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EVALUATION OF METABOLIC STATUS IN HOLSTEIN COW UNDER SHORT-TERM COLD STRESS

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ABSTRACT: The research investigates the influence of short-term cold stress on the metabolic status of Holstein cows, when they are kept in large cowsheds in the Ukrainian climate. In the winter (cold season), the air temperature in such cowsheds depends on the ambient air temperature. The temperature-humidity index of the cowshed air is less than 38 at night, which is estimated as mild cold stress. Short-term cold stress has no effect on the level of total protein, urea, cholesterol, glucose, and calcium, but it increases the total lipids in the blood plasma of second-lactation cows with a daily milk yield of 20-25 kg by 32.3%, and in those with a daily milk yield of 35-40 kg, by 1.6-fold. For third-lactation cows with a daily milk yield of 20-25 kg total lipids increase by 1.5-fold compared with the data for first-lactation cows with a daily milk yield of 20-25 kg. Cold stress has no significant effect on the activity of alanine aminotransferase (ALT) and amylase, but it significantly reduced the activity of aspartate aminotransferase (AST) in the blood plasma of second- and third-lactation cows with a daily milk yield of 20-25 kg by 14.3% and 17.8%, respectively, compared with first-lactation cows with a daily milk yield of 35-40 kg. Under short-term cold stress, the activity of plasma alkaline phosphatase decreases by 36% in second-lactation cows with a milk yield of 35-40 kg, by 44% in third-lactation cows with a milk yield of 20-25 kg, and by 38% in cows with a milk yield of 35-40 kg compared to first-lactation cows with a milk yield of 20-25 kg. It can be concluded that short-term cold stress causes changes in the metabolic profile of high-yielding Holstein cows, which can provide valuable information about the health of the animals during acclimatization and help develop corrective measures to prevent diseases and reduce milk productivity in the coldest period of the year.

Keywords: Acclimatization, Cattle, Climate, Enzymes, Lactation.

INTRODUCTION

The cattle's ability for acclimatization is of great importance, especially during importation to Ukraine. The cattle are capable of feeling cold stress in the coldest season of the year, which falls on January-February in this climatic zone (Borshch et al., 2021).

Cold stress may have an adverse effect on the metabolic and immunological status of cows (Hu et al., 2021), which in turn reduces milk production of the animals, while increasing the economic losses. These changes may be associated with differences in the low-temperature adaptation of high-producing cows, when the organism needs additional energy for maintaining body temperature and reducing heat losses. Studies conducted on the meat-type cattle have shown that a long stay in a cold environment may cause a stress reaction based on metabolic regulation of heat generation. It has been proven (Wang et al., 2023) that the behavior, digestive functions, enzyme activity, and hormone levels in the tissues of Simmental cattle changed during the winter, which increased heat generation by the cattle for maintaining a constant body temperature and eventually led to impaired growth and development.

Studies by Kim et al. (2023) have shown that when cattle experience cold stress, the metabolic rate increases, which causes an increase in the heart rate, rectal temperature, deeper breathing, and muscle tremors. In such a state, the level of cortisol and unesterified fatty acids increases in young cattle against the background of decreased blood glucose levels, which has a significant influence on the fodder intake due to changes in energy metabolism of the organism. Against the background of cold stress, the behavior pattern of cattle also changes, including the time spent standing and lying down, in particular, there has been a report of increased (Kaygusuz and Akdağ, 2021) duration of a standing and decreased duration of a lying down in the Simmental cows under cold stress. This indicates that, due to a combination of low temperatures and high humidity, the wet floor of the cowshed affects the behavior of cattle, and they prefer to stand rather than lie down in order to balance their body's heat loss.

It is noted that when the ambient air temperature decreases from -8.0 to -22.6°C within 24 hours, the air temperature in the frame-type cowshed dropped from 9.7 to -0.39 °C and during the entire period depended on the daily

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fluctuations of the ambient air temperature (Zakharenko et al., 2020). Under such conditions, the temperature of the skin on the head, neck, thoracic and hind limbs, trunk and mammary gland drops in lactating cows. It is observed that when the air temperature in the cowshed drops within 24 hours, the number of cows standing or lying in the boxes increases, but the number of cows moving, consuming fodder and water decreases.

Different stages of growth, physiological state, milk productivity and breed characteristics of cattle give a mixed response to energy needs and metabolic reactions, which leads to different levels of resistance to cold stress (Kang et al., 2020). However, the question of cow's metabolic reaction to the effects of cold stress, depending on daily milk yield and age, remain unclear. The adaptation of productive animals, particularly cattle, to environmental conditions is largely based on ensuring thermal comfort, which significantly influences the realization of their genetic productive and reproductive potential (Polli et al., 2020; Silva et al., 2021).

It had been proven that even long-term exposure to mild cold conditions in animals fosters the development of an adaptive response, which includes increased heat generation, fodder consumption, and metabolic activity in tissues, which in turn leads to changes in digestive system function (Hu et al., 2022). Studies on Sanhe and Holstein heifers exposed to -25°C for an hour have shown that the latter exhibited a more pronounced metabolic response to cold, but both breeds responded to acute cold exposure by altering volatile fatty acid and glucose metabolism (Hu et al., 2022).

Cold stress also has an influence on the hormone levels of the thyroid, pancreas, and adrenal glands (Fu et al., 2022; Lezama-García et al., 2022), which leads to adaptive changes and intensification of metabolic mechanisms aimed at generating or conserving heat. It is considered that the elevated cortisol level in cattle blood indicates the activation of the immune defense system in response to critically low ambient temperatures.

The main response of cattle to the cold stress is a change in feeding behavior (Méndez et al., 2020; He et al., 2022). The ability of dairy cows to acclimatize to environment conditions is essential for finding the best management strategy for cattle breeding, as the animals may react differently depending on the characteristics of each region. Thus, appropriate adjustments to production practices are made based on determining the effects of stress caused by seasonal environmental fluctuations (Summer et al., 2018).

Cold stress often causes reduced growth rates and increased mortality, which leads to significant economic losses in cattle breeding throughout the world (Hu et al., 2021). According to the intensity and duration of its effects on the organism, cold stress is classified as acute (short-term) or chronic (long-term) (Zhao., 2020).

Currently, the problem of restoring and increasing of the cattle population, especially dairy cows, is particularly acute in Ukraine. This can be achieved by importing heifers from Western European countries which involves their adaptation to the climatic zone of Ukraine, which is characterized by significant temperature fluctuations in the warm and cold seasons of the year (Borshch et al., 2021). Moreover, if dairy cows are kept in frame-type cowsheds made of easy-to-assemble metal structures, they are not always sufficiently protected them from winter wind and snow, which may lead to a decrease in air temperature to sub-zero levels and an increase in cold stress of cattle (Zakharenko et al., 2019).

The ambient temperature has the greatest influence on animal physiology. Therefore, the limits of the comfort zone, which are within the range of temperatures that provides a relatively stable and balanced functioning of the thermoregulatory system, have been established for each species and age-sex group (Krishnan et al., 2023). At the same time, the digestive function is inhibited, which leads to increased consumption of feed dry matter that is unable to meet heat generation needs and results in decreased productivity and growth intensity of the meat cattle.

Reports by Nakajima et al. (2019) and Abbas et al. (2020) also mention that there are physiological, metabolic and immune changes in cattle influenced by cold stress, which are aimed at maintaining homeostasis but lead to decreased productivity in cattle. At the same time, it is pointed out that the range of heat stress zone for cattle has been determined and experimentally confirmed in accordance with the temperature-humidity index (THI), while the range of cold stress and its effects on the metabolic state of dairy cows have not yet been determined.

The purpose of the study was to assess the temperature and humidity parameters of a frame-type cowshed in the coldest season of the year in Ukraine and the the metabolic response to short-term cold stress in lactating cows of the German-bred black-and-white Holstein breed, which will allow for the development of preventive measures and the reduction of possible economic losses.

MATERIALS AND METHODS

All experiments were carried out in compliance with the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes dated 1986, as well as the Law of Ukraine "On the Protection of Animals from Cruelty Treatment" dated 21.02.2006 No. 3447-IV and amended on 04.08.2017. The study was approved by the bioethics commission of the National University of Bioresources and Nature Management of Ukraine in November, 2018.

The study was carried out on the lactating cows of the black-and-white Holstein breed imported to Ukraine from Germany. It was carried out at Ukrainian Milk Company LLC in February 2021. The cows with an average weight of 550 to

600 kg were selected for the experiment. For this, 6 groups of 12 cows each, including first, second and third-lactation cows, were formed according to the scheme indicated in Table 1.

Table 1 - Distribution scheme of lactating cows into groups to study the effect of short-term cold stress, n=12

Cows (daily-average milk yield, kg)					
First lactation		Second lactation		Third lactation	
n = 12	n = 12	n = 12	n = 12	n = 12	n = 12
20-25	35-40	20-25	35-40	20-25	35-40
Sectional housing of cows in cowshed of 250 cows each					

After reaching the minimum air temperature within 10 days of -15 °C or lower, in the morning before the feeding of all cows in all groups, the blood samples were taken from the tail vein in 5 ml syringes with lyophilized heparin for analysis. The cows were kept in the frame-type cowshed made of easy-to-assemble metal structures, designed for the simultaneous housing of 1,000 cows, which was divided into 4 sections of 250 cows each. The plenum ventilation of this facility is provided by the regulation of the side curtains, the exhaust ventilation is achieved through slotted holes on the roof ridge.

The cow housing system is a confinement system with a loose cubicle housing method. The cows are milked three times a day in a milking house equipped with the “Parallel” milking machine for simultaneous milking of 50 cows. Animal manure is mechanically removed from the cowshed using a tractor with a bulldozer attachment.

The physical properties of the ambient air and the cowshed air were determined on a daily basis at intervals of 3 hours: at 9:00 a.m., 12:00 p.m., 3:00 p.m., 6:00 p.m., 9:00 p.m., 12:00 a.m. and 3:00 a.m. The temperature and relative humidity of the cowshed were measured at three points: at the ends (northern section (part 1) and southern section (part 2)) and in the center at the level of the midline of the cows’ trunks.

The temperature and relative humidity of the air were determined using of the electronic weather meter Kestrel-3000 (USA).

The temperature-humidity index 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 degree Celsius (°C) and RH represents the percentage of the relative humidity.

In the blood plasma, the concentration of glucose, total protein, urea, total lipids, cholesterol, calcium, inorganic phosphorus, as well as the activity of ALT, AST, α-amylase, alkaline phosphatase, gamma-glutamyl transpeptidase was determined using the reagent sets manufactured by Pointe Scientific Inc. (USA) and the semi-automatic analyzer Pointe 180 (Poland). The study results were statistically processed using ANOVA program, and the tabulated data are presented as x ± SD (mean ± standard deviation). The difference between the groups was considered significant at $P<0.05$ according to the Tukey test (taking into account the Bonferroni correction).

RESULTS AND DISCUSSION

The determination of the ambient air temperature in the coldest period of the year has shown that its lowest value was recorded between 9:00 p.m. and 9:00 a.m., while after 9:00 a.m. it reached its maximum at 6:00 p.m., and after sunset it decreased again (Figure 1). Such daily fluctuations in the ambient air temperature significantly affected the air temperature in different parts of the large cowshed where the lactating cows were housed. Thus, the air temperature in the southern and northern parts and in the center of the cowshed from 9:00 a.m. to 9:00 p.m. was within the range of positive temperatures, and after 9:00 p.m. it decreased and by 3:00 a.m. was within the range of negative temperatures.

As for the dynamics of the temperatures in different parts of the premise, it was higher in the southern end at 12:00 p.m., as well as in the nighttime - from 12:00 a.m. to 3:00 a.m., than in the northern end and in the center of the premise, which is associated with a slightly greater heating of this zone due to the solar energy in the daytime. It should also be stated that in the southern end of the cowshed, the daily fluctuations of the air temperature were within the range of positive temperatures, while in the northern end and the center of the cowshed the air temperature decreased starting at 9:00 p.m., reaching the minimum negative temperatures from 12:00 a.m. to 3:00 a.m., which caused freezing of the fodder mixture on the fodder table and the animal manure in the manure channels.

The fluctuations of the relative air humidity in the cowshed within 24 hours depended to some extent on the ambient air humidity. The level of the relative humidity in the center, northern and southern ends of the cowshed decreased starting from 03:00 P.M. and was minimal from 06:00 p.m. to 09:00 p.m.

After 9:00 p.m. during rest, as well as in the daytime at 12:00 p.m., in the cowshed air, especially in the northern end, the increased relative humidity was observed more than 87%, while in the southern end, the peak values of the relative humidity were observed at 3:00 P.M. and 12:00 A.M., but they did not exceed the mark of 82% (Figure 2).

An analysis of the daily dynamics of the temperature-humidity index in the coldest period of the year in the large frame-type cowshed has shown that in all parts of the premise it did not fall below the lower comfort limit from 9:00 a.m. to 9:00 p.m. It began to decrease from 09:00 p.m. and reached its minimum at 12:00 a.m., after which it stabilized somewhat, but the average values of the temperature-humidity index were outside the comfort zone for the cows in the northern end and in the center of the cowshed until 06:00 a.m., and in the southern end it was on the border of the lower comfort zone (Figure 3). In accordance with the above-mentioned classification, the level of the cold stress for the cows, which were kept in the large frame-type cowsheds for our study, is estimated as mild.

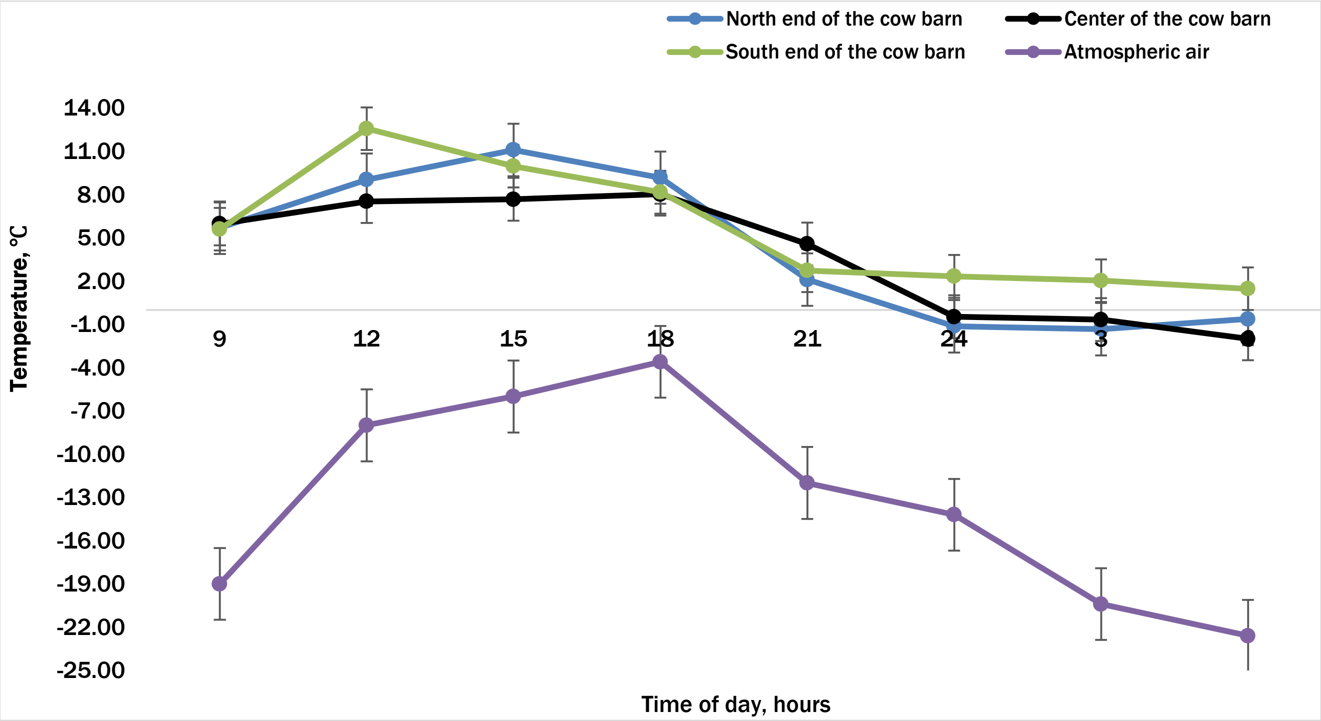

Figure 1 - Daily dynamics of air temperature in different cowshed parts in the coldest period of the year.

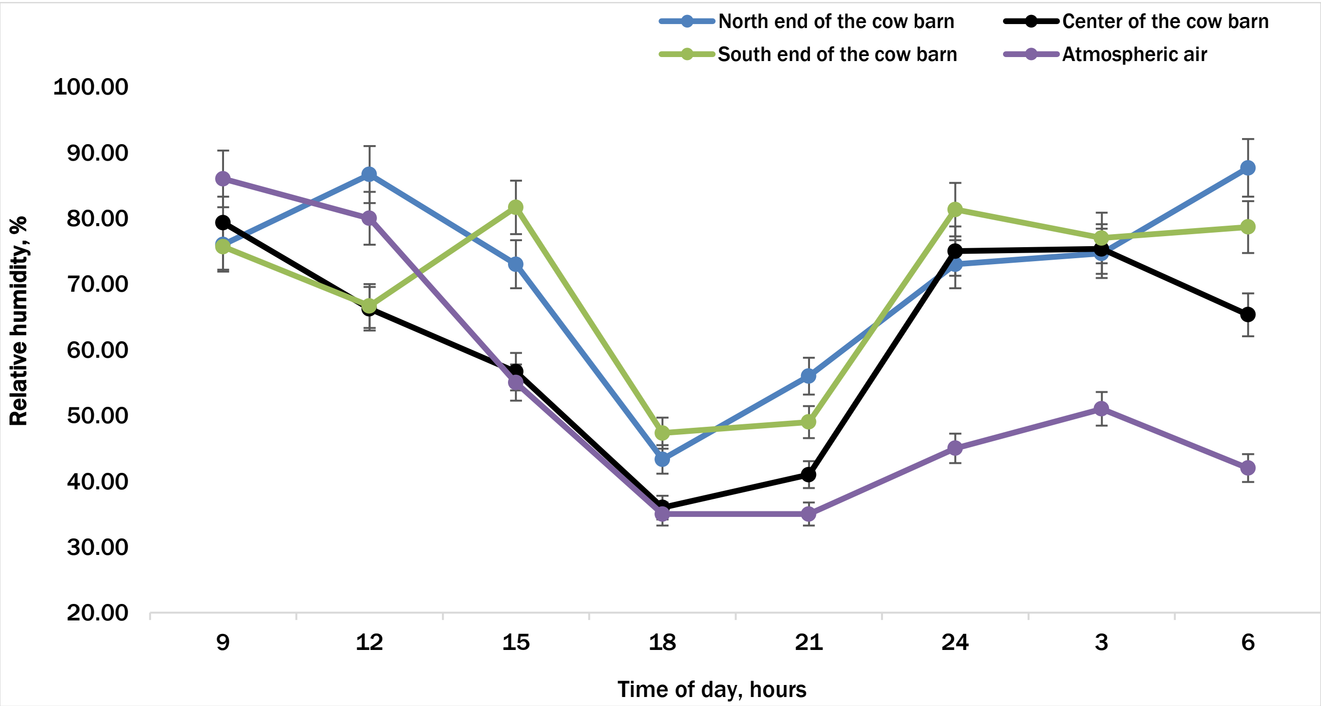

Figure 2 - Daily dynamics of relative air humidity in the coldest period of the year.

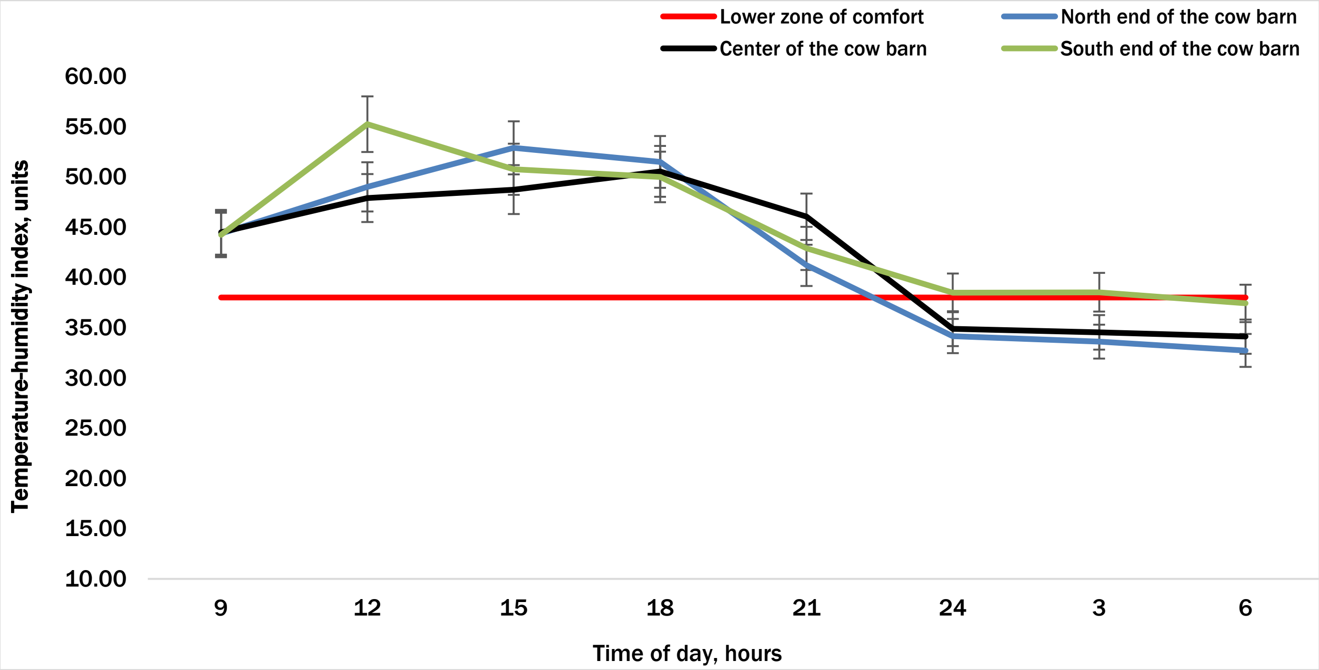

Figure 3 - Daily dynamics of air temperature-humidity index in different parts of cowshed.

It has been established that the parameters of protein metabolism in the tissues of the lactating cows, in particular, the level of total protein and urea, influenced by short-term cold stress did not differ between the groups and did not depend on the level of milk productivity and the age of the cows (Table 2).

At the same time, in second-lactation cows with a daily milk yield of 20-25 kg, the glucose level in the blood plasma was higher (P<0.05) by 16% compared to similar values in the first lactation cows with a daily milk yield of 35-40 kg. As for the parameters of lipid metabolism in the tissues of the cows affected by short-term cold stress, total lipids in the blood plasma of the second lactation cows with a daily milk yield of 20-25 kg increased by 32.3% (P<0.05), with a daily milk yield of 35- 40 kg – by 1.6-fold (P<0.05), third-lactation cows with a daily milk yield of 20-25 kg increased by 1.5-fold (P<0.05) compared to similar parameters in first lactation cows with a daily milk yield of 20 -25 kg. At the same time, no significant difference in total lipids in the blood plasma of the cows was found within each laceration based on milk productivity. Cholesterol and calcium levels in the blood plasma of first-, second-, and third-lactation cows remained within physiological norms and showed no significant differences among the cattle groups.

The study of enzymatic activity in the blood plasma of lactating cows with different milk productivity levels across different lactations under short-term cold stress showed a slight but significant difference in aspartate aminotransferase activity. In older cows, namely second- and third-lactation cows with a daily milk yield of 20-25 kg, this activity was lower by 14.3% (P<0.05) and 17.8% (P<0.05), respectively, compared to first-lactation cows with a daily milk yield of 35-40 kg (Table 3). Alanine aminotransferase (ALT) activity in the blood plasma of lactating cows, regardless of productivity level or age, did not change under short-term cold stress. No significant difference in amylase activity in the blood plasma of cows exposed to cold stress was observed (Table 3).

Table 2 - Metabolism metabolites in blood serum of lactating cows under short-term cold stress, x ± SD, n=12

Parameter	Cows (daily-average milk yield, kg)					
	First lactation		Second lactation		Third lactation	
	20-25	35-40	20-25	35-40	20-25	35-40
Total protein, g/l	97.6±3.75	94.00±1.96	89.8±2.82	93.33±2.89	98.67±3.16	98.00±2.60
Urea, mmol/l	7.30±0.45	7.33±0.48	6.80±0.56	7.67±0.37	8.42±0.43	8.40±0.60
Glucose, mmol/l	3.40±0.11[ab]	2.97±0.15[b]	3.54±0.13[a]	3.40±0.05[ab]	3.15±0.12[ab]	3.48±0.10[a]
Calcium, mmol/l	2.05±0.08	2.23±0.10	2.27±0.19	2.11±0.09	1.99±0.07	2.07±0.11
Total lipids, g/l	4.60±0.57[c]	5.73±0.39[ab]	6.80±0.50[a]	7.40±0.54[a]	6.93±0.66[a]	6.40±0.35[ab]
Cholesterol, mmol/l	5.05±0.62	4.71±0.22	5.46±0.23	5.99±0.64	4.88±0.53	5.69±0.67

*Note: different letters of the superscripts [a, b,] indicate the values that were significantly different in one table row (p < 0.05) according to the comparison results using the Tukey test.

Table 3 - Enzymatic activity of blood serum of lactating cows under short-term cold stress, x ± SD, n=12						
Parameter	Cows (daily-average milk yield, kg)					
	First lactation		Second lactation		Third lactation	
	20-25	35-40	20-25	35-40	20-25	35-40
AST, µkat/L	0.25±0.01^{ab}	0.280±0.01^{a}	0.24±0.01^{b}	0.27±0.01^{ab}	0.23±0.01^{b}	0.25±0.01^{ab}
ALT, µkat/L	0.20±0.01	0.21±0.01	0.19±0.02	0.22±0.01	0.20±0.01	0.21±0.02
ALP, µmol/h/ml	12.86±0.78^{a}	9.84±1.10^{ab}	9.95±2.04^{ab}	8.22±1.23^{b}	7.17±0.89^{b}	8.00±1.28^{b}
Amylase, g/h/l	38.40±4.80	34.56±2.63	34.56±2.63	33.60±3.59	35.20±3.51	36.00±2.77

*Note: different letters of the superscripts a, b indicates the values that were significantly different in one table row ($p < 0.05$) according to the comparison results using the Tukey test. AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase,

It has been established that the alkaline phosphatase activity of the blood plasma decreases in cows as their age and milk productivity increase. This is evidenced by the decreased alkaline phosphatase activity of the blood plasma in second-lactation cows with a daily milk yield of 35-40 kg decreasing by 36% (P<0.05), as well as in third-lactation cows with a daily milk yield of 20-25 kg decreasing by 44% (P<0.05) and those with a daily milk yield of 35-40 kg - decreasing by 38% (P<0.05) compared to similar parameters of first-lactation cows with a daily milk yield of 20-25 kg.

The analysis of the daily dynamics of ambient air temperature in the coldest period of the year for the climatic zone, where the Kyiv region is situated, has shown its maximum decrease during the night-time, but it increases during the daytime due to the sun, reaching its maximum at 6:00 p.m. Such daily changes in ambient air temperature affect the air temperature in different parts of a frame-type cowshed, which is designed for housing 1,000 cows (Figure 1). Moreover, the daily fluctuations in air temperature in the cowshed are influenced by its orientation relative to the cardinal directions. Specifically, the air temperature at the southern end of the cowshed remained above zero values throughout the 24-hour period and was, on average, 3°C higher at 12:00 p.m. and during the night compared to the center and northern end, where it dropped below zero.

The results of the present study agree with similar findings by Angrecka et al., (2020) who noted that the largest temperature difference between the ambient air and the cowshed air during the winter period was observed in the southern part of the premises, which is illuminated by the sun during the day. Additionally, the authors noted that the thermal balance of the premises depends on the wind and the location of the cowsheds relative to the cardinal directions. The studies of Cao et al. (2017) also indicated a possible decrease in air temperature inside easy-to-assemble cowsheds from -0.97 to 8.10°C during the winter period, when the ambient air temperature reached -20°C and below. Furthermore, differences in the heating of the cowshed's internal structural elements relative to the cardinal directions were reported, with the southern walls being warmer than the northern ones.

The fluctuations in air temperature in the cowshed, recorded during the night, suggest that the temperature was below the thermal comfort zone for cows, which, according to NRC (1981), should range from 13–18°C. However, studies by Butt et al. (2022) have explained the occurrence of cold stress in local cattle at temperatures ranging from 5.67±0.51 to 16.01±0.72°C. Additionally, based on their studies, Lees et al. (2019) suggest a slightly broader thermoneutral zone for cows, ranging from -0.5 to 20.0°C. In this regard, it is recommended to take into account breed, age, feeding levels, and productivity of the cattle.

The relative humidity of the ambient air depends on the weather conditions and season of the year for each climatic zone, which to a certain extent has an impact on the amount of water vapor in the cowshed air. Alongside that, the daily dynamics of the relative air humidity of the cowshed in its different parts also depended on the technological process. relative decrease in relative air humidity was observed during the daytime, when a significant amount of unorganized supply air enters the cowshed due to the technological processes, which involve the opening of gates, movement of motor vehicles, feeding and manure removal processes, as well as the movement of cattle into the milking hall and their subsequent return to the cowshed section.

The daily fluctuations of the relative air humidity in the center of the large frame-type cowshed were within the specified values and did not exceed 80% in the coldest period of the year. Yılmaz et al. (2020) consider that the relative air humidity in the cowshed should be within 40-80% in the coldest period of the year, which corresponds to most of the data obtained in the present study. Studies by Tang et al. (2019) have also shown that if the air humidity in the cowshed exceeded 80% during the winter, such a premise was unsuitable for keeping dairy cows. In contrast to these studies Jing and Jing (2021) expect that the upper limit of air humidity in cowsheds may be 85%, and when the air is almost saturated with humidity (100%), the wet surface of the skin increases heat loss from the cow's body, which fosters the development of pathogenic microflora and causes disease. However, in accordance with the data obtained in another study by Ma et al., (2017), the optimal relative air humidity in the cowshed during the winter should be maintained within a narrower range of 50-70%, which contradicts the above points of view and this discrepancy is explained by differences in cow-keeping conditions, cowshed construction, and various microclimate support systems, particularly ventilation and sewage systems. The temperature-humidity index is recommended to be used for a more objective assessment of the microclimate of livestock premises, particular, cowsheds. *It is the most widely used criterion for assessing the influence of physical environmental factors on animal thermoregulation and more objectively reflects the combination of temperature and relative humidity in the environment*) (Yan et al., 2019). Although this index is most often used to assess heat stress, it is also recommended to be used to assess cold stress in cattle. The threshold of cold stress is classified according to the temperature-humidity index (THI) as follows: THI > 38 (absence of stress); 25 < THI ≤ 38 (mild stress); 8 < THI ≤ 25 (moderate stress); −12 < THI ≤ 8 (high stress); −25 < THI ≤ −12 (extreme stress); THI ≤ −25 (dangerous stress).

The data, obtained by the authors of the present study, showed that the temperature-humidity index in the frame-type cowshed during the day and evening, namely from 9:00 A.M. to 9:00 P.M., did not fall below the lower comfort limit in the coldest period of the year. At night, it reached its minimum and in the northern end and the center of the cowshed, it remained above the lower comfort limit (Figure 3). In accordance with the above-mentioned classification, the level of cold stress for the cows, kept in the large frame-type cowsheds in the present study, is estimated as mild.

One of the important parameters, that characterizes the ability of high-producing lactating cows, depending on milk productivity and age, to adapt to cold stress is the study of their metabolic status. The changes in external environmental factors, which are often observed during the winter period, as the ambient air temperature drops to -20℃ and below, not only affects the microclimate of the premises where lactating cows are kept, but also changes affects fodder consumption and thermoregulation processes in animals, in particular, by intensifying heat generation processes. This, in turn, affects the intensity of metabolic processes in tissues, the functional state of the internal organs of the animals, as reflected in changes in blood chemistry values. The short-term cold stress did not have a significant influence on the parameters of protein metabolism in lactating cows' tissues, and changes in carbohydrate metabolism, particularly blood plasma glucose levels, were inconsistent. To a greater extent, cold stress primarily affected lipid metabolism in cows. Furthermore, total lipid levels in blood plasma increased with cow age, which is associated with the adaptive capacity of the metabolic system during cold stress and the increased use of lipids as an energy source for heat generation.

The results of the present study are difficult to compare with similar data from other researchers due to the climatic features of each geographical zone. The data obtained from Simmental cattle indicate a more pronounced effect of long-term cold stress on the metabolic status of cattle than what was observed in the present study on Holstein cows (Wang et al., 2023). In particular, this study showed that the concentration of glucose, enzymes of glucose metabolism, glucocorticoids, triiodothyronine and tetraiodothyronine in the blood plasma of Simmental cattle increased due to long-term cold stress ($P<0.05$), but the levels of triglycerides, β-hydroxybutyrate, propionate, insulin and growth hormone were reduced ($P<0.01$). The authors noted that long-term cold stress may suppress digestive function in Simmental cattle, increase energy metabolism, and disrupt stress hormone balance. The studies conducted on meat breed bulls and calves under extreme cold stress, indicate an increased ($P<0.05$) level of cortisol and non-esterified fatty acids in the blood (Kim et al., 2023). However, a decreased blood glucose levels was observed in calves under cold stress. The authors noted that this metabolic response of cattle under cold stress characterizes their physiological adaptation to maintain homeostasis regardless of growth stage. One of the important criteria for assessing liver function is the activity of transaminases, in particular, ALT and AST in cow blood plasma. The activity of aspartate aminotransferase in cow's blood decreased as cows aged compared to first-lactation cows; however, no patterns were found regarding the changes in the activity of this enzyme in the blood plasma of cows concerning milk productivity (Table 3). The short-term cold stress did not significantly affect the activity of alanine aminotransferase and amylase lactating cows' blood plasma.

During the short-term cold stress, a decrease in the activity in blood plasma alkaline phosphatase was observed in cows as age and milk productivity increased (Table 3). The results of the study agree with similar data (Butt et al., 2022), which indicate a significant ($P<0.05$) but slight effect of the cold stress on biochemical parameters in crossbred cattle, in particular, on the activity of AST, which decreased in cow's blood plasma of the cows from November to March by 7.8%, while the activity of ALT - conversely - increased by 1.3-fold during this period. The antioxidant enzyme activity in cattle was more strongly affected: despite stable superoxide dismutase activity, the activity of glutathione peroxidase decreased during the winter months 1.9-fold compared to the autumn and spring months.

CONCLUSION

The ambient air temperature in the climatic zone, where the Kyiv region is located, reaches -22.6℃ at night during the coldest period of the year. The air temperature in the large frame-type cowshed depends on the ambient air temperature during the winter. In contrast to the southern end, the air temperature in the northern end and center of the cowshed drops below zero at night, and relative humidity rises to 87% which is outside the comfort zone for cows. The temperature-humidity index of the cowshed air is below 38 at night, which is classified as slight cold stress for cows. The cold stress does not affect the levels of total protein, urea, cholesterol, glucose, or calcium, but increases total lipid levels in the blood plasma. In second-lactation cows total lipids increase by 32.3% for those producing 20-25 kg of milk daily and by 1.6-fold for those producing 35-40 kg daily. In third-lactation cows, total lipids increase by 1.5-fold for those producing 20-25 kg of

milk daily compared to first-lactation cows producing 20-25 kg daily. The cold stress does not significantly affect ALT or amylase, but reduces AST activity in the blood plasma by 14.3% in second-lactation cows and by 17.8% in third-lactation cows producing 20-25 kg of milk daily, compared to first-lactation cows producing 35-40 kg daily. The activity of blood plasma alkaline phosphatase under cold stress decreases by 36% in second-lactation cows producing 35-40 kg of milk daily, by 44% in third-lactation cows producing 20-25 kg daily, and by 38% in those producing 35-40 kg daily, compared to first-lactation cows producing 20-25 kg daily. The study results may be used to assess the impact of cold stress on cattle metabolism during acclimatization, as well as to develop preventive measures to reduce potential economic losses.

DECLARATIONS

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Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethical approval

All operations and manipulations of lactating cows during the study were humane and did not cause suffering to the animals. Any treatment of cattle was in accordance with the provisions of European legislation in the field of humane treatment of animals (Council Directive 86/609/EEC, 1986). The methodology of the experiment was agreed and verified by the Bioethical Commission of the National University of Bioresources and Nature Management of Ukraine, Kyiv (Protocol of ethical approval No. 54-17 dated 11/12/2018). The research was conducted to assess the intensity of cold stress in the climatic zone of Ukraine, which will be aimed at developing new and improving existing methods of insulating cowsheds.

Authors' contribution

The authors participated equally in data analysis and writing the manuscript.
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Competing interests

The authors declare no competing interests in this research and publication.

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