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STUDY OF SOME PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS IN DAIRY COWS BRED AT DIFFERENT TEMPERATURE AND HUMIDITY REGIMES

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Abstract

The present study examines some physiological and biochemical parameters in dairy cows depending on the temperature-humidity index (THI) in spring (May) and summer (August) in two different time zones - 10 and 15h. The temperature-humidity index (THI) in a semi-open type of building, the body temperature, pulse rate and breathing were measured twice a day (10 and 15.00 o'clock), as well as the number of ructus of the dairy cows per 24 hours in spring and summer. Changes in the biochemical parameters of the total protein, creatinine and urea were ascertained in relation to the changes in THI and the season, which are within the reference values for cattle. The high THI lowers the blood sugar levels (up to 31%), but increases the total bilirubin levels (up to 3 times) and the transaminases activity (ASAT up to 3 and ALAT - up to 4.5 times) compared to the reference values for the respective species. The changes in THI values have a statistically significant influence on the heart rate, respiration and body temperature ($p<0.001$). The month (season) of the study has a statistically significant influence on the blood sugar, total protein, urea and bilirubin ($p<0.001$) and less influence on the body temperature ($p<0.01$) and creatinine ($p<0.05$.)

***Key words**: dairy cows, physiological and biochemical parameters, season, temperature-humidity index (THI).*

INTRODUCTION

The cattle's ability to adequately respond to the high summer temperatures depends mainly on the production type and the breed. The dairy cows are more susceptible to heat stress than the meat breeds due to the fact that the former generate more metabolic heat. The highly productive cows are especially sensitive to the high temperature and this affects their blood profile (Hewett, 1974).

The high temperatures can disturb the normal physiological balance of the animal and lead to disorders in the water and protein exchange, energy, hormonal and mineral balance (Marai et al., 2000; Ivanova & Tasheva, 2020). According to Ordinance No 44/20.04.2006, the optimal temperature zone is 10-15ºC at a minimum of 5 and maximum of 28ºC. Temperatures above +18-20ºC are capable of causing heat stress to the highly lactating cows. Heat stress results from the imbalance between the ambient heat flow and the heat released from the body. The occurrence of heat stress is triggered by the high air temperature in combination with high or, the opposite, very low humidity. The comfort zone for the local cattle breeds varies between +4 and +20ºC, and for the highly lactating ones - between +9 and +16ºC. Each temperature increase above the optimum values leads to the activation of mechanisms connected to energy consumption and decrease in the adaptive abilities efficiency (Brown-Brandl & Tami, 2018). In the context of the globally altering climate, the heat stress is becoming a serious problem for the dairy cattle breeding. Mahdy et al. (2014) consider that the temperature- humidity index (THI) is one of the most important parameters reflecting the dairy cows overall comfort. The authors believe that the THI is a useful instrument in determining the heat stress occurrence. The temperature humidity index indicates the combined effect of the temperature and the relative humidity on the physiological, production, and other, parameters in cows. The plethora of studies show that the THI can be used as a heat stress indicator for dairy cows (Armstrong, 1994; Kadzere et al., 2002; Dikmen & Hansen, 2009; Hristev et al., 2020).

Mazzullo et al. (2014) prove that the environmental conditions are major stress factors influencing the animals and leading to serious disorders in their hematology-chemical and physiological parameters.

The blood indexes are sensitive to the changes happening in the organs and the cells even before the first clinical signs of a certain disease or a stress situation are exhibited. Therefore, according to Jain (1993), Otto et al. (2000), Ndlovu et al. (2007), Wood & Quiroz-Rocha (2010), the changes (deviations) in the different blood parameters values are indicators of an early organism response and may be used as a basis of early diagnostics, treatment and prevention of various pathological conditions.

The aim of the present study is to trace the changes in some physiological and biochemical blood parameters of dairy cows under the influence of different temperature-humidity regimes.

MATERIALS AND METHODS

The study was carried out in a Holstein-Friesian cattle breed farm situated in the region of Karnobat, South- East Bulgaria. The cows are bred free in separated boxes in a semi-open type of building and their feeding is unlimited with a total mixed ration. The cows in the farm are cooled by irrigation in the waiting zone of the milking area and by all-day-long ventilation in the barn during the warm months of the year. The study includes 24 cows which are the same in terms of period of calving. The monitoring of the physiological and biochemical blood parameters was performed in the spring (May) and in the summer (August). At the moment of the first examination (May), the cows are in their 1st -2nd lactating month, and during the second one (August)- respectively in 4th – 5th. The cows are in different lactation periods- from first to fifth. The heat stress level was determined by the temperature-humidity index (THI) which was measured with a Kestrel automatic measuring device. The THI recording was performed in the cows breeding area twice a day, at 10 and 15 o'clock, along with the measuring of the physiological parameters of each cow- pulse, rectal temperature and breeding intensity. The rectal temperature was measured with a digital thermometer in degrees Celsius. The breeding intensity was visually monitored by reporting the chest movement per minute, in accordance with the Zimbelman et al. (2009) method. Data regarding the activity in the rumen, expressed in the number of ructus per 24 hours, of each cow were taken from the farm management software. This activity was reported by straps, with microphones of the SCR by Allflex system fixed around the neck, which register each burp of the animals. The blood samples were taken once on the day of THI and physiological parameters of the cows reporting. The biochemical blood analysis was performed via Biomed ready-to-use tests. THI is incur-porated in the models as an average of the values reported for the respective month- May and August, and displayed as an effect of the month. The data were statistically processed via SPSS-21.

The following model was used for the assessment of the controlled factors influence on the physiological parameters: $\mathbf{Y_{ijkl} = \mu + M_i + H_j + M{*}H_k + e_{ijkl}}$, where: $\mathbf{Y_{ijkl}}$ - dependent variable (each of the physiological parameters examined), $\mathbf{\mu}$ - mean effect, $\mathbf{M_i}$ - effect of the average THI for the month (classes), $\mathbf{H_j}$ -THI effect at the time of reporting, $\mathbf{M{*}H_k}$ - the related effect of the detection time in the month of reporting and $\mathbf{e_{ijk}}$ is the random residual effect. The THI effect on the ructus and the biochemical parameters examined is reported by using a single factor analysis $\mathbf{Y_{ij} = \mu + THI_i + e_{ij}}$, Where: $\mathbf{Y_{ijkl}}$ - dependent variable (each of the biochemical parameters examined and number of ructus), $\mathbf{\mu}$ e mean effect, $\mathbf{THI_i}$ - effect of the average THI for the month (classes) and $\mathbf{e_{ij}}$ is the random residual effect. The average of the least square (LSM) is calculated in fixed factors classes with the use of the analysis of variance (ANOVA) for the model.

RESULTS AND DISCUSSIONS

The THI values are reported twice a day for a period of 4 months (Table 1). The data exhibited shows that the average THI values are below 72 only in May. During the rest of the summer months the average THI values are above 74, and the lower values reported at 10 o'clock are bordering and almost 72-71.6 and

71.8, respectively. Armstrong (1994) presents a heat stress level in dairy cows classification depending on the THI values. The author states that there is zero stress in cows with values below 72, mild - from 72-79, temperate - from 80-89, severe - 90-98 and emergent above 98. According to this classification, the cows in the farm examined have been under mild to temperate heat stress during the daytime of the summer months from June to August. The results of our studies show that only the THI values reported at 10 o'clock in May are within the heat comfort zone. The THI values at 15 o'clock and those in both time frames in August are above 72 which comes to indicate that the cows experience mild to temperate heat stress during almost the entire daytime irrespective of the ventilators working at the same time. In a one-year study of the climatic conditions in South Bulgaria, Dimov et al. (2017) ascertain that the average daily THI values of above 75 reported in the summer provide conditions for heat stress in the dairy cows bred in semi-open type of buildings.

Table1. Average values and variation of THI in months of reporting

Reporting month	THI		
	x ± SE	min	max
May	$71.72{\pm}0.30^{a}$	69.3	74.2
June	$75.06{\pm}0.26^{b}$	71.8	81.5
July	$74.32{\pm}0.16^{c}$	71.6	77.6
August	$77.35{\pm}0.33^{d}$	74.5	81.5
Average	74.62±0.16	69.3	81.5

Note: The differences between the values indicated with the same letters are significant only upon P<0.05.

There is a certain risk of similar conditions during the spring, too, when the average daily values are above 69. Analogous results are also reported by Hristev et al. (2020). This proves the observation that the registered temperatures and humidity in both seasons might be the reason for temperature homeostasis disorders in cows during the daytime. According to Grant (2009) the indexes values are not fixed but change throughout the day. The temperature homeostasis maintenance in dairy cows is possible upon THI values of up to 70 when the thermoregulation mechanisms function normally and allow the maintenance of a normal body temperature (Kadzere et al., 2002). Therefore, most of the researchers consider the index 72 as corresponding to temperature of 25ºC and relative humidity of 50%, and the index values between 77 and 87 as critical. The lethal cases in cows increase when the values are above the aforementioned ones (Vitali et al., 2009). Many authors point that the short- term (several-day) high values of THI do not have such a serious effect as the month-and-more-continuing influence of THI values associated with heat stress, especially on the biochemical, production and reproduction parameters of dairy cows (Silanikove, 2000; Bouraoui et al., 2002; Kadzere et al., 2002; Spiers et al., 2004; Chase, 2006). Taking these notions into account, the biochemical blood parameters of the cows were reported in August- after a two-month influence of conditions with THI daily values predisposing to heat stress levels (mild to moderate) and compared with the biochemical status under conditions without heat stress in May. Due to the fact that the cows included in the study are in the same stage of lactation and are bred and fed under the same conditions, the main factor is THI, and the month of study effect overlaps with that of the THI. The physiological parameters and THI are reported twice a day at the same time - at 10 and 15 o'clock, with the exception of the ruminations index. It is displayed as a daily average and it is impossible the THI effect at 10 and 15 o'clock to be reported. The analysis of variance (Table 2) shows that there is a considerable effect of THI at the time of reporting (P<0.001) and the related effect THI for the month and time of reporting (P<0.01) on the pulse of the animals. When it comes to the respiratory movements, significance is reported only with reference to the related effect of THI for the month and hour of reporting (P<0.001). Both the average THI value for the month of study (P<0.01) and the THI at the time of reporting (P<0.001) but not the related effect of the two factors influence the rectal temperature. No considerable influence of the THI values for the month of examination is reported regarding the average daily ructus values. Table 3 displays the results regarding the THI influence on the physiological parameters examined.

It is noticeable that the pulse frequency during the spring and the summer is either at the upper physiological borderline or exceeds it in both time intervals. The monitoring of the frequency and the properties of the pulse provides additional information about the heart rate, the blood vessels condition as well as about the blood circulation as a whole.

Table 2. Analysis of variance on the influence of the controlled factors on the physiological parameters examined

Parameters	Number of reports	Mean THI per month		Reporting time (THI)		Mean THI per month *Reporting time (THI)	
	n	MS	F P	MS	F P	MS	F P
Puls n/min	24	0.75	0.329 -	30.08	13.193***	18.75	8.223**
Breath n/min	24	320.33	3.96 -	147.00	1.816 -	1587.00	19.607***
R. temper C^0	24c	1.47	12.30**	2.00	16.8***	0.27	2.3 -
Ructus n/24 h	12	11.48	0.375	-	-	-	-

Note: F - Fisher's criterion ***P<0.001; **P<0.01; *P<0.05; - no significance.

Table 3. LSM values of the physiological parameters depending on the average THI for the month and time of reporting

Parameters	Number of reports	Mean THI per month		Reporting time		May		August	
		May THI=71,75	August THI=78,30	10 o'clock THI=72,85	15 o'clock THI=77,20	10 o'clock THI=69,30	15 o'clock THI=74,20	10 o'clock THI=76,40	15 o'clock THI=80,20
	n	LSM ± SE	LSM ± SE	LSM ± SE	LSM ± SE	LSM ± SE	LSM ± SE	LSM ± SE	LSM ± SE
Puls n/min	24	20.92±0.31	20.67±0.31	20.00±0.31	21.58±0.31	20.75±0.44	21.08±0.44	19.25 ±0.44	22.08±0.44
Breath n/min	24	40.0±1.83	45.17±1.83	40.83±1.83	44.33±1.83	44.0 2.60	36.0±2.60	37.66±2.60	52.67±2.60
R. temper C^0	24	38.07±0.07	38.72±0.07	38.34±0.07	38.75±0.07	38.24±0.10	38.50±0.10	38.44±0.10	39.00±0.10
Ructus n/24h	12	555.0±15.97	541.17±15.97						

The change of pulse is heavily influenced by the THI values (Table 3), the time of measurement has a weaker effect, while the month does not affect this parameter at all. According to Atkins et al., (2018) the breathing may be considered a more precise criterion than the different indexes measuring the heat stress. This parameter may vary in wide ranges depending on whether the cow is in standing or lying position, its productivity and other factors. The momentary reporting, however, should not be always regarded as a norm due to the fact that the animal might have been stressed from the manipulation itself. The results of our study show a significant increase in the breathing rate during the 15 o'clock measurement in August with values almost twice as high as the upper reference value.

The accelerated lungs ventilation leads to a decrease in the carbon dioxide levels which causes disorder in their balance with the blood bicarbonates and the pH levels in the organism. According to Brouk (2003), upon increase in the outer temperature above 21.2^{O}C, the evaporation cooling becomes the main method for releasing heat from the body. Regardless of the double- accelerated breathing of the cows examined, their body temperature remains within the reference values (38-39^{O}C). Similarly to the pulse, the breathing rate and the body temperature are dependent on the THI (p<0.001). The month and the time of examination have a temperate to strong influence on the body temperature (p<0.01) but small on the breathing. Upon heat load increase, the breathing rate also increases, the food consumption decreases and the water intake rises (Bernabucci et al., 2010). Along with the pulse rate, the rectal temperature also goes up (Avendono-Reyes et al., 2012). The body temperature, however, is unstable. Its values change throughout the day in relation to the metabolic processes and the thermoregulation abilities. Therefore, the THI can be considered the major factor influencing both the heat production and heat release. The rumination monitoring may be used for assessment of the feeding results, the breeding conditions and the cows health. Through the ructus cows release gases formed in the forestomach as a result of the fermentation processes happening there. The accumulated gases are released as a reflex following the activity of the reticulo-rumen and the abdominal contraction. The ructus rate depends on the speed and the degree of gas formation in the forestomach. This is connected to the food content, microbiological and chemical processes in the rumen. Their number in cows ranges from 15 to 90 per hour (Gabrashanski et al., 1989). No influence of the THI, month and time of examination on the rumination was ascertained. According to Bernabucci et al. (1999), upon heat stress, the initial reaction observed is decrease in the rumen movement and the ructus, but upon its continued influence, the animals tend to acclimatize and the ruminations are recovered and reach their levels of prior the heat stress. This is also what we observed upon gathering our data for the months May and August. The acclimatization is a process during which the animals adapt to the environmental conditions triggering behavioral, hormonal and metabolic changes which allow the organism to survive in the new 'physiological condition'.

Table 4. Analysis of variance of the influence of the THI average values on the blood biochemical parameters

Parameters	Number of reports n	Mean THI per month	
		MS	F P
Glucose mmol/L	12	6.33	22.44***
Total protein g/L	12	836.4	20.58***
Creatinine μmol/L	12	100.0	1.98 -
Urea m 45 mol/L	12	18.38	43.20***
Total bilirubin μmol/L	12	878.22	30.11***
ASAT U/L	12	66.7	0.13 -
ALAT U/L	12	170.67	1.35 -

Note: ***P<0.001; **P<0.01; *P<0.05; - no significance.

This load which is provoked by the heat in an attempt to maintain the homeostasis affects the lipid and protein metabolism, leads to disorders in the liver functioning, causes oxidative stress, endangers the immune response and decreases the reproduction and productive parameters. The analysis of variance performed indicates a noticeable effect (P<0.001) of the THI average values for the respective month of examination on the values of the following blood biochemical parameters- Glucose, Total protein, Urea and Total bilirubin. No significant effect of the THI values on the Creatinine, ASAT and ALAT values is reported. Table 5 displays the LSM values of all biochemical blood parameters examined irrespective of the THI effect.
The blood sugar is one of the main biochemical parameters characterising the carbohydrate exchange. Its levels in ruminants are low which is connected to their specific exchange (Gromyko, 2005). The glucose levels in the blood in August are 34% lower than those in May. Similar conclusions were reached by Gorski & Saba (2012) under an analogous experiment.

Table 5. LSM values of the blood parameters depending on the THI average values for the month of reporting

Parameters	Number of reports n	Mean THI per month	
		May THI=71.75 LSM ± SE	August THI=78.30 LSM ± SE
Glucose mmol/L	12	3.00±0.195	1.97±0.094
Total protein g/L	12	76.27±2.09	88.07±1.54
Creatinine μmol/L	12	88.42±1.94	84.33±2.49
Urea mmol/L	12	2.96±0.23	4.71±0.13
Total bilirubin μmol/L	12	6.68±1.22	18.78±1.84
ASAT U/L	12	117.42±8.60	120.75±1.37
ALAT U/L	12	26.58±4.38	31.92±3.25

As it was underlined, the blood sugar levels are not the main source of energy for the ruminant organisms, however, at the end of the pregnancy and the beginning of the lactation, a big part of it is used for lactose and milk fat synthesis, and therefore, its levels are indicative upon some pre-pathological and pathological conditions. Darul & Kruczynska (2005) point out that the blood sugar levels decrease after birth and initiation of active lactation due to the change in the energy balance of cows. Due to the rising decrease in the fatty acids oxidation upon chronic heat stress, the stressed animals become more and more dependent on the glucose for their energy needs. When taking the reduced feed intake into account, it can be seen that there has been a discrepancy between the energy received and that consumed upon lactation and metabolism. (Baumgard & Rhoads, 2007). Subsequently, the cows subjected to moderate heat stress (Moore et al., 2005) are in a negative energy balance condition. The results indicated in table 5 show that the blood sugar levels are influenced by both the THI and the season (p<0.001). The levels of the total protein reported in May are within the reference values, but those in the summer tend to increase. The hyperproteinemia combined with hypoglycemia are probably a sign of a threatened ketosis and early stages of liver damage. The feeding is the factor which has a major influence on the protein levels in the blood. They are also affected by the liver, kidneys, gastro-intestinal system condition, the stress, water loss, and so on. In their efforts to improve the dairy cows productivity, farmers increase the proteins in the blood at the expense of the crude fibre content, which affects both the rumen homeostasis and the blood protein levels. Our study ascertained a reasonable THI influence (Table 5) on the albumin levels ($p<0.001$). The changes in the blood urea levels are primarily related to the functional condition of the liver. A bigger part of the proteins contained in the feeds are hydrolised to amino acids, and after their degradation, the extra ammonia is absorbed in the blood, then goes into the liver and turns into urea (Holodov & Ermolaev, 1988). The results which we achieved show that the ammonia levels in the blood in May are below the reference values for the respective type of animals. In August the levels rise to 44% but are still within the reference values. During pregnancy, all metabolic processes activate to meet the growing needs of the fetus and the cow. The increased urea levels show a high degree of protein feeds assimilation (Holodov & Ermolaev, 1988). Our studies display that the urea levels are statistically influenced by the THI ($p<0.001$). Creatinine, along with the urea, is a product of the protein exchange. It is formed during the metabolism in the muscle tissue and is excreted by the kidneys. Table 5 displays the effect of the THI on the creatinine levels. The average levels after all tests are within the reference values for the respective kind. It is only the season that reasonably affects the creatinine levels ($p<0.001$) (Table 5). Taking the creatinine levels into account, we can make an assessment of the kidney excretory functions and its metabolism speed in the muscle tissue of the dairy cows. The heat stress mostly affects the organs with high metabolism speed-liver, kidneys and the epithelium layer of the digestive tract mucous membrane. This is due to the redistribution of the blood towards the skin. In this case the internal organs experience nutrient deficiency and accumulate metabolism products whose free radicals damage the organs cell walls. The bilirubin levels in the blood serum of the healthy cattle are negligible. They are often increased after calving or during food deprivation. Its excretion is used for the diagnosis of steatosis in cows. The bilirubin concentration in our studies displays an upward trend from 6.7 in May to 18.8 in August. These values are very high and must be accompanied by severe icterus or acetonaemia which were not observed. Probably they were the result of a mass invasion of haemosporidia which are going to be a subject of a future study. Table 5 shows that the bilirubin levels are reasonably affected by the THI and the season ($p<0.001$). The metabolic processes in the animal organisms speed up in conditions endangering the maintenance of the organism homeostasis. The enzyme systems play a huge role in these processes. The enzyme levels are one of the fast-reacting units of the biochemical homeostasis and reveal even the slightest changes in the metabolism of the animals, they help for the identification of pathological processes prior the display of clinic signs (Yarovan & Novikova, 2012). The ASAT and ALAT enzymes are important for the amino acids metabolism as they catalyze the transfer of amino-groups to the keto acids. They are present in all organs and tissues and so their increased activity in the blood serum is indicative with reference to many diseases (Kazartsev & Ratoshny, 1986). The results of the present study show a drastic increase in both transferases. The average ASAT levels in May are 117 U/L, and 121 U/L in August, and the ALAT levels are respectively 27-32 U/L. No significant THI influence is indicated due to its low variation. Moore (1997) came to the conclusion that the increased ALAT serum levels are usually indicative of liver damage in the milk cattle. The hepatocytes are highly sensitive upon degenerative processes, and therefore, the ALAT plasma activity increase is rarely an indicator with a clinical importance unless it is twice the upper reference values, as is the case. The increased ASAT (three times the reference value!) must be carefully considered because this enzyme is contained not only in the liver but also in other tissues (heart and skeleton muscles, the brain, kidneys, pancreas and the lungs). The slighter ALAT increase is due to the fact that a big part of the enzyme is in the mitochondria and is excreted only upon severe degenerative cell damage. According to one of our previous studies (Ivanova & Tasheva, 2020), the high transaminase levels might be a result of the high productivity of these animals, which is the so called productive stress (Cozzi, 2011) The ASAT and ALAT activities are accelerated even upon moderate heat stress when there is an accelerated lactation and metabolism (Gorski & Saba, 2012).

CONCLUSIONS

The changes ascertained in the biochemical parameters of the total protein, creatinine and urea related to the changes in the THI and the season are within the reference values for cattle.

When the THI is high, the blood sugar decreases (up to 31%), the total bilirubin levels increase (up to 3 times), and the transaminase activities also go up (ASAT up to 3 and ALAT- up to 4.5 times) the reference values.

The THI changes statistically affect the pulse rate, breathing and the body temperature ($p<0.001$).

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