



Acid-base balance and morphological composition of blood in high-producing dairy cows under cold stress

V. I. Oliynyk, M. O. Zacharenko, L. V. Shevchenko, V. M. Mykhalska, V. M. Poliakovskiy,
N. M. Slobodyanyuk, A. O. Ivaniuta, T. V. Rozbytska, O. S. Pylypchuk

National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine

Article info

Received 19.08.2024

Received in revised form

01.10.2024

Accepted 16.10.2024

*National University of Life
and Environmental Sciences
of Ukraine, Heroiv Oborony
st., 15, Kyiv, 03041, Ukraine.
Tel.: +38-050-193-10-29.*

E-mail:

shevchenko_laris@ukr.net

Oliynyk, V. I., Zacharenko, M. O., Shevchenko, L. V., Mykhalska, V. M., Poliakovskiy, V. M., Slobodyanyuk, N. M., Ivaniuta, A. O., Rozbytska, T. V., & Pylypchuk, O. S. (2024). Acid-base balance and morphological composition of blood in high-producing dairy cows under cold stress. Regulatory Mechanisms in Biosystems, 15(4), 723–727. doi:10.15421/0224104

The efficiency of milk production is based on the use of high-yielding dairy cows, particularly the black-and-white Holstein breed, which are kept using a loose-box housing system in large frame-type barns. The microclimate of these barns is significantly influenced by climatic conditions, which, during the winter period, can cause cold stress in the cattle. This stress leads to diseases and a decrease in milk productivity. The experiment assessed the temperature-humidity parameters of the macro- and microclimate of a large frame-type barn designed for 1,000 cows during the coldest period of the year. It also examined the impact of cold stress on the morphological composition and acid-base balance of blood in high-producing cows depending on their lactation stage and daily milk yield. It was found that the greatest cooling of the atmospheric air occurs between midnight and 6:00 AM, reaching -21.6 degrees. This results in a decrease in barn air temperature to subzero levels and a temperature-humidity index of 35 units, which corresponds to the zone of mild cold stress for cows. The temperature of the feed and water depends on the air temperature in the barn and is considered uncomfortable for cows during the coldest period of the year. During short-term cold stress, cows in their first lactation with a daily milk yield of 35–40 kg, as well as cows in their second and third lactations regardless of milk productivity, experience a decrease in leukocyte count by 34.4–41.7%. With the increase in the age of cows up to the third lactation, short-term cold stress causes a reduction of 12.8–17.1% in segmented neutrophils in the blood. Mild cold stress leads to a decrease in the partial pressure of CO₂ in the blood of first-lactation cows regardless of milk productivity. In cows, regardless of milk productivity and lactation stage, cold stress results in a negative shift in buffer base, which, considering the blood pH level, indicates the development of metabolic acidosis under a high-energy feeding regimen. The obtained research results can serve as the basis for developing measures aimed at adapting high-yielding Holstein cows during the coldest period of the year in frame-type barns with sharp daily temperature fluctuations.

Keywords: adaptation; acclimatization; hematological indicators; housing; temperature-humidity index.

Introduction

Ambient temperature is one of the most critical factors influencing an animal's body. The comfort zone (thermoneutral zone) is the range of temperatures that do not trigger changes in the body's regulation of heat production or heat loss through evaporation (Dos Santos et al., 2021; Li et al., 2021). This is particularly important when housing high-producing dairy cows in easily assembled frame-type barns, especially during critical periods of the year when high or low air temperatures are recorded in the summer and winter seasons (Mylostyvyi et al., 2023a, 2023b). Most studies by domestic and foreign scientists focus on the impact of global warming on the body of cattle, particularly lactating cows (Hempel et al., 2019; Ruban et al., 2020). In contrast, the effects of low temperatures are less studied, especially regarding high-yielding dairy cows.

During the winter housing period, when atmospheric air temperatures are extremely low, heat loss from the body outweighs heat production, causing an imbalance in heat regulation and leading to cold stress in animals (Wang et al., 2023). The impact of low temperature on the animal's body is primarily based on the activation of peripheral receptors responsible for sensing cold and heat. These signals are then transmitted through the spinal cord, leading to two types of reactions. First, there is a reflex constriction of blood vessels, which helps reduce heat loss from the animal's body. An additional response is shivering, which increases heat production in the skeletal muscles. Cold stress can also alter hormone

levels in tissues, particularly in the thyroid, pancreas, and adrenal glands (Fu et al., 2022; Lezama-Garcia et al., 2022), aimed at generating or conserving heat. Cold stress is one of the causes of diseases, slowed growth, and increased animal mortality, leading to significant economic losses for livestock farming worldwide (Bayer et al., 2021; Shevchenko et al., 2023).

Cold stress can be categorized into acute and chronic (long-term) based on its intensity and duration of impact on the body (Hu et al., 2021). It is known that prolonged exposure to even mild cold conditions can lead to physiological adaptation in animals. This adaptation includes increased energy production, higher feed intake, enhanced tissue metabolism, and changes in the digestive system's function.

Currently, the most common technologies in dairy farming involve housing high-yielding herds in frame-type barns with loose-box systems. In such facilities, temperature fluctuations depending on the season are often observed, affecting feed intake, rest time, and the cows' physical activity, which in turn influences the herd's productivity (Zakharenko et al., 2019). It is well-documented that low ambient temperatures in winter create an unfavorable microclimate in the barn. Additionally, strong winter winds and snow contribute to increased heat loss from the body, which can exacerbate cold stress in cattle. Dairy cows during the cold winter may reduce their milk production, and it has been noted that Holstein cattle are more sensitive than local breeds (Hu et al., 2022). Prolonged exposure to cold environmental conditions significantly reduces average daily weight gain, feed conversion efficiency, and immune function in

cattle, both within breeds (Nakajima et al., 2019) and in crossbred cattle (Butt et al., 2022).

The impact of low ambient temperatures on the body of beef or dairy cattle has primarily been studied under year-round grazing conditions on natural pastures (Toghiani et al., 2020), while similar research under housing conditions in frame-type barns is insufficient. Given the global climate changes over the past few decades, there have been increasing temperature fluctuations during the winter in Central and Eastern Europe, which to some extent affect the productivity and adaptability of dairy cows (Borshch et al., 2021). In this study, it was noted that in the central part of Ukraine (Kyiv region), during January-February, low average daily temperatures ranging from -12.2 to -18.7 °C were recorded, accompanied by strong winds and daily snowfall. The authors of this publication even suggest insulating frame-type barns to better accommodate cows.

To develop effective, scientifically grounded recommendations for improving the microclimate in modern frame-type barns, it is essential to consider the clinical, morphological, and biochemical characteristics of cows, depending on their productivity levels and age.

Thus, the aim of this study is to determine the impact of cold stress on the morphological indicators and acid-base balance of blood in high-yielding lactating Holstein black-and-white cows.

Materials and methods

The research on lactating cows was conducted in accordance with the requirements of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (1986). National regulatory documents were also followed, including the Law of Ukraine "On the Protection of Animals from Cruelty" of 2006, with amendments made in 2017. The research program was approved by the Bioethics Committee of the National University of Life and Environmental Sciences of Ukraine on October 9, 2020, under Protocol No. 46.

The cows were provided with appropriate housing conditions in a frame-type barn with easily assembled structures: an area of 7.5 m² per cow, a stall with a rubber mat for resting measuring 220 x 110 x 115 cm, and feeding was carried out considering the daily requirements for nutrients and biologically active substances, with free access to the feed table and water.

For the study, high-yielding lactating cows of the German-bred black-and-white Holstein breed, with an average live weight of 550–600 kg, were used. The experiment was conducted at the Ukrainian Dairy Company LLC in Kyiv region from February 1 to February 28, 2021. Lactating cows at 40–60 days postpartum were selected and divided into groups based on productivity and lactation according to the scheme in Table 1.

Table 1

Scheme for dividing lactating cows into groups under loose-box housing in the winter period

Cows, lactation number	I		II		III	
	12	16	15	13	13	12
n	12	16	15	13	13	12
Average daily milk yield, kg	20–25	35–40	20–25	35–40	20–25	35–40

After the ambient temperature reached a minimum of below -15 °C for 10 days, and before the main feeding in the morning, clinical indicators were assessed, and anaerobic blood samples were collected from the tail vein of lactating cows in all groups. Blood samples were taken in 10 mL tubes and 2 mL polypropylene syringes with heparin for analysis.

The study was conducted in a facility constructed with easily assembled metal structures with the following dimensions: length – 310 m, width – 38 m, height – 11 m. The barn's roof is insulated with thermal insulation panels. Ventilation is provided through unorganized air intake via side curtains, and polluted air is expelled through 42 exhaust ducts located at the roof's peak.

The barn is designed to house 1,000 lactating cows simultaneously, divided into 4 sections with 250 cows each, using a system of free access to feed and water with resting boxes. The housing system is a loose-box system with no outdoor exercise. Feeding is provided year-round with high-energy feed mixtures from a feed table. The ration for lactating cows consisted of corn silage, oat silage, alfalfa haylage, corn grain, crushed corn, sunflower meal, brewers' grains, soybean meal, feed yeast, sodium

bicarbonate, salt, and a vitamin-mineral premix. The feeding ration per 100 kg of body weight included: 3.7 kg of dry matter, 41.2 MJ of metabolizable energy, 582 g of crude protein, 526 g of digestible protein, 416 g of crude fiber, 222 g of crude fat, 26.3 g of calcium, and 14.0 g of phosphorus.

Milking is performed three times a day in a milking parlor equipped with a "Parallel" milking system from DeLaval (Sweden), designed to milk 50 cows simultaneously.

Manure removal from the facility is done mechanically using a mini-tractor with a bulldozer attachment. The liquid fraction of the manure is moved to storage tanks for subsequent processing at a biogas facility.

The facility includes two manure channels, three rows of resting boxes on either side of the barn, and a feed table. Cows are watered using group drinkers. The water for drinking is supplied from an artesian well with a temperature of 4–6 °C.

Physical properties of the ambient air, barn air, feed, and drinking water were measured daily four times with a 6-hour interval for 5 consecutive days. In the barn, microclimate parameters were measured at three points diagonally: at the center, in the northern and southern ends at the height of the average horizontal line of the cows' bodies. Temperature and relative humidity of the air were measured with an electronic weather meter Kestrel 3000 (USA), and temperature of water and feed was measured with an infrared thermometer Thermo Spot (Laserliner, Germany).

Table 2

Average daily dynamics of air temperature during the coldest period of the year ($x \pm SD$)

Time interval, hours	Barn (n = 15)	Ambient air (n = 5)
0.00 – 6.00	1.2 ± 1.9^a	-21.6 ± 3.1^a
6.00 – 12.00	3.6 ± 1.7^a	-16.4 ± 2.4^b
12.00 – 18.00	6.9 ± 1.2^b	-9.8 ± 1.5^c
18.00 – 24.00	3.7 ± 1.3^a	-15.5 ± 1.9^b

Note: different letters in superscripts indicate values that are significantly different within a column of the table ($P < 0.05$) according to Tukey's test.

During the coldest period of the year, the ambient air temperature was lowest during the night, from 00:00 to 06:00 hours, and increased from 06:00 to 12:00 hours, reaching its maximum from 12:00 to 18:00 hours (Table 2). The temperature of the air in the barn for housing lactating cows was significantly influenced by fluctuations in ambient air temperature. The average daily values of barn air temperature did not drop below 0 °C during different periods of the study; however, in certain areas of the barn, particularly at one of the ends during the night period from 00:00 to 06:00 hours, the temperature fell to negative values, reaching -3.5 °C. During the day, even at its peak, the barn air temperature did not exceed 7 °C.

Table 3

Average daily dynamics of feed mixture and water temperature for cows during the coldest period of the year ($x \pm SD$)

Time interval, hours	Feed mixture (n = 15)	Water (n = 15)
0.00 – 6.00	0.1 ± 2.8^a	1.6 ± 0.4^a
6.00 – 12.00	0.8 ± 2.3^a	2.6 ± 1.2^a
12.00 – 18.00	2.9 ± 2.1^a	3.0 ± 1.9^a
18.00 – 24.00	0.5 ± 1.7^a	1.5 ± 0.7^a

Note: see Table 2.

The temperature of the feed mixture for lactating cows depended on the barn air temperature and dropped to negative values during certain times of the day. From 12:00 to 18:00 hours, the temperature of the feed mixture at the feed table was at its maximum, approaching the temperature of the water used for drinking (Table 2). Throughout the entire day during the coldest period of the year, the temperature of the water in the drinkers and the feed mixture provided to the cows was below 8 °C, which was outside the optimal temperature range.

The relative humidity of the barn air during the coldest period of the year was not significantly affected by the ambient air humidity and fluctuated within the optimal range for cows, which is 60–80% (Table 4).

The Temperature-Humidity Index (THI) was calculated using the following formula: $THI = 0.8 \times AT + (RH(\%) / 100) \times [(AT - 14.4) + 46.4]$ (Mader et al., 2006), where AT is the air temperature in Celsius (°C) and RH denotes the percentage (%) relative humidity.

Table 4
Average daily dynamics of relative humidity of air during the coldest period of the year ($x \pm SD$)

Time interval, hours	Barn (n = 15)	Ambient air (n = 5)
0.00 – 6.00	69.1 ± 1.5 ^a	47.1 ± 2.9 ^a
6.00 – 12.00	70.9 ± 2.2 ^a	70.3 ± 3.4 ^b
12.00 – 18.00	57.8 ± 1.1 ^b	56.3 ± 3.7 ^c
18.00 – 24.00	57.5 ± 1.0 ^b	37.3 ± 2.1 ^d

Note: see Table 2.

Table 5
Average daily dynamics of temperature-humidity index during the coldest period of the year ($x \pm SD$)

Time interval, hours	Barn (n = 15)	Ambient air (n = 5)
0.00 – 6.00	35.3 ± 2.1 ^a	12.2 ± 0.8 ^a
6.00 – 12.00	43.2 ± 2.6 ^b	11.4 ± 1.3 ^a
12.00 – 18.00	50.7 ± 2.3 ^c	25.0 ± 2.9 ^b
18.00 – 24.00	43.5 ± 1.3 ^b	22.8 ± 1.5 ^b

Note: see Table 2.

The temperature-humidity index of the ambient air did not rise above 25 units throughout the study period, while in the barn for lactating cows of the frame type, it remained within this norm, except during the coldest period of the day from 00:00 to 06:00, when it dropped to 35.3 units (Table 5). Combined with cold feed and water, this is considered as short-term cold stress.

Blood parameters such as the number of erythrocytes, leukocytes, hemoglobin content, and leukogram were determined using the Vet Auto Hematology Analyzer BC-2800 Vet (Mindray, China). The study measured the following acid-base balance indicators in the blood: pH; PO₂ – partial pressure of oxygen; PCO₂ – partial pressure of carbon dioxide; HCO₃⁻ – bicarbonate concentration; BE – base excess; total CO₂ – total

Table 6
Hematological parameters of lactating cows during untied-box housing in the coldest period of winter ($x \pm SD$)

Indicator	Cows, lactation number					
	I		II		III	
	Daily milk yield, kg					
	20–25	35–40	20–25	35–40	20–25	35–40
Hemoglobin, g/L	116.0 ± 3.7 ^a	120.7 ± 2.6 ^a	115.6 ± 2.3 ^a	119.2 ± 5.7 ^a	107.4 ± 6.5 ^a	113.2 ± 2.4 ^a
Red blood cells, 10 ¹² /L	5.17 ± 0.24 ^a	5.55 ± 0.24 ^a	5.48 ± 0.18 ^a	5.33 ± 0.14 ^a	5.08 ± 0.12 ^a	5.42 ± 0.13 ^a
Leukocytes 10 ⁹ /L	8.42 ± 1.08 ^a	5.40 ± 0.17 ^b	4.91 ± 0.41 ^b	4.83 ± 0.51 ^b	5.52 ± 0.45 ^b	5.25 ± 0.64 ^b
Neutrophils:						
Band forms, %	2.67 ± 0.54 ^a	3.01 ± 0.89 ^a	3.02 ± 0.79 ^a	4.02 ± 1.06 ^a	2.80 ± 0.65 ^a	2.50 ± 0.79 ^a
Segmented forms, %	32.2 ± 4.0 ^{ab}	23.3 ± 3.3 ^{ab}	21.8 ± 6.2 ^{ab}	35.5 ± 1.6 ^a	18.4 ± 4.7 ^b	22.7 ± 2.1 ^b
Lymphocytes, %	58.2 ± 4.8 ^a	63.8 ± 3.4 ^a	62.2 ± 6.7 ^a	50.0 ± 2.6 ^a	63.1 ± 4.9 ^a	58.8 ± 2.3 ^a
Monocytes, %	5.67 ± 1.22 ^a	6.33 ± 1.49 ^a	10.83 ± 2.65 ^a	7.33 ± 1.57 ^a	9.43 ± 1.64 ^a	9.33 ± 0.97 ^a
Eosinophils, %	1.33 ± 0.37 ^a	3.50 ± 1.46 ^a	2.21 ± 0.42 ^a	3.17 ± 0.66 ^a	6.41 ± 3.60 ^a	4.67 ± 1.93 ^a

Note: different letters in the superscripts indicate values that are significantly different within the same row of the table ($P < 0.05$) according to Tukey's test; for values of n, see Table 1.

Table 7
Acid-base balance in the blood of lactating cows under free-box housing in winter ($x \pm SD$)

Indicator	Cows, lactation number					
	I		II		III	
	Daily milk yield, kg					
	20–25	35–40	20–25	35–40	20–25	35–40
pH	7.38 ± 0.01 ^a	7.38 ± 0.01 ^a	7.38 ± 0.01 ^a	7.38 ± 0.01 ^a	7.39 ± 0.01 ^a	7.39 ± 0.01 ^a
PO ₂ , mm. Hg	62.6 ± 2.6 ^a	62.1 ± 6.1 ^a	59.5 ± 3.2 ^a	62.5 ± 3.2 ^a	59.6 ± 1.2 ^a	60.2 ± 1.6 ^a
PCO ₂ , mm. Hg	42.3 ± 1.6 ^a	41.0 ± 2.1 ^a	57.1 ± 3.1 ^b	58.1 ± 1.2 ^b	57.4 ± 5.6 ^b	58.6 ± 5.1 ^b
HCO ₃ ⁻ , mM	23.40 ± 0.35 ^a	23.9 ± 0.55 ^a	23.34 ± 0.24 ^a	23.12 ± 0.36 ^a	23.58 ± 0.25 ^a	23.62 ± 0.41 ^a
Total CO ₂ , mM	24.66 ± 0.38 ^a	25.14 ± 0.61 ^a	25.04 ± 0.19 ^a	24.24 ± 0.39 ^a	25.28 ± 0.19 ^a	25.38 ± 0.38 ^a
BE, mM	-0.58 ± 0.38 ^a	-0.40 ± 0.62 ^a	-0.52 ± 0.21 ^a	-0.86 ± 0.33 ^a	-0.46 ± 0.14 ^a	-0.82 ± 0.29 ^a

Note: see Table 6; values of n are given in Table 1.

The partial pressure of CO₂ in the blood of first-lactation cows, regardless of milk yield, was lower ($P < 0.05$) than in cows of second and third lactations. During cold stress, cows of first, second, and third lactations, regardless of milk yield, showed negative values for the buffer base shift, which corresponds with the blood pH values and indicates the development of metabolic acidosis in the animals.

carbon dioxide concentration using the microanalyzer "Radelkis-OP-210P" (Hungary).

Statistical analysis of the research results was performed using one-way ANOVA. Data were compared between groups of cows using the Tukey test. Data in tables are presented as $x \pm SD$ (mean ± standard deviation). Differences between group values were considered significant at $P < 0.05$ with Bonferroni correction.

Results

One of the important criteria that characterize the ability of high-producing lactating cows to adapt to low air temperatures is changes in the morphological composition and acid-base balance indicators of the blood. Hematological parameters of high-producing lactating cows in their first, second, and third lactations with varying milk yields during the coldest period of winter housing did not differ from each other except for the number of leukocytes (Table 6). In cows of the first lactation with a daily milk yield of 35–40 kg, as well as in cows of the second and third lactations with milk yields of 20–25 kg and 35–40 kg respectively, the number of leukocytes in the blood was lower by 34.4–41.7% ($P < 0.05$) compared to cows of the first lactation with a daily milk yield of 20–25 kg.

Regarding the leukogram of the cows' blood, the proportion of band neutrophils did not change based on the level of milk production, while the proportion of segmented neutrophils was lower ($P < 0.05$) only in third-lactation cows with varying levels of milk production compared to the same indicators in second-lactation cows with a daily milk yield of 35–40 kg. The proportions of lymphocytes, monocytes, and eosinophils in the blood of cows under cold stress did not depend on the number of lactations or the level of milk production. Short-term cold stress did not significantly affect the pH, partial pressure of oxygen, bicarbonate levels, or total carbon dioxide concentration in the blood of lactating cows with different levels of milk production across different lactations (Table 7).

Discussion

As expected, during the coldest period of the year in Kyiv Oblast, Ukraine, the lowest atmospheric temperature was observed at night, while the highest was during the day. These fluctuations in atmospheric temperature are largely due to its heating by solar energy during the day and cooling after sunset. This decrease in atmospheric temperature led to a

drop in the air temperature inside the barn, reaching sub-zero values. Although the barn temperature increased during the day, it did not exceed 7 °C, which was significantly below the thermoneutral zone for cows, recommended by the National Research Council (1981) as 13–18 °C. It should also be noted that there is no unanimous agreement among researchers regarding the thermoneutral zone for cows. For example, Lees et al. (2019) suggest that a temperature range of –0.5 to 20.0 °C can ensure the well-being of dairy cows. However, breed and physiological characteristics of cattle, their breeding area, feeding level, productivity, and other environmental factors such as wind and precipitation must be considered.

Our research results align with those from Angrecka et al. (2020), conducted in Poland, which showed a dependency of indoor temperature in light-frame structures on atmospheric temperature during the winter period.

The temperature of the feed table and feed for cows depended on the barn's air temperature. Since the feed mixture used for cows is close in temperature to the atmospheric air, it warms up after being distributed, nearly reaching the barn's air temperature. The feed temperature was lower than the water temperature in the drinking troughs, indicating that it requires some time to heat up. During the daytime, when the maximum barn temperature is recorded, the feed temperature almost reaches that of the water. It should be noted that during the coldest period of the year, the temperature of the feed mixture and drinking water was lower than recommended by most researchers and was considered uncomfortable. For instance, Golher et al. (2021) argue that water consumption by dairy cows is directly or indirectly influenced by several factors, including breed, body weight, physiological state, age, stress, ecological adaptation, and environmental factors such as season, ambient temperature, humidity, wind speed, and precipitation. They emphasize that for adequate water consumption, the temperature difference between the water and the cows' body temperature should be minimal. They also demonstrated that warm water can improve dairy cattle performance. The basis for overcoming cold stress is the ability of warm water to reduce the heat cost for warming the feed mixture and conserving energy in dairy cattle. In cold winters, especially in highland areas, Golher et al. (2015) even recommend providing cows with water heated to 35–40 °C. Petersen et al. (2016) also noted that water at 8.2 °C is considered cold, which reduces its consumption, decreases ruminal content temperature, and impairs digestion in the stomach compartments of ruminants.

It should also be noted that the relative humidity of the air in the barn during the coldest period of the year was within the normative range for cows, which is 40–80% (Yilmaz et al., 2020). The highest values of relative humidity in the air of the frame-type barn were recorded during the night from 00:00 to 06:00 and from 18:00 to 24:00. During the period from 06:00 to 18:00, the relative humidity of the atmospheric air and the barn air were almost at the same level. This is primarily due to the technological operations performed during this time, including feed distribution, manure removal, and milking, which involve frequent opening and closing of gates and more intensive air exchange in the building due to the movement of vehicles, service personnel, and the animals themselves.

The THI threshold for cold stress usually occurs at THI = 38, which can cause a reduction in milk production in Holstein cows (Hu et al., 2018). The THI of the atmospheric air during the coldest period of the year in Kyiv Oblast was below 38 units and did not vary with the time of day. In the frame-type barn, the THI was close to the comfort zone for most of the day, except during the night when it was 35 units. This is considered a short-term cold stress, which, combined with the low temperature of the feed and water, can cause certain changes in the adaptive capacity of lactating cows.

Cold stress induces physiological, metabolic, and immune changes in cattle to maintain homeostasis (Kim et al., 2023; Mota-Rojas et al., 2024). In our experiment, the response of high-producing cows to short-term cold stress was primarily reflected in changes in leukocyte count. The number of leukocytes in the blood of cows during the coldest period of the year depended on lactation and decreased with age, indicating specific adaptation features. Most of the leukogram data we obtained align with the norms established for Holstein cattle depending on lactation and age (Moretti et al., 2017). Specifically, the authors found higher values for red and white blood cells in first-lactation cows compared to older cows, which is

also evident in our research results influenced by cold stress. Our research findings somewhat align with the data from Butt et al. (2022), who studied the effect of cold stress assessed by Wind Chill Temperature (WCT) on hematological parameters in crossbred cattle. Specifically, their study found no effect of WCT on hemoglobin concentration, erythrocyte sedimentation rate, and hematocrit in the temperature range from 13.51 ± 1.09 to 5.67 ± 0.51 °C over 14 weeks, although other hematological profile indicators were not considered. However, it is noted in their study that cold stress is evident in crossbred cattle at WCT temperatures from 5.67 ± 0.51 to 16.01 ± 0.72 °C. Shephard & Maloney (2023) indicate an increase in the number of erythrocytes in calves during cold stress, although similar studies regarding lactating dairy cows are scarce.

The indicators of acid-base balance in the blood of lactating cows with different milk production levels and lactations indicate metabolic shifts in the body as a response to a significant amount of high-protein concentrated feed in the cows' diet. Considering that most studies focus on the impact of heat stress on the acid-base balance in cows (Afzaal et al., 2004), comparing our results on the effect of cold stress on these parameters is not feasible. Therefore, to compare our results, we used physiological parameters for dairy cattle blood such as pH, partial pressure of carbon dioxide (PCO₂), base excess (BE), and standard bicarbonate (HCO₃⁻), which should be 7.38–7.43; 5.2–6.4 kPa; 0.5 – 4.5 mmol/L; and 23.5–27.0 mmol/L, respectively (Indrova et al., 2017). It should be noted that while the pH and standard bicarbonate levels in the blood of cows during short-term cold stress were within normative values, the base excess was more pronounced in older cows with a daily milk yield of 35–40 kg, particularly in second and third lactations. This is largely explained by the depletion of alkaline equivalents in the bodies of lactating cows with age and their increased excretion with milk in high-yield cows.

Habibu et al. (2019) indicate that the preservation of heat in cow tissue during cold stress is associated with a decrease in the partial pressure of oxygen, which is related to a respiratory effect achieved through reduced breathing frequency and consequently, increased oxygen saturation in the arterial blood. This somewhat aligns with the results of our studies, which show an increase in the partial pressure of carbon dioxide in the blood of older lactating cows, specifically those in their second and third lactations, compared to first-lactation cows. However, to confirm this, it is necessary to compare the acid-base status of cow blood during periods of thermal comfort or thermal stress, as well as indicators of energy metabolism in tissues.

Conclusions

Keeping high-yielding Holstein cows in large prefabricated frame-type barns with a loose-box system in a temperate climate zone is associated with significant temperature fluctuations, which can cause short-term cold stress during the winter period. It has been established that a decrease in atmospheric temperature to –21.6 °C leads to a drop in the air temperature in the large frame barn during the night in some areas to –3.5 °C. Daily temperature fluctuations of feed and water during the coldest period of the year depend on the barn's air temperature and are outside the thermal comfort zone for cows. The relative humidity of the air in the frame-type barn is not significantly affected by the atmospheric humidity and remains within normative parameters. During the night in the frame-type barn during the coldest period of the year, the temperature-humidity index drops to 35 units, which corresponds to a mild cold stress zone. Cold stress causes a decrease in the number of leukocytes in the blood of first-lactation cows with a daily milk yield of 35–40 kg, and in cows of the second and third lactations regardless of yield, ranging from 34.4% to 41.7%. Cold stress also reduces the percentage of segmented neutrophils by 12.8–17.1% in the blood of third-lactation cows, while no such changes are observed in younger cows. Cold stress leads to a decrease in the partial pressure of CO₂ in the blood of first-lactation cows. In cows, regardless of milk production level and lactation, a negative base excess value is registered, which aligns with the blood pH level and indicates the development of metabolic acidosis in the body. The obtained experimental data can be used to justify measures to reduce heat loss in barns and to adjust the conditions for keeping high-yielding dairy cows during the winter period.

The authors declare that they have no potential conflict of interest.

References

- Afzaal, D., Nisa, M., Khan, M. A., & Sarwar, M. (2004). A review on acid base status in dairy cows: Implications of dietary cation-anion balanced. *Pakistan Veterinary Journal*, 24(4), 199–202.
- Angerecka, S., Herbut, P., Godyń, D., Vieira, F. M. C., & Zwolenik, M. (2020). Dynamics of microclimate conditions in freestall barns during winter – a case study from Poland. *Journal of Ecological Engineering*, 21(5), 129–136.
- Bayer, O. V., Bondarets, O. V., Mykhalska, V. M., Shevchenko, L. V., Stupak, O. M., Fesenko, J. V., Dovgopoli, Y. V., Liniichuk, N. V., Kovalenko, V. L., Galka, I. V., Kryvenok, M. J., & Poliakovskiy, V. M. (2021). Otsinka prydatnosti metodu pry zastosuvanni QuEChERS pidkhotu dlia vyznachennia zalyshkiv benzimidazoliv u miaci tvaryn za dopomohoi UPLC-MS/MS [Evaluation of QuEChERS sample preparation for determination of benzimidazole residues in meat by UPLC-MS/MS]. *Methods and Objects of Chemical Analysis*, 16(1), 32–40 (in Ukrainian).
- Borshch, O. O., Borshch, O. V., Mashkin, Y., Malina, V., & Fedorchenko, M. (2021). Behavior and energy losses of cows during the period of low temperatures. *Scientific Horizons*, 24(5), 46–53.
- Butt, J. H., Konwar, D., Brahma, B., Khan, A., & Chakraborty, D. (2022). Assessment of cold stress in crossbred cattle by wind chill temperature index. *The Indian Journal of Animal Sciences*, 91(12), 1109–1111.
- Dos Santos, M. M., Souza-Junior, J. B. F., Dantas, M. R. T., & de Macedo Costa, L. L. (2021). An updated review on cattle thermoregulation: Physiological responses, biophysical mechanisms, and heat stress alleviation pathways. *Environmental Science and Pollution Research International*, 28(24), 30471–30485.
- Fu, X., Zhang, Y., Zhang, Y. G., Yin, Y. L., Yan, S. C., Zhao, Y. Z., & Shen, W. Z. (2022). Research and application of a new multilevel fuzzy comprehensive evaluation method for cold stress in dairy cows. *Journal of Dairy Science*, 105(11), 9137–9161.
- Golher, D. M., Patel, B., Bhoite, S. H., Syed, M. I., Panchbhai, G. J., & Thirumurugan, P. (2021). Factors influencing water intake in dairy cows: A review. *International Journal of Biometeorology*, 65(4), 617–625.
- Golher, D. M., Thirumurugan, P., Patel, B. H., Upadhyay, V. K., Sahu, S., Gaur, G. K., & Bhoite, S. H. (2015). Effect of drinking water temperature on physiological variables of crossbred dairy cattle at high altitude temperate region of Himalayas. *Veterinary World*, 8(10), 1210–1214.
- Habibu, B., Yaqub, L., Dzenda, T., & Kawu, M. (2019). Sensitivity, impact and consequences of changes in respiratory rate during thermoregulation in livestock – a review. *Annals of Animal Science*, 19(2), 291–304.
- Hempel, S., Menz, C., Pinto, S., Galan, E., Janke, D., Estellés, F., Müschner-Siemens, T., Wang, X., Heinicke, J., Zhang, G., Amon, B., del Prado, A., & Amon, T. (2019). Heat stress risk in European dairy cattle husbandry under different climate change scenarios – uncertainties and potential impacts. *Earth System Dynamics*, 10, 859–884.
- Hu, L., Brito, L. F., Abbas, Z., Sammad, A., Kang, L., Wang, D., Wu, H., Liu, A., Qi, G., Zhao, M., Wang, Y., & Xu, Q. (2021). Investigating the short-term effects of cold stress on metabolite responses and metabolic pathways in Inner-Mongolia Sanhe cattle. *Animals*, 11(9), 2493.
- Hu, L., Brito, L. F., Zhang, H., Zhao, M., Liu, H., Chai, H., Wang, D., Wu, H., Cui, J., Liu, A., Xu, Q., & Wang, Y. (2022). Metabolome profiling of plasma reveals different metabolic responses to acute cold challenge between Inner-Mongolia Sanhe and Holstein cattle. *Journal of Dairy Science*, 105(11), 9162–9178.
- Hu, L., Kang, L., Wang, S., Li, W., Yan, X., Luo, H., Dong, G., Wang, X., Wang, Y., Xu, Q. (2018). Effects of cold and heat stress on milk production traits and blood biochemical parameters of Holstein cows in Beijing area. *Scientia Agricultura Sinica*, 51(19), 3791–3799.
- Indrova, E., Dolezel, R., Novakova-Mala, J., Pechova, A., Zavadilova, M., & Cech, S. (2017). Impact of acute metabolic acidosis on the acid-base balance in follicular fluid and blood in dairy cattle. *Theriogenology*, 89, 41–46.
- Kim, W. S., Ghassemi Nejad, J., & Lee, H. G. (2023). Impact of cold stress on physiological, endocrinological, immunological, metabolic, and behavioral changes of beef cattle at different stages of growth. *Animals*, 13(6), 1073.
- Lees, A. M., Sejian, V., Wallage, A. L., Steel, C. C., Mader, T. L., Lees, J. C., & Gaughan, J. B. (2019). The impact of heat load on cattle. *Animals*, 9(6), 322.
- Lezama-García, K., Mota-Rojas, D., Martínez-Bumes, J., Villanueva-García, D., Domínguez-Oliva, A., Gómez-Prado, J., Mora-Medina, P., Casas-Alvarado, A., Olmos-Hernández, A., Soto, P., & Muns, R. (2022). Strategies for hypothermia compensation in altricial and precocial newborn mammals and their monitoring by infrared thermography. *Veterinary Sciences*, 9(5), 246.
- Li, H., Zhang, Y., Li, R., Wu, Y., Zhang, D., Xu, H., Zhang, Y., & Qi, Z. (2021). Effect of seasonal thermal stress on oxidative status, immune response and stress hormones of lactating dairy cows. *Animal Nutrition*, 7(1), 216–223.
- Mader, T. L., Davis, M. S., & Brown-Brandl, T. (2006). Environmental factors influencing heat stress in feedlot cattle. *Journal of Animal Science*, 84(3), 712–719.
- Moretti, P., Paltrinieri, S., Trevisi, E., Probo, M., Ferrari, A., Minuti, A., & Giordano, A. (2017). Reference intervals for hematological and biochemical parameters, acute phase proteins and markers of oxidation in Holstein dairy cows around 3 and 30 days after calving. *Research in Veterinary Sciences*, 114, 322–331.
- Mota-Rojas, D., Ghezzi, M. D., Hernández-Ávalos, I., Domínguez-Oliva, A., Casas-Alvarado, A., Lendez, P. A., Ceriani, M. C., & Wang, D. (2024). Hypothalamic neuromodulation of hypothermia in domestic animals. *Animals*, 14(3), 513.
- Mylostyvyi, R., Izhboldina, O., Midyk, S., Gutyj, B., Marenkov, O., & Kozyr, V. (2023a). The relationship between warm weather and milk yield in Holstein cows. *World's Veterinary Journal*, 13(1), 134–143.
- Mylostyvyi, R., Midyk, S., Izhboldina, O., Chemiy, N., & Komiienko, V. (2023b). Changes in the qualitative composition of the milk of Holstein cows during summer chronic heat stress. *Jurnal Ilmu Ternak dan Veteriner*, 28(2), 112–121.
- Nakajima, N., Doi, K., Tamiya, S., & Yayota, M. (2019). Effects of direct exposure to cold weather under grazing in winter on the physiological, immunological, and behavioral conditions of Japanese Black beef cattle in Central Japan. *Animal Science Journal*, 90(8), 1033–1041.
- Petersen, M. K., Muscha, J. M., Mulliniks, J. T., & Roberts, A. J. (2016). Water temperature impacts water consumption by range cattle in winter. *Journal of Animal Science*, 94(10), 4297–4306.
- Ruban, S., Borshch, O. O., Borshch, O. V., Orischuk, O., Balatskiy, Y., Fedorchenko, M., Kachan, A., & Zlochevskiy, M. (2020). The impact of high temperatures on respiration rate, breathing condition and productivity of dairy cows in different production systems. *Animal Science Papers and Reports*, 38(1), 61–72.
- Shephard, R. W., & Maloney, S. K. (2023). A review of thermal stress in cattle. *Australian Veterinary Journal*, 101(11), 417–429.
- Shevchenko, L., Mitsevsky, M., Shulyak, S., Mykhalska, V., Poliakovskiy, V., Boiarchuk, S., Ivaniuta, A., Kondratiuk, V., Nosevych, D., & Gruntovskiy, M. (2023). The effect of colloidal silver on clinical, morphological parameters and mineral composition of blood of calves. *Acta Fytotechnica et Zootechnica*, 26(1), 8–14.
- Toghiani, S., Hay, E. H., Roberts, A., & Rekaya, R. (2020). Impact of cold stress on birth and weaning weight in a composite beef cattle breed. *Livestock Science*, 236, 104053.
- Wang, S., Li, Q., Peng, J., & Niu, H. (2023). Effects of long-term cold stress on growth performance, behavior, physiological parameters, and energy metabolism in growing beef cattle. *Animals*, 13, 1619.
- Yılmaz, H., Gelaw, F., & Speelman, S. (2020). Analysis of technical efficiency in milk production: A cross-sectional study on Turkish dairy farming. *Revista Brasileira de Zootecnia*, 49, e20180308.
- Zakharenko, M. O., Olynyk, V. I., Polyakovskiy, V. M., & Solomon, V. V. (2019). Temperatur-moisture mode of the modern browner at low air temperatures. *Ukrainian Journal of Veterinary Sciences*, 10(4), 56–69 (in Ukrainian).