	79.6 ± 15.48	66.0 ± 5.45	0.0555
AST (U/L)	104.9 ± 11.17	117.9 ± 8.95	0.0489

ALT (U/L)	38.9 ± 3.02	49.1 ± 9.06	0.0425
De Ritis ratio (AST/ALT)	2.70 ± 0.34	2.45 ± 0.54	0.1985
Alkaline phosphatase (U/L)	68.5 ± 14.38	101.9 ± 45.51	0.0933
Glucose (mmol/L)	2.47 ± 0.13	2.71 ± 0.55	0.2923
Calcium (mmol/L)	2.11 ± 0.36	2.26 ± 0.09	0.0393
Inorganic phosphorus (mmol/L)	2.06 ± 0.19	1.96 ± 0.20	0.3785
Ca/P ratio	1.02 ± 0.14	1.16 ± 0.16	0.1046
β-Carotene (µg/dL)	603.4 ± 200.51	322.5 ± 24.90	0.0240
Total lipoproteins (mg/dL)	1297 ± 177.26	830 ± 64.92	0.0015
Thymol turbidity test (S-H units)	3.99 ± 1.08	3.45 ± 0.62	0.3226
Vitamin A (µg/100 mL)	52.9 ± 13.46	54.7 ± 7.22	0.3225
Vitamin E (µg/mL)	5.27 ± 2.14	4.62 ± 0.36	0.5244

Total protein levels were 6.0 % higher in HYP than in CON, but this difference was insignificant. However, albumin concentration increased by 21.8 % ($P < 0.05$), suggesting an adaptive hepatic response to maintain osmotic balance. The albumin-to-globulin ratio was 33.8 % higher in HYP ($P < 0.05$), indicating an imbalance between albumin and globulin fractions.

Nitrogen metabolism showed marked alterations. Urea levels were 77.7 % higher in HYP ($P < 0.05$), with a similar increase in blood urea nitrogen (BUN) (+77.5 %, $P < 0.05$), indicating increased protein catabolism. Although creatinine levels tended to be higher in HYP (+20.6 %), the difference was insignificant.

Regarding enzyme activity, AST was 11.0 % lower ($P < 0.05$), while ALT decreased by 20.9 % ($P < 0.05$). These changes likely reflect metabolic adjustments rather than hepatic dysfunction. The De Ritis ratio remained unchanged, confirming the absence of significant liver damage.

In mineral metabolism, calcium levels were 6.5 % lower in HYP ($P < 0.05$), while inorganic phosphorus and the Ca/P ratio remained stable, suggesting that mineral balance was maintained mainly despite heat stress.

β-Carotene concentrations were 87.1 % higher in HYP ($P < 0.05$), likely due to increased mobilization as part of the stress response. However, vitamins A and E levels remained stable across groups, indicating preserved antioxidant status.

Lipid metabolism was significantly affected, with total lipoprotein levels being 56.3 % higher in HYP ($P < 0.05$), suggesting increased lipid mobilization to meet energy demands under heat stress.

Overall, acute heat stress-induced marked changes in protein, nitrogen, and lipid metabolism, along with alterations in enzymatic activity and calcium homeostasis. These findings highlight physiological adaptations to maintain homeostasis, but prolonged heat exposure may lead to metabolic imbalances that could affect dairy cows' productivity and health.

3.2 Discussion

Hematological Adaptations to Heat Stress

Heat stress induces significant alterations in hematological parameters, reflecting physiological adaptations to maintain homeostasis. In our study, hemoglobin concentration decreased by 8.8 % in heat-stressed cows compared to the control group ($P < 0.05$), accompanied by a non-significant 3.0 % increase in hematocrit. The reduction in hemoglobin levels aligns with findings by Davidson et al. (2021), who also observed declines in hemoglobin concentration and erythrocyte counts in heat-stressed cattle, attributing these changes to haemodilution and altered erythropoiesis. Similar decreases have been reported in prolonged heat stress conditions in sheep (Rashid et al., 2013) and dairy cattle (Nabi et al., 2022).

Conversely, studies on Katjang and Boer goats demonstrated increased hemoglobin and hematocrit levels under heat stress, likely due to dehydration-induced hemoconcentration (Syafiqa et al., 2023). This discrepancy highlights species-specific differences in hematological responses, where dairy cattle predominantly experience haemodilution, whereas other ruminants may exhibit compensatory erythrocytosis.

Our study's significant reduction in platelet count (−30.2 %, $P < 0.05$) suggests disruptions in haemostasis, possibly due to endothelial dysfunction or decreased thrombopoiesis. Similar reductions have been documented in heat-stressed cattle (Sukandi et al., 2023), emphasizing the impact of thermal stress on coagulation processes. Additionally, leukocyte counts decreased by 25.1 % ($P < 0.05$), reflecting a potential immunosuppressive effect of heat stress. This finding is consistent with studies by Dahl et al. (2020) and Chen et al. (2023), who reported that heat stress modulates immune cell dynamics by reducing circulating lymphocytes and increasing neutrophil-to-lymphocyte ratios.

Biochemical Responses to Acute Heat Stress

The biochemical alterations observed in heat-stressed cows reflect metabolic adjustments essential for physiological stability. Total protein levels were 6.0 % higher in the HYP group, although this difference was insignificant. However, albumin levels increased by 21.8 % ($P < 0.05$), suggesting a hepatic response to maintain osmotic balance. This finding is supported by Mylostyva et al. (2022), who noted increased albumin synthesis under heat stress as an adaptive response to altered fluid dynamics. Similarly, our study's elevated albumin-to-globulin ratio (+33.8 %, $P < 0.05$) suggests an imbalance in protein metabolism, potentially driven by dehydration or altered hepatic function.

Heat stress significantly impacted nitrogen metabolism, with blood urea nitrogen (BUN) and urea levels increasing by 77.7 % and 77.5 %, respectively ($P < 0.05$). These findings align with studies by O'Brien et al. (2010) and Gao et al. (2017), who reported increased urea levels under heat stress, suggesting enhanced protein catabolism as an alternative energy source. Similarly, Hou et al. (2021) observed elevated BUN levels in dairy cows exposed to long-term heat stress, reinforcing that thermal stress redirects metabolic pathways toward protein degradation.

Enzymatic activity displayed notable changes, with AST and ALT levels decreasing by 11.0 % and 20.9 %, respectively ($P < 0.05$). While some studies report increased AST and ALT activity as indicators of hepatic stress (Nabi et al., 2022; Blond et al., 2024), our findings suggest an alternative

metabolic adaptation. The stable De Ritis ratio indicates that these enzyme reductions likely reflect shifts in metabolic priorities rather than direct hepatic dysfunction.

Mineral and Lipid Metabolism Adjustments

Heat stress notably affected calcium homeostasis, with serum calcium levels decreasing by 6.5 % ($P < 0.05$). This trend aligns with previous findings by Hou et al. (2021) and Rathwa et al. (2017), who observed reductions in calcium levels under heat stress, likely due to impaired absorption or mobilization. However, inorganic phosphorus levels remained stable, indicating that significant disruptions in phosphorus metabolism did not occur.

Lipid metabolism was significantly altered in heat-stressed cows, as evidenced by a 56.3 % increase in total lipoprotein levels ($P < 0.05$). This finding suggests enhanced lipid mobilization as a compensatory mechanism to meet energy demands under thermal stress. Belhadj Slimen et al. (2015) reported similar increases in lipid utilization under heat stress, highlighting the metabolic trade-offs required for thermoregulation. Additionally, β-carotene levels increased by 87.1 % ($P < 0.05$), potentially reflecting enhanced mobilization of carotenoids to counteract oxidative stress (Kozyr et al., 2019; Mary et al., 2021).

Physiological Implications and Future Perspectives

The observed hematological and biochemical changes underscore the systemic impact of heat stress on dairy cows, emphasizing the physiological trade-offs necessary for adaptation. While increased albumin synthesis and lipid mobilization may serve short-term protective roles, prolonged heat exposure may exacerbate metabolic imbalances, ultimately compromising productivity and immune resilience.

Further research is required to explore the long-term consequences of acute heat stress on hematological and biochemical homeostasis. Additionally, future studies should investigate nutritional and environmental interventions, such as electrolyte supplementation, antioxidant support, and optimized cooling systems, to enhance thermotolerance in dairy cattle.

4. Conclusions

The present study demonstrates that acute heat stress induces significant alterations in the hematological and biochemical parameters of Brown Swiss cows, reflecting physiological adaptations aimed at maintaining homeostasis under hyperthermic conditions. The most pronounced changes included a reduction in haemoglobin concentration (−8.8 %), mean corpuscular haemoglobin (−11.2 %), and mean corpuscular haemoglobin concentration (−12.3 %), suggesting impaired oxygen transport capacity. Additionally, a 30.2% decrease in platelet count and a 25.1 % reduction in leukocytes indicate disruptions in haemostasis and immune function, respectively.

Biochemical alterations further highlight metabolic adaptations to heat stress. A significant increase in albumin concentration (+21.8 %) and albumin-to-globulin ratio (+33.8 %) suggests a shift in hepatic protein metabolism. Elevated blood urea nitrogen (+77.5 %) and urea (+77.7 %) levels indicate intensified protein catabolism, likely compensating for reduced energy availability. Moreover, lipid metabolism was affected, with total lipoproteins increasing by 56.3 %, suggesting enhanced lipid mobilization. Notably, β-carotene levels rose by 87.1 %, potentially as an adaptive antioxidant response.

The observed changes emphasize the systemic impact of heat stress on Brown Swiss cows, particularly in terms of oxygen transport, immune function, protein turnover, and metabolic regulation. These findings underscore the importance of targeted management strategies, including nutritional interventions, electrolyte and antioxidant supplementation, and enhanced cooling measures, to mitigate the adverse effects of thermal stress on dairy cattle. Further research is warranted to explore long-term adaptations and develop optimized mitigation strategies for improved thermotolerance in high-producing Brown Swiss cows.

Conflict of interest

The author declare that there is no conflict of interest.

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