Article

Influence of Heat Stress on Some Biochemical Indicators in the Blood of Dairy Cows

Toncho Penev [*] and Martin Stojnov

Article

Influence of Heat Stress on Some Biochemical Indicators in the Blood of Dairy Cows

Toncho Penev * and Martin Stojnov

Department of Ecology and Animal Hygiene, Faculty of Agriculture, Trakia University,
6000 Stara Zagora, Bulgaria
* Correspondence: toncho.penev@trakia-uni.bg

Abstract: Heat stress (HS) is a strong stress factor that significantly affects the metabolism of dairy cows. The aim of the present study was to investigate how HS affects some biochemical indicators in the blood of dairy cows reared in Bulgaria. The study was conducted on a cattle farm with a capacity of 500 Holstein cows, loose housed in a free stall barn shed type. The research was carried out in three seasons - summer, autumn and winter, and a total of 77 samples were taken from the cows of the farm - 30 blood samples for each season. The cows included in the study were from 30 to 60 days in milk (DIM). Temperature and humidity data were taken from the closest to the farm weather station. Based on the conducted research, it was found that the HS reported during the summer season had a significant impact on the following blood parameters: glucose, urea, total protein and cortisol. In summer, blood glucose values were low and ranged from 1.32 to 1.63 mmol/l in cows on different lactations. Probably because of these low glucose levels, high levels of total protein, ranging from 76.92 to 86.10 g/l, were reported in cows from the first to third lactation. This protein was used by cows to provide energy from non-carbohydrate sources and as a residual product, higher blood urea levels were reported in summer, ranging from 4.79 to 5.23 mmol/l. As proof of the stressful influence of heat on the metabolism of the examined cows, the cortisol values in the summer were in the upper reference norms - 38.44 nmol/l or higher and reached 59.09 nmol/l.

Keywords: dairy cows; heat stress; blood; biochemical parameters

1. Introduction

Nowadays, we are witnessing a noticeable global warming, resulting in an increase in ambient temperature even in more northern latitudes with a moderate climate [1–3]. An intergovernmental report on climate change indicates that the Earth's temperature has risen by 1.2 ºC since the Industrial Revolution to the present day [4]. On the other hand, in recent decades, there has been increasingly intensive selection in dairy cattle farming to increase productivity. The lactation period also significantly affects the metabolism of dairy cows [5]. All of this makes dairy cows increasingly susceptible to the impending climate changes associated with global warming [6], and the welfare of these animals increasingly depends on strategies to alleviate heat stress [7].

Studying the biological mechanisms occurring during heat stress (HS) in cows is of crucial importance for making the right decisions to mitigate the negative impact of HS on animal productivity [6]. Modern dairy cows are highly productive animals with a very intensive metabolism. Therefore, an in-depth analysis of serum parameters can provide explanations for the physiological changes that occur under HS conditions [8]. Blood biochemical parameters provide objective information about the biophysical and biochemical processes occurring in the cows' bodies [8]. Mylostyvyi & Sejian [9] demonstrate that environmental conditions are one of the main stress factors affecting hematological and chemical parameters in cows' blood. Blood tests are valuable because they provide information about the cows' condition before clinical signs of disease or different types of stress occur and can be used for the diagnosis and prevention of these conditions [10].

Taking into account the above and the relatively small number of similar studies in recent years on dairy cows in Bulgaria, it gives us a reason to conduct a study of the influence of heat stress on some biochemical indicators in the blood of dairy cows reared in Bulgaria.

2. Material and Methods

The study was conducted in a cattle farm located in Central South Bulgaria. The farm had a capacity of 500 Holstein cows. During the study period (March – October 2022) the number of cows in milk ranged from 220 to 250. Cows were housed loose in free stall open barn (shed type). They were divided into groups depending on the DIM and physiological state. Feeding was ad libitum with a total mixed ration, according to the physiological state and the level of milk production and was not changed during the entire period of the study. The average milk yield from a cow for a 305 day lactation was 10000 kg.

The research was done in three seasons, summer, autumn and winter, and a total of 77 samples were taken from 77 different cows reared on the farm and calved during the three seasons studied. For the summer season in the conditions of a temperate continental climate, the months of June, July and August are accepted. The autumn season includes the months of September, October and November, and the winter season includes December, January and February. Cows from 30 to 60 DIM were included in the study.

The cows were from the first to the third lactation, respectively on the 1st - 28, on the 2nd - 24, and on the 3rd - 25 cows.

The blood samples were taken once for each season from the tail vein during routine veterinary monitoring of the health status of the cows on the farm, respectively on 17.02, 26.07 and 21.10.2022.

The data on the climate indicators for the farm area, temperature and air humidity were taken from the nearest Meteorological Station, the city of Plovdiv and were for the year 2022.

From the data on the temperature and humidity of the air, the values of the Temperature-Humidity Index (THI) were also calculated according to the formula of Thom [11]:

$$0.8 \times T0 + (B0/100) \times (T0 + 14.4) + 46.4,$$

where: T0 is the air temperature in ºC, B0 is the percentage of air humidity.

The blood samples were examined in a specialized laboratory Zinvest (Stara Zagora). The following indicators were examined:

- Glucose - hexokinase method,
- Triglycerides - GPO-PAP method,
- Urea – Uryase/GLDH method
- Total protein - Biuret End Point method
- ASAT, ALAT, GGT and alkaline phosphatase - enzymatic IFCC method at 37oC
- Cortisol - ECLIA method
- Inorganic phosphorus and calcium - ammonium phosphomolybdate UV method and Arsenaso III method.

Roche Cobas Integra 400+ and Roche Cobas E 411 devices were used for this purpose.

To determine the influence of HS on some hormones, a study was done to determine the concentrations of prolactin and cortisol. Roche Cobas E 411 device and ECLIA method were used.

2.1. Statistical Data Analysis

MS Excel and IBM® SPSS® Statistics 26.0 statistical packages were used for basic statistical data processing. Levene's Test was used to check the homogeneity of the data. Univariate ANOVA analysis with Post Hoc procedure by LSD or Dunnett T3 tests depending on the results of Levene's test for homogeneity was used to evaluate the influence of the factors season and number of lactations on the studied blood indicators.

3. Results

Table 1 presents the average values and variation of the main climate indicators included in the study for the farm area for summer, autumn and winter seasons. It can be seen that these values were typical for a temperate continental climate. In the variation of the average daily temperature, only in the summer season values at the temperature comfort limit of 25 °C were reported. There are no significant differences in the percentage of air humidity by season. The daily average values for the three seasons were from 63.21 to 77%, with the highest average value for the winter season, Table 1.

The climate indicator that more accurately characterizes the conditions of the environment is the THI, which was calculated by including the two indicators - air temperature and percentage of air humidity. From the average daily values of THI, it was found that during the summer season the cows included in the study were in conditions of HS - about 72. This indicates that this season was the main one in which cows would experience heat stress.

Table 1. Average values and variation of the main climate indicators for the farm area.

Indicator	Number of reports	Statistical data			
		x ± Se	SD	min	max
Average daily air temperature, ºC					
Summer	92	24.27 ± 0.25	2.40	18.0	28.6
Autumn	90	14.80 ± 0.52	4.95	4.70	24.9
Winter	90	4.84 ± 0.36	3.42	-4.0	12.7
Average daily air humidity, %					
Summer	92	63.21 ± 1.12	10.70	45	93
Autumn	90	69.16 ± 1.20	11.38	50	96
Winter	90	77.00 ± 1.37	12.99	46	97
Average daily values of THI					
Summer	92	71.78 ± 0.30	2.89	64.15	76.96
Autumn	90	58.23 ± 0.80	7.55	41.29	72.52
Winter	90	42.91 ± 0.56	5.36	30.97	55.37

The summarized values of THI from one month before and for the month during the study are presented in Table 2. For the samples taken in February, the daily average values for THI, on which the cows were exposed were from 44.2 (in January) to 44.6 (February), and the maximum was 52.0. For the July samples, they were from 70.1 (in June) to 72.8 (July), and the maximum from 82 to 85.6, respectively. For October samples, respectively, they were from 50.00 to 58.1, in September and October for daily averages and maximums from 70.2 to 59.8. Due to the coming cooling in October, it was logical that THI values in September were higher than those in October.

Table 2. Values of THI with effect on the cows by the month of blood examination.

THI values	Samples from February		Samples from July		Samples from October	
	January	February	June	July	September	October
Daily	44.17±0.87	44.63±1.39	70.09±0.50	72.81±0.48	58.07±0.65	50.95±0.97
Maximum	52.02±1.30	52.01±1.98	82.04±0.96	85.64±1.04	70.18±0.74	59.81±1.61
Minimum	38.22±0.77	39.38±0.84	60.78±0.49	62.33±0.40	49.98±0.76	44.95±0.81

Table 3 presents the reference, average values and variations of the results for the biochemical indicators of the blood during the summer season of the cows on the first, second and third lactation. From the data, it can be seen that statistically significant differences between lactations (at $P < 0.05$) were found only in a few indicators - total protein, ASAT, ALAT and GGT. Regarding the total protein in the blood, significant differences were found between the cows on first and those on third lactation. In ASAT, significant differences were found between cows on second and third lactation. Statistically significant differences were reported in the summer season for the ALAT indicator

between cows on first and second lactation. A statistically significant model was registered only for the GGT indicator, as statistically significant differences were reported between cows он first and third lactation. This shows that within the summer season the lactation factor did not have a significant impact on the studied blood indicators. For all other listed indicators with significant differences between lactations, a significance of the model was not reported. It is noteworthy that the values of glucose and triglycerides in the blood were below the reference values in all three lactations. Concentrations of GGT were above normal in cows on third lactation, of total protein in cows on second and third lactation, and of cortisol in cows on first and second lactation.

Table 3. Basic statistics and Univariate ANOVA of the studied blood indicators during summer season in cows on first, second and third lactation.

Indicator. (n=77)	Reference values	First lactation $\bar{x} \pm SD$	Second lactation $\bar{x} \pm SD$	Third lactation $\bar{x} \pm SD$	Sig. (p)	R^2
Glucose	2-3 (mmol/l)	2.82±0.37	2.63±0.56	2.77±0.38	0.729	0.028
Triglycerides	0.2-0.5 (mmol/l)	0.09±0.045	0.09±0.023	0.07±0.025	0.340	0.093
Urea	2.8-8.5 (mmol/l)	2.57±0.59 [a]	3.49±0.54 [a]	3.05±0.59	0.029	0.276
Total protein	65-80 (g/l)	72.41±8.97	75.82±2.49	73.53±6.57	0.692	0.033
ASAT	45-110 (U/I)	94.45±18.01	123.98±34.91	111.07±29.94	0.156	0.156
ALAT	7-35 (U/I)	16.28±5.39	23.22±5.88	20.85±7.49	0.141	0.163
GGT	4.9-26 (U/I)	22.23±6.23 [a]	42.82±15.75 [ab]	28.77±11.23 [ab]	0.009	0.348
ALP	18-153 (U/I)	69.98±17.60	53.60±16.89	54.83±15.22	0.098	0.190
Prolactin	-	0.90±0.00	0.90±0.00	0.90±0.00	--	--
Cortisol	40-50 (nmol/l)	14.95±8.40	10.56±9.38	16.50±12.82	0.599	0.046
Inorganic phosphorus	1.52-2.25 (mmol/l)	1.85±0.25	1.93±0.23	1.92±0.18	0.737	0.029
Calcium	2.3-3.2 (mmol/l)	2.31±0.10	2.39±0.08	2.27±0.12	0.157	0.155

* Same superscripts within the same rows represent significant differences at the level of significance p < 0.05 as follows: a-a; b-b between first, second and third lactation; Post Hoc test: LSD or Dunnett T3 depend on the Levene's Test of Equality of Error Variances; SD – Standard deviation; R^2 – Coefficient of determination; n – number of the observations.

Table 4 presents the reference values, average values and variations of the results for the blood biochemical indicators of the cows on first, second and third lactation during the autumn season. From the data, it can be seen that statistically significant differences between different lactations (at P < 0.05) were found only for the urea and GGT indicators. For urea, significant differences were found only between first and second lactation cows. For the GGT indicator, significant were only the differences between cows on first and second and between first and third lactation. For both blood indicators, the model was statistically significant (at P < 0.05). Deviations from the reference values were observed for triglycerides, which were below the norm in all three lactations. Subnormal values were observed for urea in first lactation cows, cortisol in all three lactations, and blood calcium in third lactation cows. ASAT values were above normal in second and third lactation cows.

Table 4. Basic statistics and Univariate ANOVA of the studied blood indicators during autumn season in cows on first, second and third lactation.

Indicator (n=77)	Reference values	First lactation	Second lactation	Third lactation		
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	Sig. (p)	R^2
Glucose	2-3 (mmol/l)	1.58±0.35	1.61±0.62	1.32±0.48	0.41	0.065
Triglycerides	0.2-0.5 (mmol/l)	0.08±0.026	0.09±0.036	0.08±0.025	0.54	0.046
Urea	2.8-8.5 (mmol/l)	4.63±0.97	5.0±1.09	5.23±1.29	0.49	0.053
Total protein	65-80 (g/l)	76.82±9.26 [a]	83.76±7.86	86.10±9.06 [a]	0.063	0.192
ASAT	45-110 (U/I)	87.79±17.53	108.57±41.00 [a]	78.02±32.18 [a]	0.121	0.150
ALAT	7-35 (U/I)	14.74±3.68 [a]	19.27±5.73 [a]	18.45±3.96	0.062	0.192
GGT	4.9-26 (U/I)	18.41±6.64 [a]	23.90±3.79	44.20±38.47 [a]	0.032	0.233
ALP	18-153 (U/I)	46.92±15.36	79.31±52.48	61.00±39.18	0.158	0.132
Prolactin	-	0.97±0.07	0.95±0.08	1.00±0.00	0.414	0.066
Cortisol	40-50 (nmol/l)	56.87±29.05	59.09±35.85	38.43±29.03	0.340	0.080
Inorganic phosphorus	1.52-2.25 (mmol/l)	1.86±0.31	1.81±0.26	1.75±0.22	0.685	0.029
Calcium	2.3-3.2 (mmol/l)	2.35±0.10	2.35±0.10	2.32±0.09	0.868	0.011

* Same superscripts within the same rows represent significant differences at the level of significance $p < 0.05$ as follows: a-a; b-b between first, second and third lactation; Post Hoc test: LSD or Dunnett T3 depend on the Levene's Test of Equality of Error Variances; SD – Standard deviation; R2 – Coefficient of determination; n – number of the observations.

Table 5 presents the reference values, average values and variations of the results for the blood biochemical indicators of the cows on first, second and third lactation during the winter season. In the indicator glucose, statistically significant differences were found between cows on first and second and between those on first and third lactation. For this indicator, the model was statistically significant (at $P < 0.05$). It must be noted that for glucose the values for all three lactations are above the reference values for the species. Deviation from the reference values was observed for some indicators, as for triglycerides they were below the norm for all three lactations, while for total protein and GGT they were above the norm for all three lactations. The ASAT in the blood was above the norm only in cows on third lactation.

Table 5. Basic statistics and Univariate ANOVA of the studied blood indicators during winter season in cows on first, second and third lactation.
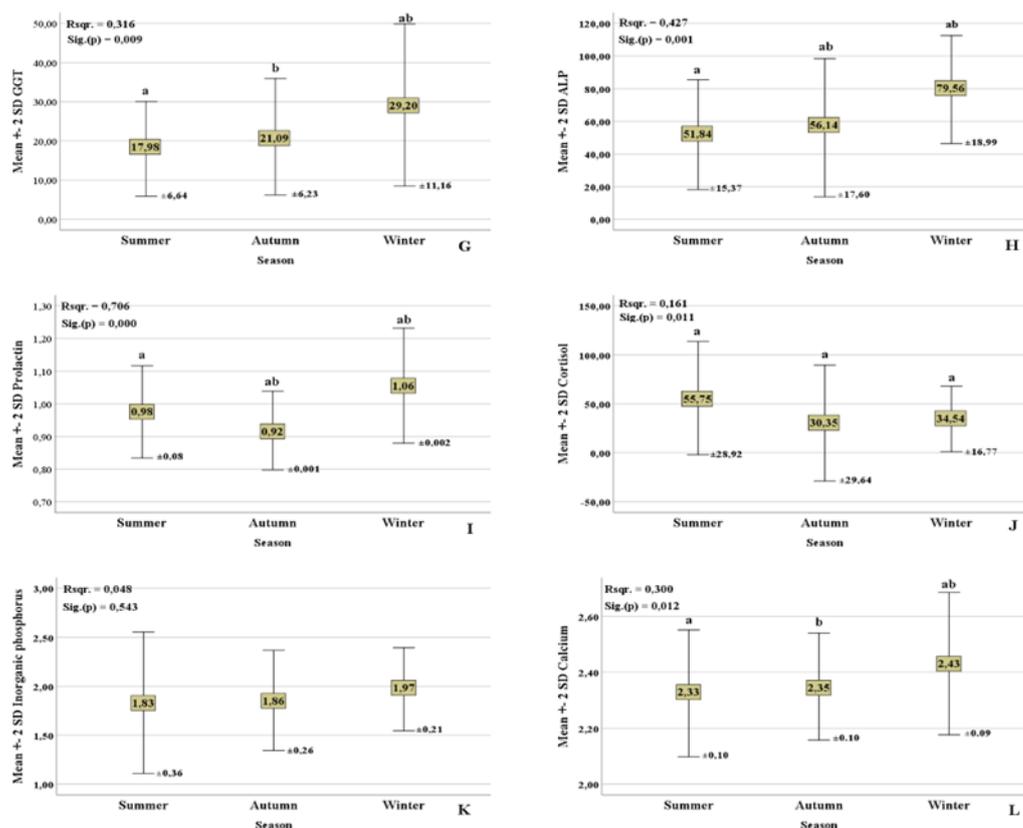
Indicator (n=77)	Reference values	First lactation	Second lactation	Third lactation		
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	Sig. (p)	R^2
Glucose	2-3 (mmol/l)	3.57±0.18 [a]	3.06±0.30 [ab]	3.26±0.24 [a]	0.003	0.444
Triglycerides	0.2-0.5 (mmol/l)	0.09±0.012	0.08±0.043	0.08±0.033	0.942	0.006
Urea	2.8-8.5 (mmol/l)	3.66±1.14	3.63±0.46	3.30±1.07	0.724	0.032
Total protein	65-80 (g/l)	89.77±6.49	89.47±7.93	87.51±6.39	0.828	0.019
ASAT	45-110 (U/I)	85.84±17.74	106.58±26.57	113.36±26.87	0.119	0.192
ALAT	7-35 (U/I)	18.91±4.06	19.33±5.03	20.75±4.64	0.763	0.027
GGT	4.9-26 (U/I)	31.07±11.16	39.36±47.17	39.56±20.87	0.863	0.015
ALP	18-153 (U/I)	79.32±18.98	63.19±20.88	61.18±11.03	0.149	0.173

Prolactin	-	1.10±0.00	1.10±0.00	1.10±0.00	--	--
Cortisol	40-50 (nmol/l)	39.09±14.41	27.46±23.90	25.94±20.84	0.439	0.079
Inorganic phosphorus	1.52-2.25 (mmol/l)	1.97±0.24	1.85±0.23	1.81±0.21	0.444	0.078
Calcium	2.3-3.2 (mmol/l)	2.47±0.09	2.44±0.10	2.43±0.08	0.722	0.032

* Same superscripts within the same rows represent significant differences at the level of significance $p < 0.05$ as follows: a-a; b-b between first, second and third lactation; Post Hoc test: LSD or Dunnett T3 depend on the Levene's Test of Equality of Error Variances; SD – Standard deviation; R2 – Coefficient of determination; n – number of the observations.

Figure 1 presents the data of the studied blood indicators in the first lactation cows by season. Significant differences were found in the indicators: glucose; urea; total protein; GGT; alkaline phosphatase; prolactin; cortisol and calcium during the different seasons. For glucose, significant differences were observed between summer and autumn, summer and winter and between autumn and winter. The lowest blood glucose values were recorded in summer and the highest in winter. For the urea indicator, significant differences were observed between summer and autumn, summer and winter and between autumn and winter seasons. In the summer, the highest blood urea values were reported. For total protein, the highest values were recorded in winter. Significant differences were registered between summer and winter and between autumn and winter. For the GGT indicator, significant differences were registered between summer and winter and between autumn and winter. The lowest values were recorded in summer and the highest in winter. In the case of alkaline phosphatase, significant differences were reported between the seasons summer and autumn, summer and winter and between autumn and winter. The lowest values were recorded in summer and the highest in winter.
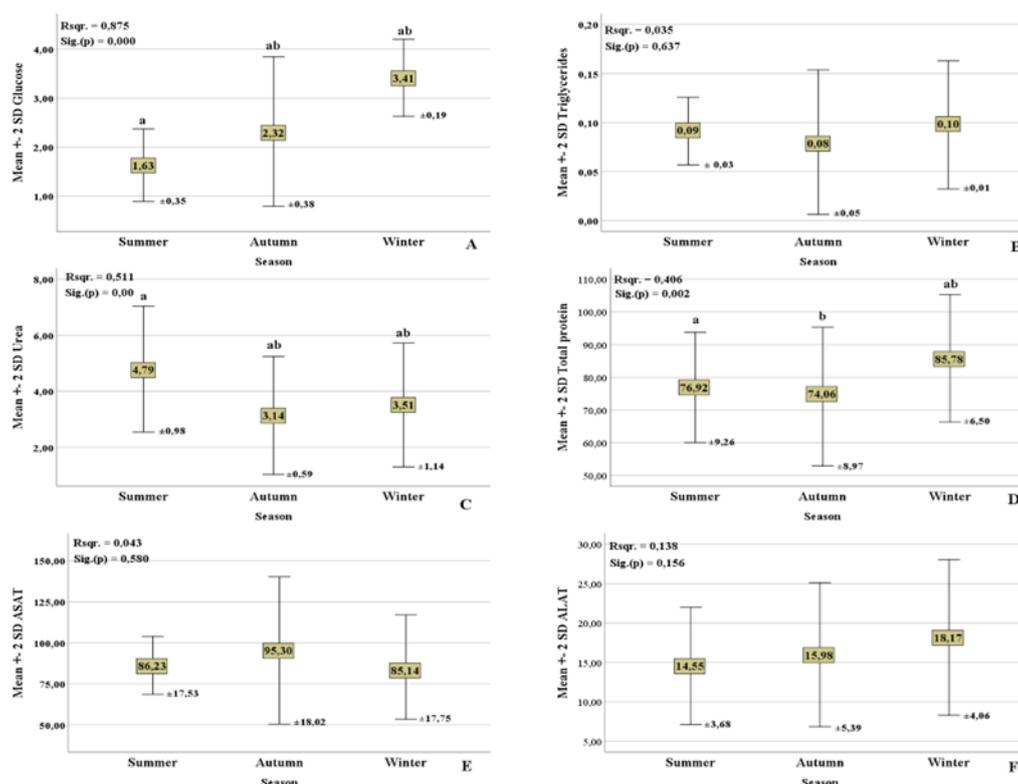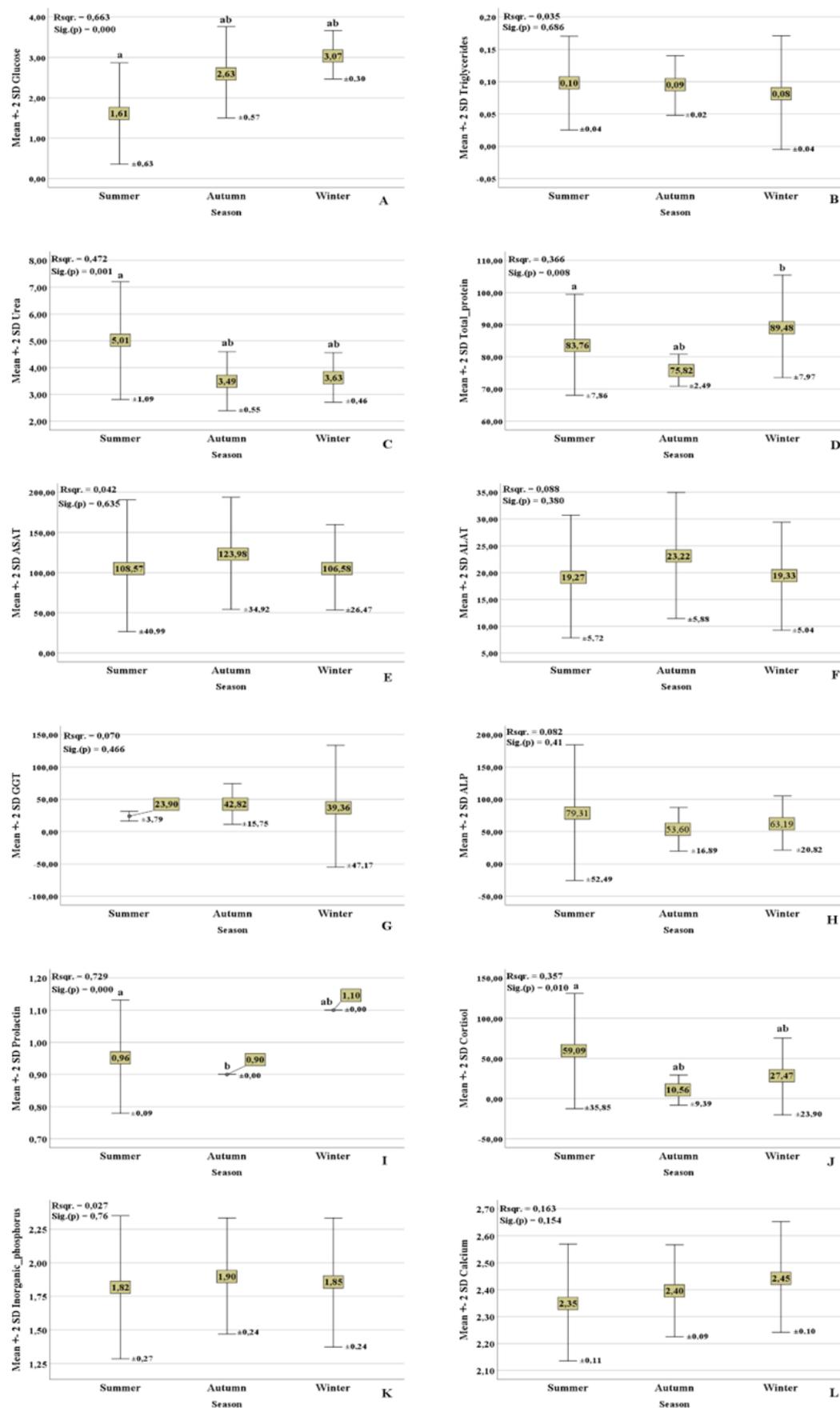
Figure 1. Values of blood indicators in first lactation cows by season. Statistically significant differences of the studied blood indicators by seasons are marked with the same letters - a-a; b-b at P < 0.05.

For prolactin, significant differences were reported between the seasons summer and autumn, summer and winter and autumn and winter. The lowest values were recorded in autumn, and the highest in winter. For the blood cortisol indicator, significant differences were reported between summer and autumn and between summer and winter. The values of cortisol in the blood in summer differ significantly from those registered in autumn and winter. The lowest values of this indicator were reported in autumn, and the highest in summer. For the blood calcium indicator, significant differences were reported between summer and winter and between autumn and winter. The lowest values were in summer and the highest in winter.
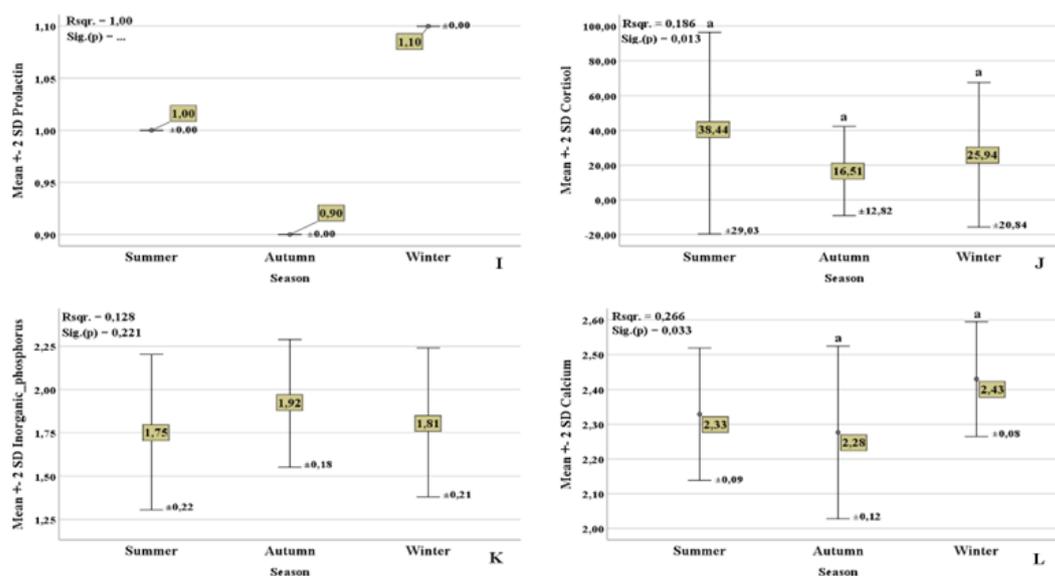
Figure 2 presents the data for the studied blood indicators by season in cows on second lactation. From the data, it can be seen that significant differences were found in the indicators glucose, urea, total protein, prolactin and cortisol. For glucose, statistically significant differences were reported between summer and autumn, summer and winter and between autumn and winter. Lowest values as found in first lactation cows were reported in summer, followed by values in autumn, and highest in winter. For urea in the blood, significant differences were established between summer and autumn, summer and winter and between autumn and winter. The highest values of this indicator were recorded in summer, and the lowest in autumn. Statistically significant differences were registered between the summer and autumn and between autumn and winter seasons for the indicator total protein in the blood. The highest protein values were recorded in winter and the lowest in autumn. A similar trend was also found in first lactation cows. Regarding the prolactin, statistically significant differences were found between the summer and winter and between autumn and winter seasons. The lowest values of this indicator in the blood were found in autumn, and the highest in winter. The blood cortisol of cows on second lactation maintained the trends observed in cows on first lactation. The highest values were recorded in the summer season, and the lowest in autumn. Statistically significant differences are reported between summer and autumn, summer and winter and between autumn and winter.

Figure 2. Values of blood indicators in second lactation cows by season. Statistically significant differences of the studied blood indicators by seasons are marked with the same letters - a-a; b-b at P < 0.05.

   Figure 3 presents the data of the studied blood indicators by season in the cows on third lactation. Significant differences were reported for the following indicators: glucose, urea, total protein, ASAT, cortisol and calcium. Similar to the trends observed in first and second lactation cows, blood glucose values were lowest in the summer. Statistically significant differences on this indicator are established between summer and autumn, between summer and winter, and between autumn and winter. For the blood urea indicator, statistically significant differences were again established between the values recorded in summer and autumn, summer and winter, autumn and winter. The highest values of urea were recorded in summer. For the indicator of total protein in the blood, significant differences were reported between summer and winter and between autumn and winter. The lowest values for this indicator were recorded in autumn, and the highest in winter. The values of the ASAT indicator in the blood have statistically significant differences between summer, compared to those measured in autumn and winter. For the blood cortisol indicator, significant differences were reported between the values recorded in summer, autumn and winter. The highest values of this indicator were again recorded in the summer, and the lowest in the autumn, as in the case of cows on first and second lactation. Statistically significant differences were registered between the values recorded in autumn and winter for the blood calcium indicator. The lowest values of this indicator were recorded in autumn, and the highest in winter.
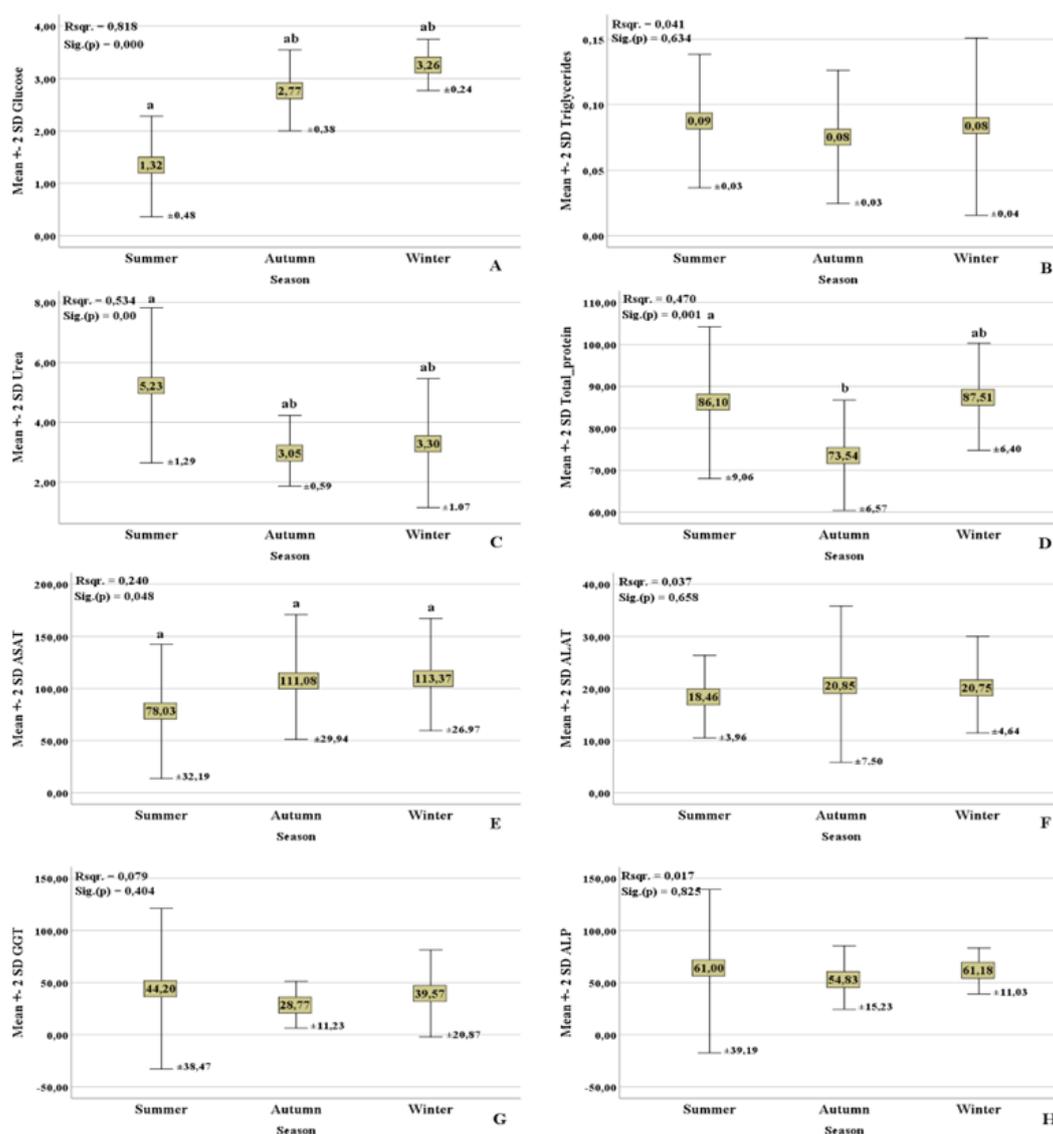
Figure 3. Values of blood indicators in third lactation cows by season. Statistically significant differences of the studied blood indicators by seasons are marked with the same letters - a-a; b-b at P < 0.05.

4. Discussion

From the data presented in Table 1, it can be seen that the highest temperatures were recorded in summer and the lowest in winter. The winter was relatively mild – average daily temperatures were around 4 ℃, varying from -4.0 to 12.7 ℃. The most serious was the problem with air temperatures in the summer season. The average daily temperature for the season was 24.27 ℃, reaching 28.6 ℃. An ambient temperature between 0°C and 25°C has been found to be optimal for dairy cows [12], but above 25°C cause rise to their body temperature, leading to to HS.

The environmental conditions are determined more accurately by THI, calculated on the basis of air temperature and percentage of air humidity. Solar radiation, air temperature and relative humidity percent are the most important climatic variables that determine HS [13].

It is obvious that during the summer season the daily average THI values were 71.78, with a deviation up to 76.96 High values were also recorded in the autumn - up to 72.52.

Gantner et al. [14] indicated that THI values in spring and summer in the three regions of Croatia (Eastern, Mediterranean and Central) exceed values of 72, during which cows are at risk of HS. During the autumn and winter, when THI values are usually lower than 72, cows rarely experience heat stress.

Armstrong [15] classified HS into three types according to the THI: mild (72 to 78 units), moderate (79 to 89 units), and severe (90 to 98 units). According to a number of authors, a THI above 72 is accepted as the threshold for inducing heat stress in the tropics [16,17], while in temperate zones, high-producing cows may be affected by heat stress and in more low THI values of 60 [18,19]. According to Blond et al. [20] in Serbia, a neighboring country with similar climatic conditions to Bulgaria, in June and July THI values range between 72 and 90, which represents moderate to severe heat stress.

According to the reported THI values in the farm area, in the summer it can be said that the cows were exposed to HS almost 24 hours a day, and such conditions were also reported during separate periods in the spring and autumn. Considering the fact that the building (shed type) provides almost no protection from the external conditions, the animals were exposed to the risk of HS.

The values presented in Table 2 show that the cows from which samples were taken in July were exposed to HS (mild to moderate) long enough to expect consequences not only on physiological indicators, but also on indicators related to the homeostasis of the animals. According to Zimbelman et al. [21] high yielding dairy cows decrease their milk yield at a THI of approximately 68. Cattle have been shown to become more sensitive to HS as productivity increases [17]. Indeed, as daily milk yield increases from 35 kg to 45 kg, sensitivity to TC increases and threshold temperature decreases by 5°C [22]. This increased sensitivity may be explained by the additional heat associated with the synthesis of more milk.

In other studies [23,24] reported that a THI of 68 is already a critical value at which dairy cows will start to show HS symptoms. Another study [25] found symptoms of HS in high-producing dairy cows when the average daily THI was above 68 units or when the minimum THI was above 65 units. In our study, such values for maximum THI were reported in the month of September and may therefore affect the cows studied for the autumn season.

Table 3 presents reference values, average values and variations of the studied blood indicators in cows on first, second and third lactation during the summer season. It can be seen that the blood glucose values were lower than the reference values for the species in all three lactations. In early lactation, lipolysis and insulin resistance increase to direct glucose to the udder to maintain lactation, and during heat stress, insulin sensitivity increases and lipolysis decreases, diverting glucose to tissues [6]. In confirmation of this hypothesis and the results obtained by us, there are also the studies of Ivanova et al. [8]. The authors found glucose values of 1.38 to 1.60 mmol/l under conditions of moderate heat stress. It has been proven that, under the influence of HS, the basal concentrations of insulin in the blood increase, as well as high values of the glucose tolerance test [26,27]. It is believed that this is an adaptive-protective mechanism that occurs under the influence of stress factors [28]. According to Shahzad et al. [29] glyconeogenesis in lactating cows is much more intense during warm periods compared to cold seasons of the year. Higher values of THI (respectively HS), according to the authors, forces the cows to increase their metabolism, while at the same time they have a low feed intake. In addition, the mammary gland needs glucose for the synthesis of lactose, and the body also uses energy to control its body temperature - cooling. All of the above fully explains the low blood glucose values in cows during the summer season. From the data thus presented (Table 3), it can be seen that the values of triglycerides were significantly lower than the accepted norms. In a study by Blond, et al. [20] a tendency for lower values of this indicator was demonstrated in cows at the beginning of lactation at different THI values. The results presented in Tables 3, 4 and 5 fully confirm this statement. A similar tendency is also proven by other authors [20,30]. In the summer season, the total protein in the blood was slightly higher in cows on second and third lactation (83.76 g/l and 86.10 g/l) at norms up to 80 g/l. Our results support the hypothesis of Abbas et al. [31], according to which HS causes cows to catabolize proteins, releasing amino acids that the body uses to convert to glucose and thus energy. This is probably the reason for the higher values in this indicator.

Another indicator that shows a deviation from the norms in the summer season was GGT in cows on third lactation. A significant influence of the model was found only for this indicator (table 3). This enzyme is involved in protein metabolism by splitting C-terminal glutamyl groups from amino acids and transferring them to another peptide or amino acid [32]. It is possible that the increase in GGT in third lactation cows is related to the higher milk yield and the need for amino acids in the blood for milk protein synthesis. Studies by other authors show conflicting trends. Blond et al. [20] found that HS increased GGT values, but according to Joo et al. [33], HS does not affect this blood indicator. We believe that these high GGT values were due to the higher milk yield and the need for amino acids for milk protein synthesis. An increase in blood cortisol levels was also observed in first and second lactation cows during the summer season. A similar tendency to increase the levels of cortisol in the blood under HS was also demonstrated by Blond et al. [20]. However, the values found by us for the month of July (conditions under HS) were approximately two times higher than the values reported by Blond et al. [20.] Cortisol is known as a stress hormone, also having a major role in lactopoiesis and lactation maintenance [34,35].

It is well known that HS reduces the immune response of cows [36,37] and increases the secretion of cortisol and prolactin [38]. According to the authors, it is not yet known whether the negative effect of HS on immunity is related to a change in the secretion of cortisol and prolactin or to their immunomodulatory effects on the proliferation or anti-inflammatory response of immune cells or both together.

From the data presented in table 4, it can be seen that the model had a significant influence on the indicators urea and GGT in the blood. Urea in the autumn season was lower only in cows on first lactation, and GGT values were higher in cows on second and third lactation. Higher than normal values of the ASAT enzyme were also found in cows on second and third lactation, but the deviations

were not substantial. Considerably lower blood cortisol values were reported in the autumn examination of cows from all lactations.

Table 5 presents the results of the studied blood indicators during the winter season of the all lactations cows. A significant influence of the model was proven only for the indicator of glucose in the blood. It is observed that in this season, for this indicator, the values in cows on all lactations were slightly above the accepted norms. This shows the energy supply in the ration and its digestibility by the cows in conditions of temperature comfort in winter (Table 2). As previously noted, triglycerides were below normal, probably due to the initial stage of lactation. Total protein and GGT in the blood were above the norm in all three lactations. Cortisol levels, although not deviating considerably, were below the accepted reference values, which was probably due to the absence of stress factors. It is noticed that cortisol iwas highest in cows in the first lactation, which was probably due to the fact that for them there were the most changes of a technological and physiological nature (calving, change of barn, milking, change of ration, etc.).

Figure 1 shows the values of blood indicators in first lactation cows by seasons with statistical processing. Significant differences were found in the indicators: glucose; urea; total protein; GGT; alkaline phosphatase; prolactin; cortisol and calcium during the different seasons. The lowest glucose values were recorded in summer and the highest in winter (Figure 1A). These results confirm the previously stated hypothesis about the influence of HS on blood glucose levels. Lack of energy in the form of blood glucose in summer forces cows to use non-carbohydrate sources for this purpose. This leads to an increase in total protein levels in the blood, and in first lactation cows these levels were lower than the values found in winter (Figure 1D). This is probably because these proteins in summer were used to a greater extent as a source of energy, for the synthesis of milk. In confirmation of this are the results for the levels of urea in the blood - a waste product of the metabolism of proteins in the process of gluconeogenesis. In summer, urea values were highest (Figure 1C). Similar to our results, Shwartz et al. [39], also reported higher urea values in cows under HS. Under HS, there is a change in nitrogen metabolism, which leads to decreased milk protein values and increased blood urea values [40]. As a confirmation of the influence of the season as a stress factor for the cows metabolism are also the levels of cortisol in the blood, which were the highest in the summer (Figure 1J).

Figure 2 presents the data for the studied blood indicators by season in the cows on second lactation. Differences between seasons were observed in glucose, urea, total protein, prolactin and cortisol indicators. Similar to the trends observed in first lactation cows, the data show a shortage of energy in the summer (Figure 2A), which was provided at the expense of protein, and leads to high levels of urea (Figure 2C). Total blood protein levels were high in summer (Figure 2D), but were lower than in winter, probably because they were used as an energy source in summer instead of carbohydrates. This trend was also observed in third lactation cows (Figure 3D). As evidence for the influence of season as a stressor in second lactation cows were high cortisol levels in summer (Figure 2J).

Figure 3 presents the data of the studied blood indicators by season in the cows on third lactation. Similar to the trends found in first and second lactation cows, low blood sugar levels (Figure 3A), high urea values (Figure 3C), high total protein levels (Figure 3D) and high blood cortisol levels were also found (Figure 3J) in summer.

5. Conclusions

In the present study, it was found that heat during the summer season was a stress factor that affected some biochemical blood indicators in dairy cows. As proof of the stress influence are the high values of the hormone cortisol in cows in the summer in all studied groups. Based on the values of the studied indicators, it can be stated that HS leads to a shortage of energy from carbohydrate sources, which triggers mechanisms to provide it from non-carbohydrate sources. These processes are associated with a change in the concentration of glucose, urea, total protein and cortisol in the blood of dairy cows.

13

References:

1. Renaudeau, D.; Collin, A; Yahav, S; de Basilio, V; Gourdine, J.L.; Collier, R.J. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal* **2012,** May;6(5):707-28. doi: 10.1017/S1751731111002448. PMID: 22558920.

2. Segnalini, M.; Bernabucci, U.; Vitali, A. et al. Temperature humidity index scenarios in the Mediterranean basin. *Int. J Biometeorol*. **2013**, 57, 451–458. https://doi.org/10.1007/s00484-012-0571-5

3. Fournel, S.; Ouellet, V.; Charbonneau, É. Practices for Alleviating Heat Stress of Dairy Cows in Humid Continental Climates: A Literature Review. *Animals* **2017**, 7, 37. https://doi.org/10.3390/ani7050037

4. Wankar, A.K.; Rindhe, S.N.; Doijad, N.S. Heat stress in dairy animals and current milk production trends, economics, and future perspectives: The global scenario. Trop. Anim. Health Prod. **2021**, 53, s11250–s020

5. Mezzetti, M.; Cattaneo, L.; Passamonti, M.M.; Lopreiato, V.; Minuti, A.; Trevisi, E. The transition period updated: A review of the new insights into the adaptation of dairy cows to the new lactation. *Dairy* **2021**, 2, 617–636.

6. Sammad, A; Wang, Y. J.; Umer, S.; Lirong, H.; Khan, I.; Khan, A.; Ahmad, B. and Wang. Y. Nutritional Physiology and Biochemistry of Dairy Cattle under the Influence of Heat Stress: Consequences and Opportunities. *Animals* **2020**, 10, 793; doi:10.3390/ani10050793

7. Frigeri, K.D.M.; Kachinski, K.D.; Ghisi, N.d.C.; Deniz, M.; Damasceno, F.A.; Barbari, M.; Herbut, P.; Vieira, F.M.C. Effects of Heat Stress in Dairy Cows Raised in the Confined System: A Scientometric Review. *Animals* **2023**, 13, 350.

8. Ivanova, R.; Hristev, H. & Gergovska, Zh. Influence of the level of daily milk yield on some blood biochemical parameters in dairy cows reared under the same temperature and humidity conditions. *Bulg. J. Agric. Sci*. **2022**, 28 (Supplement 1), 55–64.

9. Mylostyvyi, R.V.& Sejian, V. Welfare of dairy cattle in conditions of global climate change. *Theor. Appl. Vet. Med*. **2019**, 7, 47–55.

10. Ndlovu, T.; Chimonyo, M.; Okoh, A. I.; Muchenje, V.; Dzama, K. & Raats, J. G.. Assessing the nutrititional status of beef cattle: current practices and future prospects. *Afr. J. Biotechnol*. **2007**, 6, 2727–273

11. Thom, E.C. Cooling degree days. *Air Conditioning, Heating and Ventilating* **1958**, 55:65-69

12. Correa-Calderon, A.; Armstrong, D.; Ray, D.; DeNise, S.; Enns, M.; Howison C. Thermoregulatory responses of Holstein and Brown Swiss Heat-Stressed dairy cows to two different cooling systems. *Int. J Biometeorol.* **2004**, 48:142-8. https://doi.org/10. 1007/s00484-003-0194-y

13. Turner, M. G.; Dale, V. H.; & Gardner, R. H. Predicting across scales: theory development and testing. *Landscape ecology* **1989**, 3, 245-252.

14. Gantner, V.; Mijić, P.; Kuterovac, K.; Solić, D.; Gantner, R. Temperature-humidity index values and their significance on the daily production of dairy cattle. *Mljekarstvo* **2011**, 61:56-63.

15. Armstrong, D. V. Heat stress interaction with shade and cooling. *J. Dairy Sci*. **1994**, 77:2044–2050.

16. Ravagnolo, O.; Misztal, I. and Hoogenboom, G. Genetic component of heat stress in dairy cattle, development of heat index function. *J. Dairy Sci*. **2000**, 83:2120–2125.

17. Kadzere, C.T.; Murphy, M.R.; Silanikove, N.; Maltz, E. Heat stress in lactating dairy cows: a review. *Livest. Prod. Sci.* **2002**, 77:59-91.

18. Brügemann, K.; Gernand, E.; König von Borstel, U.; & König, S. Defining and evaluating heat stress thresholds in different dairy cow production systems. *Archives Animal Breeding* **2012**, 55(1), 13-24.

19. Gorniak, T.; Meyer, U.; Südekum, K.H.; Dänicke, S. Impact of mild heat stress on dry matter intake, milk yield and milk composition in mid-lactation Holstein dairy cows in a temperate climate. *Arch Anim Nutrit* **2014**, 68:358-369.

20. Blond, B.; Majkić, M.; Spasojević, J.; Hristov, S.; Radinović, M.; Nikolić, S.; Anđušić, L.; Čukić, A.; Došenović, Marinković, M.; Vujanović, B. D.; et al. Influence of Heat Stress on Body Surface Temperature and Blood Metabolic, Endocrine, and Inflammatory Parameters and Their Correlation in Cows. *Metabolites* **2024**, 14, 104. https://doi.org/10.3390/metabo14020104

21. Zimbelman, R. B.; Rhoads, R. P.; Rhoads, M. L.; Duff, G. C.; Baumgard, L. H. and Collier, R. J. A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. Pages 158–169 *in Proceedings of the Southwest Nutrition Conference.* Accessed Feb. 2, **2009**.

22. Berman, A. Estimates of heat stress relief needs for Holstein dairy cows. *J. Anim. Sci*. **2005**, 83: 1377–1384

23. De Rensis, F.; Garcia-Ispierto, I.; Lopez-Gatius, F. Seasonal heat stress: Clinical implications and hormone treatments for the fertility of dairy cows. *Theriogenology* **2015**, 84:659-666.

24. Avendaño-Reyes L. Heat stress management for milk production in arid zones. In: Narongsak Chaiyabutr editor. Milk production - An up-to-date overview of animal nutrition, management and health. *Intech Open, London* UK **2012**, 165-184.

25. Carter, B.H.; Friend, T.H.; Sawyer, J.A.; Garey, S.M.; Alexander, M.B.; Carter, M.J. *et al*. Effect of feed-bunk sprinklers on attendance at unshaded feed bunks in drylot dairies. *Prof. Anim. Sci*. **2011**, 27:127-132.

26. Fox, D.G.; Tylutki, T.P. Accounting for the Effects of Environment on the Nutrient Requirements of Dairy Cattle. *J. Dairy Sci*. **1998**, 81, 3085–3095.

27. Wheelock, J.B.; Rhoads, R.P.; VanBaale, M.J.; Sanders, S.R.; Baumgard, L.H. Effects of heat stress on energetic metabolism in lactating Holstein cows. *J. Dairy Sci*. **2010**, 93, 644–655.

28. Baumgard, L.H.; Rhoads, R.P. Effects of Heat Stress on Postabsorptive Metabolism and Energetics. *Annu. Rev. Anim. Biosci*. **2013**, 1, 311–337.

29. Shahzad, K.; Akbar, H.; Vailati-Riboni, M.; Basirico, L.; Morera, P.; Rodriguez-Zas, S. L. & Loor, J. J. The effect of calving in the summer on the hepatic transcriptome of Holstein cows during the peripartal period. *J. Dairy Sci*. **2015**, 98(8), 5401-5413.

30. Turk, R.; Rošić, N.; Vince, S.; Perkov, S.; Samardžija, M.; Beer-Ljubić, B.; Belić, M.; Robić, M. The influence of heat stress on energy metabolism in Simmental dairy cows during the periparturient period. *Vet. Arh*. **2020**, 90, 1–10.

31. Abbas, Z.; Sammad, A.; Hu, L.; Fang, H.; Xu, Q.; Wang, Y. Glucose Metabolism and Dynamics of Facilitative Glucose Transporters (GLUTs) under the Influence of Heat Stress in Dairy Cattle. *Metabolites* **2020**, 10, 312.

32. Hanigan, M.H. Gamma-glutamyl transpeptidase: redox regulation and drug resistance. *Adv. Cancer Res*. **2014**; 122:103-41. doi: 10.1016/B978-0-12-420117-0.00003-7. PMID: 24974180; PMCID: PMC4388159.

33. Joo, S.S.; Lee, S.J.; Park, D.S.; Kim, D.H.; Gu, B.-H.; Park, Y.J.; Rim, C.Y.; Kim, M.; Kim, E.T. Changes in Blood Metabolites and Immune Cells in Holstein and Jersey Dairy Cows by Heat Stress. *Animals* **2021**, 11, 974. https://doi.org/10.3390/ani11040974

34. Tucker, H. A. Hormones, mammary growth, and lactation: A 41-year perspective. J. Dairy Sci. 2000, 83:874–884. https://doi.org/10.3168/jds.S0022-0302(00)74951-4.

35. Lacasse, P.; Zhao, X.; Vanacker, N.; and Boutinaud, M. Review: Inhibition of prolactin as a management tool in dairy husbandry. *Animal* **2019**, 13:s35–s41. https://doi.org/10.1017/S1751731118003312.

36. do Amaral, B. C.; Connor, E. E.; Tao, S.; Hayen, J.; Bubolz, J. and Dahl G. E. Heat stress abatement during the dry period influences prolactin signaling in lymphocytes. *Domest. Anim. Endocrinol*. **2010**, 38:38–45. https://doi.org/10.1016/j.domaniend.2009.07.005.

37. do Amaral, B. C.; Connor, E. E.; Tao, S.; Hayen, J.; Bubolz, J. and Dahl G. E. Heat stress abatement during the dry period influences metabolic gene expression and improves immune status in the transition period of dairy cows. *J. Dairy Sci*. **2011**, 94:86–96. https://doi.org/10.3168/jds.2009-3004.

38. Marins, N.T.; Gao, J.; Yang, Q.; Binda, R. M.; Pessoa, C. M. B.; Orellana Rivas R. M.; Garrick, M.; Melo, V. H. L. R.; Chen, Y.; Bernard, J. K.; Garcia, M.; Chapman, J. D.; Kirk, D. J. and Tao, S. Impact of heat stress and a feed supplement on hormonal and inflammatory responses of dairy cows. *J. Dairy Sci*. **2011**, 104:8276–8289

39. Shwartz, G.; Rhoads, M.L.; Vanbaale, M.J.; Rhoads, R.P.; Baumgard, L.H. Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows. *J. Dairy Sci*. **2009**, 92, 935–942.

40. Cowley, F.C.; Barber, D.G.; Houlihan, A.V.; Poppi, D.P. Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. *J. Dairy Sci*. **2015**, 98, 2356–2368.