

The effect of animal-related and some environmental effects on daily milk production of dairy cows under the heat stress conditions

DOI: 10.15567/mljekarstvo.2022.0406

Nenad Mičić¹, Dragan Stanojević^{2}, Ljiljana Samolovac¹, Veselin Petričević¹, Nenad Stojiljković¹, Vesna Gantner³, Vladan Bogdanović²*

¹Institute for Animal Husbandry, Department of Cattle Breeding and Genetics, Autoput 16, 11080 Belgrade, Serbia

²University of Belgrade, Faculty of Agriculture, Department of Animal Science, Nemanjina 6, 11080 Belgrade, Serbia

³University of J. J. Strossmayer in Osijek, Faculty of Agrobiotechnical Sciences Osijek, Department for animal production and biotechnology, Vladimira Preloga 1, 31000 Osijek, Croatia

Received: 19.04.2022. Accepted: 24.09.2022.

*Corresponding author: stanojevic@agrif.bg.ac.rs

Abstract

The study aimed to determine the effect of different fixed factors (breed, region, calving year, calving season, parity and lactation stage) on daily milk production (yield and content) of four dairy cattle breeds under the heat stress conditions ($THI \geq 72$). The research was conducted on a set of test-day records of four dairy cattle breeds (Simmental, Holstein-Friesian, Red Holstein and Brown Swiss) reared in the Republic of Serbia (Region of Mačva, Podunavlje and Šumadija). The statistical analysis included test-day records collected in the period from the beginning of the year 2012 to the end of the year 2019. Based on the performed analysis, it could be demonstrated that all the effects included in the statistical model (breed, region, calving year, calving season, parity and lactation stage) statistically highly significant ($p < 0.001$) affected daily milk production (yield and content) of cows reared in the terms of the heat stress conditions. The highest daily milk production was determined in the region of Podunavlje, and by the Holstein-Friesian breed. Furthermore, dairy cows calved during the spring season, in the third lactation and from the 61st to the 120th day of lactation, had the highest milk production during the summer period in an environment characterised by heat stress. Also, daily milk yield of cows in a heat stress environment decreased in the period from June to September. The results obtained indicate different animals' responses to heat stress concerning the animal's breed, rearing region, calving year, calving season, parity and lactation stage.

Key words: dairy cows; cattle breeds; milk production; heat stress; test-day records

Introduction

Heat stress (HS) can be defined as the sum of all environmental effects that affect an animal and induce an increase in its body temperature causing a physiological response (Dikmen and Hansen, 2009). In dairy cows, HS reduces milk yield and quality of milk, fertility, welfare and results in changes in behaviour (Ravagnolo et al., 2000; Bouraoui et al., 2002; Casa and Ravelo, 2003; Freitas et al., 2006; Bohmanova et al., 2007; Bernabucci et al., 2010; Hammami et al., 2013; Smith et al., 2013; Lambertz et al., 2014; Gantner et al., 2017; Collier et al., 2017; Polsky and Von Keyserlingk, 2017; Summer et al., 2018; Nordlund et al., 2019; Chavez et al., 2020; Ramón-Moragues et al., 2021). The emergence of HS is primarily impacted by the current climate changes that significantly affect livestock production (Das et al., 2016), with different implications in different regions (Bernabucci et al., 2014; Carabano et al., 2016). Dairy cows can adapt to a wide temperature range, with the comfort zone in interval from 0 to 20 °C, while from the aspect of milk production, a narrower interval of 0 to 15 °C is optimal (Dejanović et al., 2015). In the terms of heat stress, when the temperature of the hypothalamus is above the thermoneutral zone, that makes the temperatures which are comfortable for the animal, the mechanisms of heat loss are activated (Collier et al., 2019). Consequently, with an increase in temperature above the optimum, the response of the organism changes and milk production decreases (Gantner et al., 2011; Dunn et al., 2014; Gantner et al., 2017).

The first equations of the Temperature Humidity Index (THI), as a measure of HS, involving ambient temperature (AT) and relative air humidity (RH) were presented by Thom (1958), Kibler (1964), Berry et al. (1964) and the National Research Council (1971). Using THI is a non-invasive way of detecting HS that can be easily calculated (Ouellet et al., 2019; Maggiolino et al., 2020) and provides opportunities for early detection of HS in dairy cows (Yan et al., 2021). Further development of THI indicators represents an important aspect of numerous studies (Sahin and Ugurlu, 2017; Wang et al., 2018; Ouellet et al., 2019). Researchers have tried to predict the effect and level of heat stress in dairy cows based on the effect of THI values on the daily yield and composition of milk during the milk recording (Ekine-Dzivenu et al., 2020). Furthermore, numerous researches indicated that the average thermoneutral value of THI for cows is 57, while heat stress occurs when the THI reaches values at of 72 and above (Wheelock et al. 2010; Zimelman et al. 2010; Hall et al. 2016; Hall et al. 2018; Collier et al. 2019). A THI value of 72 corresponds to a temperature of 22 °C at a humidity of 100 % and 25 °C at a humidity of 50 % or 28 °C at a humidity of 20 % (Fiore et al. 2009).

Animal stress according to THI values was classified among the first by McDowell et al. (1976) as Mild: THI (72-78), Moderate: THI (79-88), Strong: THI (89-98) and Very Strong: THI (above 98). Furthermore, Bernabucci et al. (2010) classified THI (68-71) as stress threshold values, then mild to moderate THI (72-79), moderate to strong THI (80-89) and strong THI (above 90). The highest THI values were recorded during the summer months in the research of

Bohmanova et al. (2007) conducted in the USA, with the largest decline in milk production at a THI value of 76-81. Bouraoui et al. (2002) in the Mediterranean climate determined mean THI values in the spring of 68 and in the summer of 78. Research by Herbut et al. (2012) conducted in Poland reported THI values in the range of 76-82 during July, August, and September. Furthermore, the analysis of climatic conditions in Serbia shows that from May to September the THI values were around 72, while the highest values were determined in July and August and were above 80 (Vujanac, 2010).

The effect of the lactation stage on the response to heat stress was determined by Cincović and Belić (2011) who point out that at THI higher than 72 in the middle third of lactation there was a significant decrease in the quantity and quality of milk (milk fat and proteins). Furthermore, Veysel and Atakan (2019) and Mylostyvyi et al. (2021) confirmed that cow breeds affect heat resistance and concluded that Brown Swiss cows are more resistant to higher temperatures and higher relative humidity levels than Holstein Friesian and Simmental breeds.

Gaafar et al. (2011) stated that with an increase in THI from 59.82 in the winter season to 78.53 in the summer season, heat stress reduced both total lactation (at 305 days) and daily milk yield by 39.00 % and 31.40 %, respectively. Furthermore, Joksimović-Todorović et al. (2011) state that the total milk production per cow was significantly ($p < 0.05$) higher in the spring period compared to the summer. In addition, the same authors found that the decline in milk production due to heat stress was 14 % in the first third lactation and around 36 % in the middle third lactation. The average milk production during the period of early lactation (in the first 60 days of lactation) was significantly ($p < 0.05$) higher in spring than in summer seasons (Joksimović-Todorović et al., 2011; Shock et al., 2016). Furthermore, Collier et al. (2012) stated that the heat stress has a negative effect in the first 24-48 hours to four days after exposure. The effect of HS levels may also depend partly on the characteristics of the animal's body cover (Bohmanova et al. 2007). Also, cows sheltered from direct heat radiation (in the shade) have a milder consequence of the effects of heat stress on the organism (Collier and Gebremedhin, 2015). Bohmanova et al. (2008) and Nguyen et al. (2017) state that animals with lower milk production are predisposed to heat tolerance. The selection of dairy cows for thermotolerance represents a permanent, cumulative and most cost-effective approach to alleviating the effects of heat stress in dairy cattle (Biffani et al., 2016). Furthermore, by choosing adequate genetics (Luo et al., 2021), facility planning (Bakony and Jurkovich, 2020; Cardoso et al., 2021), optimizing technology and farm management, it is possible to eliminate in advance some of the preconditions for the negative effects of HS (Wang et al., 2020).

Since in the Republic of Serbia, as well as globally, heat stress is becoming an increasingly pronounced problem, the study aimed to determine the effect of different fixed factors on daily milk yield and content (milk fat and protein) of four dairy cattle breeds under the heat stress conditions.

Materials and methods

Materials

The research was conducted on a set of test-day records of dairy cows of four breeds (Simmental, Holstein-Friesian, Red Holstein and Brown Swiss) reared on the territory of three regions in the Republic of Serbia (Region of Mačva, Podunavlje and Šumadija). The research referred to the period from the beginning of the year 2012 to the end of the year 2019. Data on the production of dairy cows that were under the selection following the rules defined by the Main Breeding Program in Cattle Breeding for the area of Central Serbia were used. Milk samples were collected during the regular milk recording accordingly to the referent milk recording method (AT₄ method). According to the rules of the ICAR (International Committee for Animal Recording), for implementing milk control the A₂ method, which involves measuring the amount of milk from every cow, is regarded as the reference of milk in the control day in a period of an average of 4 weeks. At each milk recording, measuring and sampling of milk were performed during the evening or morning milkings on the control day. The milk samples were analyzed in the laboratory and the milk fat and protein content was determined following accredited laboratory methods, i.e. using infrared spectrophotometry on the MilkoScan analyzer. Furthermore, during the milk recording, records on air temperature and relative humidity in barns on an hourly basis were measured and recorded using a Data Logger (Datalogger AMTAST, AMT116, Qingdao Shandong China). Based on the calving date (beginning of lactation), test-day records were divided into four calving seasons: winter (December, January and February), spring (March, April and May), summer (June, July and August) and autumn (September, October and November). According to the parity, cows were divided into six classes: I.; II.; ...; and VI. (cows in the sixth and higher lactation), while according to the stage of lactation test-day records were divided into five classes: I. (≤60 days) lactation); II. (61-120 days of lactation); III. (121-200 days of lactation); IV. (201-300 days of lactation); and V. (>300 days of lactation). Furthermore, based on the measured microclimate parameters, ambient temperature and relative humidity, THI was calculated using the following equation (Dunn et al., 2014):

$$\text{THI} = (1.8 \times T + 32) - (0.55 - 0.0055 \times \text{RH}) \times (T - 26.8) \quad (1)$$

where:

T = ambient temperature (°C),

RH = relative humidity (%).

THI values calculated for each hour (based on the measured ambient temperature and relative humidity) were reduced to daily values as an average of 24-hour measurements. The calculated THI values were observed as an effect within the model for estimation of the variability of the analyzed milk yield traits (daily milk yield, daily fat and protein content). The THI values used in the analysis were recorded three days

before milk control (Bohmanova et al., 2008; Wildridge et al., 2018), as a confirmed way that the current level of heat stress refers to milk production on average three days after. Furthermore, only test-day records with THI ≥ 72 recorded during the four summer months of milk recording (June, July, August, and September) were used for the statistical analysis. Basic statistics of analysed traits (daily milk yield and composition) are presented in the Table 1.

Table 1. Basic statistics of analysed test-day traits records for THI ≥ 72 (n = 58.005)

Trait	Abr	Mean	Min	Max	Var	SD	CV
Daily milk yield, kg	DMY	15.51	3.60	47.80	25.03	5.00	32.27
Daily fat content, %	DFC	4.04	2.22	5.97	0.04	0.20	4.99
Daily protein content, %	DPC	3.23	2.01	3.99	0.02	0.14	4.39

*Var - variance; SD - standard deviation; CV - coefficient of variation;

Statistical analyses and models

The variability of daily milk traits (daily milk yield and composition) due to breed, region, calving season, parity and lactation stage classes were tested using least square means in GLM procedure in SAS (SAS Institute Inc., 2019). Following statistical model was used:

$$y_{ijklmnop} = \mu + B_i + R_j + C_k + S_l + P_m + L_n + H_o + e_{ijklmnop} \quad (2)$$

Where:

$y_{ijklmnop}$ = daily milk trait;

μ = overall mean;

B_i = fixed effect of breed i ($i = 1, \dots, 4$),

R_j = fixed effect of region j ($j = 1, \dots, 3$),

C_k = fixed effect of calving year k ($k = 1, \dots, 8$),

S_l = fixed effect of calving season l ($l = 1, \dots, 4$),

P_m = fixed effect of parity m ($m = 1, \dots, 6$);

L_n = fixed effect of lactation stage classes n ($n = 1, \dots, 5$),

$e_{ijklmnop}$ = residual.

Scheffe's multiple comparisons in PROC GLM (SAS/STAT) were used to test the significance ($p < 0.001$) of the differences in daily milk traits due to analysed effects.

Results and discussion

Ekine-Dzivenu et al. (2020) assumed that in the period from October to May there is a low probability of expression of heat stress in dairy cows, therefore, the data collected in this period were not included in the statistical analysis. The test-day records obtained during the four summer months

(June, July, August, and September) when the microclimate was characterized by $THI \geq 72$, were used for the statistical analysis. Analysis of variance showed that the analysed daily milk traits (daily milk yield, daily fat content, and daily protein content), were statistically significantly ($p < 0.001$) affected by all effects considered in the statistical model. Similarly, Petrovic et al. (2015) found that the effects of a farm, lactation groups and calving seasons on milk traits were statistically significant ($p < 0.01$). Other authors (Popovac et al., 2020; Straczek et al. 2021) found that the lactation stage statistically significant ($p < 0.05$) affects milk yield per cow. Popovac et al. (2020) also reported a statistically highly significant ($p < 0.001$) effect of the lactation stage on the milk fat and protein yield. Contrary to the results found in this study, Popovac et al. (2020) found that the year of calving had no significant ($p > 0.05$) effect on milk and protein yield, while the calving season statistically significantly affected the same traits ($p < 0.05$). Furthermore, analysing the decline in daily milk production, Wildridge et al. (2018), found a statistically highly significant ($p < 0.001$) association between the THI levels and the milk yield per cow.

Table 2 shows the LSMs (*least square means*) of the analyzed daily milk traits depending on the breeding region and cows' breed.

The regional variation in the daily milk yield measured during the summer months in heat stress conditions ($THI \geq 72$) shows that the highest daily milk production of cows was achieved in the predominantly lowland Region of Podunavlje (18.70 kg/day), followed by the region of Šumadija (17.75 kg/day) and Mačva (17.00 kg/day). Furthermore, the content of milk fat (%) was higher in the region of Šumadija (4.00 %) compared to the other two (3.95 %), while the protein content (%) was lowest in cattle reared in the region of Mačva (3.18 %) compared to the other two regions (3.24 %). LSMs of the analysed daily milk traits depending on the cow's breed show that the highest daily milk yield was achieved by Holstein-Friesian cows with an average of 19.30 kg/day, then Red Holstein 19.23 kg/day, Simmental cows with 16.82 kg/day and Brown Swiss with 15.93 kg/day. The highest daily content of milk fat was found in Brown Swiss cows (4.02 %), followed by two Holstein populations (3.92 % to 3.91 %), with the lowest value determined in Simmentals (3.89 %). Analysis of daily protein content indicates the highest content in Brown Swiss 3.25 % compared to other breeds.

Higher productivity of Holstein populations comparable to Simmental and Brown Swiss was confirmed by other studies (Straczek et al., 2021; Knob et al., 2021). Furthermore, daily

fat content varies due to breed (Soyeurt et al., 2011; Woolpert et al., 2016), lactation stages and parity (Stoop et al., 2009). Knob et al. (2021) confirmed the existence of differences in both, milk fat and protein content between different breeds. Atakan and Veysel (2019) and Mylostyvyi et al. (2021) found that the Brown Swiss breed was more tolerant to higher temperatures and relative humidity than the Holstein Friesian and Simmental breeds. This corresponded to the findings of Bohman et al. (2005) and Nguyen et al. (2017) who indicated that the lower daily milk production was a predisposition to heat tolerance. Bohmanova et al. (2007) determined that the level of heat stress may also depend on the animal's body cover, with lower resistance in black cows. The highest daily milk production traits determined in Holstein breeds indicate that high productive animals will tend to maintain high production even in heat stress conditions which are in agreement with the results of Vučković et al. (2020).

Table 3 shows LSMs depending on the effects of calving season, parity and lactation stage. These three factors represent the related effects of fertility and milk yield that accompany the course of milk production. The highest daily production, during the hot period characterised by heat stress ($THI \geq 72$), in the amount of 18.69 kg/day, was achieved by cows calved during the spring season, which is in line with the basics of the natural seasonality of cattle. Furthermore, the daily milk yield of cows under the heat stress differed regarding the calving season, with the lowest determined daily milk yield in cows calved during the autumn season (16.90 kg/day). The daily milk fat content varied from a maximum of 3.99 % in cows calved during the autumn season, to a minimum of 3.94 % in cows calved during the spring. The protein content has the same value of 3.21 % in cows calved during the spring and summer seasons, while it is slightly higher (3.22 %) in cows calved during the autumn and winter. Gaafar et al. (2011), Joksimović-Todorović et al. (2011), and Shock et al. (2016) also noted the impact of the summer season on declining milk quantity and quality. The summer season, compared to the winter, first reduces the animal's appetite, and then consequently milk production (Rauba et al., 2019).

Analysis of the effect of parity showed that daily milk production increases until the third lactation, after which it gradually decreases (in the first 17.09 kg/day, the third 18.56 kg/day, and the sixth 17.43 kg/day). The content of milk fat was the highest in the fifth lactation (3.97 %), while the protein content was constant until the completed fifth, and decreased in higher lactations (sixth and higher). Wang et al.

Table 2. Least square means of daily milk traits in regard to clustering cows' breed and region ($THI \geq 72$)

Effect / Trait	Region			Breed			
	Mačva	Podunavlje	Šumadija	Simmental	Holstein-friesian	Red Holstein	Brown Swiss
DMY, kg	17.00 ^A	18.70 ^B	17.75 ^C	16.82 ^A	19.30 ^B	19.23 ^B	15.93 ^A
DFC, %	3.95 ^A	3.95 ^A	4.00 ^B	3.89 ^A	3.92 ^B	3.91 ^B	4.02 ^C
DPC, %	3.18 ^A	3.24 ^B	3.24 ^B	3.21 ^A	3.21 ^A	3.20 ^A	3.25 ^B

*DMY - daily milk yield, DFC - daily fat content; DPC - daily protein content; Least square means within the same row marked with different letters differ statistically highly significant ($p < 0.001$)

(2020) pointed out that younger cows (in the first lactation) in comparison to older ones, generate less metabolic heat and have a larger body surface area compared to body weight, which effectively dissipates body heat on the body surface and is therefore considered more tolerant to heat stress than older cows (cows in higher lactations).

Analysis of the effect of the lactation stage showed, as expected, that cows had the highest milk production in the period from 61-120 days of lactation (21.78 kg/day); and the lowest at the end of lactation, after 300 days (11.97 kg/day). Straczek et al. (2021) determined the maximum daily production on day 65, while Knob et al. (2021) the maximum milk yield determined in the fourth week of lactation (up to the 28th day). The daily content of milk fat and protein showed a progression from the beginning to the end of lactation. The daily fat content increased from 3.83 % to 4.11 %, while daily protein content increased from 3.15 % in the first to 3.28 % in the fifth class of lactation stage. Stoop et al. (2009) confirmed that both fat yield and content vary depending on parity and lactation stage. Cincović and Belić (2011) found a significant decline in milk production and quality in the second third of the lactation (100-200 days) taking place in an environment characterised by a THI value higher than 72.

Table 4 shows the LSmeans of daily milk yield traits in regard to the month of milk recording, with the constant value of THI \geq 72. The effect of heat stress on milk production was evident, especially in the daily milk yield and daily fat content, the values of which constantly decrease in the analyzed period from June to September. Daily milk yield decreased from 18.01 kg/day in June to 17.92 kg/day in July, then 17.78 kg/day in August to 17.55 kg/day in September. The daily fat content also decreased from 3.97 % in June to 3.96 % in the following months, while the daily protein content was the lowest during July and August (3.21 %). Renna et al. (2010) reported a significant decline in milk yield, fat and protein content during the summer months, starting in June, similarly to the results of this study. Also, Tamami et al. (2018) determined that at values of THI \leq 60 the highest daily milk production per animal was recorded. Ekine-Dzivenu et al. (2020) stated that cows were under the influence of heat stress when THI is in the interval from 67-76. Bernabucci et al. (2010) quantify the loss of production by stating a decrease in the daily milk yield by 0.27 kg with an increase in the THI value by 1.

The effect of heat stress on daily milk fat content is unclear, and different results have been reported. Bernabucci et al. (2015) analysed the effect of recording months and found a significant decrease in daily milk fat content during the summer months (3.20 %) compared to the values recorded during the winter months (3.80 %) and spring months (3.61 %). The differences recorded in this study were significantly lower (3.96 % and 3.97 %). Cowley et al. (2015) reported that they did not find significant differences in milk fat content between cows under the normal conditions and during the heat stress conditions. Bohmanova et al. (2007) found that the highest values of the THI index (76-81) evident during the summer months (June, July and August) caused the highest decline in milk production. Kadzere et al. (2002) observed that moderate levels of heat stress occur at THI values \geq 72 when

Table 3. Least square means of daily milk traits in regard to calving season, parity and lactation stage classes (THI \geq 72)

Trait/Effect	Daily milk yield, kg	Daily fat content, %	Daily protein content, %
Calving season			
I. Winter	18.31 ^A	3.96 ^A	3.22 ^A
II. Spring	18.69 ^B	3.94 ^B	3.21 ^B
III. Summer	17.38 ^C	3.95 ^C	3.21 ^B
IV. Autumn	16.90 ^D	3.99 ^D	3.22 ^A
Parity			
I.	17.09 ^A	3.95 ^A	3.22 ^A
II.	17.89 ^B	3.96 ^B	3.22 ^A
III.	18.56 ^C	3.96 ^B	3.22 ^A
IV.	18.20 ^D	3.96 ^B	3.22 ^A
V.	17.72 ^E	3.97 ^C	3.22 ^A
VI.	17.43 ^F	3.96 ^B	3.21 ^B
Lactation stage (days)			
I. (\leq 60)	21.34 ^A	3.83 ^A	3.15 ^A
II. (61-120)	21.78 ^B	3.87 ^B	3.17 ^B
III. (121-200)	18.73 ^C	3.95 ^C	3.22 ^C
IV. (201-300)	15.26 ^D	4.03 ^D	3.25 ^D
V. ($>$ 300)	11.97 ^E	4.11 ^E	3.28 ^E

*LSMeans within the same column marked with different letters differ statistically highly significant ($p < 0.001$)

Table 4. Least square means of daily milk traits in regard to milk recording month (THI \geq 72)

Effect/Trait	Milk recording month			
	June	July	August	September
Daily milk yield, kg	18.01 ^A	17.92 ^B	17.78 ^C	17.55 ^D
Daily fat content, %	3.97 ^A	3.96 ^B	3.96 ^B	3.96 ^B
Daily protein content, %	3.22 ^A	3.21 ^B	3.21 ^B	3.22 ^A

*LSMeans within the same row marked with different letters differ statistically highly significant ($p < 0.001$)

the first physiological adaptations, decreased productivity and pathophysiological changes occur, while other studies confirmed that it also occurs at THI values \geq 68 (Bernabucci et al., 2010; Brugemann et al., 2012; Hammami et al., 2015). Some authors define a threshold at which dairy cows begin to physiologically adapt to the harmful effects of heat stress when THI reaches 68 (Allen et al., 2015), so the values of 68 and 72 were defined as the THI threshold for heat stress in lactating dairy cows (Anderson et al., 2013; Vučković et al., 2020). Globally, scientists report ubiquitous climate changes and the negative effects of heat stress on cattle (Nardone et al., 2010), where, additionally to temperature changes, solar radiation can significantly contribute to the accumulation of body heat. Solar radiation is not easy to measure and its effects may depend on the characteristics of the animal's body covering (Bohmanova et al., 2007). The gene responsible for hair length in the bovine genome and

the "heat shock gene" as a candidate thermotolerance gene in dairy breeds of cattle may affect the maintenance of lower animal body temperature levels (Xiong et al., 2013; Das et al., 2016). Carabaño et al. (2019) stated that the selection for high milk production, due to the negative correlation between the milk production traits and the resistance of cattle to heat stress, caused a decrease in the adaptation of cattle to high temperatures. Considering that heat tolerance is genetically determined, the application of adequate selection measures can increase the tolerance of dairy cattle to heat stress (Ravagnolo and Misztal, 2000; Garner et al., 2017).

Conclusions

Based on the conducted research, it could be emphasised that all the effects included in the statistical model (breed, region, calving year, calving season, parity and lactation stage) statistically highly significant ($p < 0.001$) affected daily milk production (yield and content) of cows reared in the terms of the heat stress conditions ($THI \geq 72$). Regarding the breeding region, the highest daily milk production was determined in the region of Podunavlje, while the highest daily content of fat and protein was in the region of Šumadija. Regarding the breed, the highest daily milk yield was produced by the Holstein-Friesian breed, with the highest content determined in Brown Swiss cows. Furthermore, dairy cows calved during the spring season, in the third lactation and from the 61st to the 120th day of lactation, had the highest milk production

during the summer period in an environment characterised by heat stress ($THI \geq 72$).

The results obtained indicate that the daily milk yield of cows in a heat stress environment decreased in the period from June to September. The lowest daily fat content was determined in cows calved in the spring season, during the first lactation and in the first phase of lactation (<60 days). Furthermore, the daily milk fat content was lower during July, August and September compared to June. The daily protein content decreased during the summer period and increased regarding the parity and lactation stage. Obtained results indicate different animals' responses to heat stress concerning the animal's breed, rearing region, calving year, calving season, parity and lactation stage. Therefore, all of these effects should be taken into account when optimising the environmental and genetic variability related to the response to heat stress.

Acknowledgements and funding

This study research was funded by the Agreements on the financing of scientific research work in 2022 between:

- The Faculty of Agriculture in Belgrade and the Ministry of Education, Science and Technological Development, Republic of Serbia, No: 451-03-68/2022-14/200116,
- The Institute for Animal Husbandry and the Ministry of Education, Science and Technological Development, Republic of Serbia, No: 451-03-68/2022-14/200022.

Učinak utjecaja vezanih uz pojedinu životinju i nekih okolišnih utjecaja na dnevnu proizvodnju mlijeka mliječnih krava u uvjetima toplinskog stresa

Sažetak

Cilj istraživanja bio je utvrditi učinak različitih fiksnih utjecaja (pasmina, regija uzgoja, godina teljenja, sezona teljenja, redosljed te stadij laktacije) na dnevnu proizvodnju mlijeka (količinu i sastav) četiri mliječne pasmine goveda u uvjetima toplinskog stresa ($THI \geq 72$). Istraživanje je provedeno na bazi podataka kontrole mliječnosti (test-day records) četiri različite pasmine mliječnih goveda (simentalska, holstein-frizijska, crveni holstein i smeđa) uzgajanih u Republici Srbiji (Mačva, Podunavlje i Šumadija). Statističkom analizom obuhvaćeni su podaci prikupljeni tijekom kontrole mliječnosti u razdoblju od početka 2012. godine do kraja 2019. godine. Provedena analiza pokazuje da su svi utjecaji uključeni u statistički model (pasmina, regija uzgoja, godina teljenja, sezona teljenja, redosljed te stadije laktacije) statistički visoko značajno ($p < 0,001$) utjecali na dnevnu proizvodnju mlijeka (količinu i sastav) krava uzgajanih u uvjetima toplinskog stresa. Najveća dnevna proizvodnja mlijeka utvrđena je u regiji Podunavlje i to kod holstein-frizijske pasmine. Nadalje, najveću proizvodnju mlijeka tijekom ljetnog razdoblja u uvjetima toplinskog stresa imale su mliječne krave koje su se telile tijekom proljetne sezone, te u trećoj laktaciji i od 61. do 120. dana laktacije. Također, dnevna količina mlijeka krava u uvjetima toplinskog stresa smanjivala se u razdoblju od lipnja do rujna. Dobiveni rezultati indiciraju različite reakcije pojedinih životinja na toplinski stres ovisno o pasmini, uzgojnom području, godini i sezoni teljenja, te redosljedu i stadiju laktacije.

Ključne riječi: mliječne krave; pasmine goveda; proizvodnja mlijeka; toplinski stres; podaci na kontrolni dan

References

1. Allen, J.D., Hall, L.W., Collier, R.J., Smith, J.F. (2015): Effect of core body temperature, time of day, and climate conditions on behavioural patterns of lactating dairy cows experiencing mild to moderate heat stress. *Journal of Dairy Science* 98 (1), 118-127.
<https://doi.org/10.3168/jds.2013-7704>
2. Anderson, S.D., Bradford, B.J., Harner, J.P., Tucker, C.B., Choi, C.Y., Allen, J.D., Hall, L.W., Rungruang, S., Collier, R.J., Smith, J.F. (2013): Effects of adjustable and stationary fans with misters on core body temperature and lying behavior of lactating dairy cows in a semiarid climate. *Journal of Dairy Science* 96 (7), 4738-4750.
<https://doi.org/10.3168/jds.2012-6401>
3. Atakan, K., Veysel, A.Ü. (2019): Monthly changes of behavioral characteristics in holstein-friesian, brown swiss and simmental bulls. *Animal Science. Series D. LXII* (1).
4. Bakony, M., Jurkovich, V. (2020) Heat stress in dairy calves from birth to weaning. *Journal of Dairy Research*, 87 (S1), 53-59.
<https://doi.org/10.1017/s0022029920000618>
5. Bernabucci, U., Lacetera, N., Baumgard, L.H., Rhoads, R.P., Ronchi, B., Nardone, A. (2010): Metabolic and hormonal acclimation to heat stress in domesticated ruminants. *Animal* 4, (7) 1167-1183.
<http://dx.doi.org/10.1017/s175173111000090x>
6. Berry, I.L., Shanklin, M.D., Johnson, H.D. (1964): Dairy shelter design based on milk production decline as affected by temperature and humidity. *Transaction of Am. Soc. Ag. Eng.* 7 (3) 329-331.
<https://doi.org/10.13031/2013.40772>
7. Biffani, S., Bernabucci, U., Vitali, A., Lacetera, N., Nardone, A. (2016): Short communication: Effect of heat stress on nonreturn rate of Italian Holstein cows. *Journal of Dairy Science*, 99 (7), 5837-5843.
<https://doi.org/10.3168/jds.2015-10491>
8. Bohmanova, J., Misztal I., Tsuruta S., Norman H.D., Lawlor T.J. (2008): Short communication: Genotype by environment interaction due to heat stress. *Journal of Dairy Science* 91 (2), 840-846.
<https://doi.org/10.3168/jds.2006-142>
9. Bohmanova, J., Misztal, I., Cole, J. (2007): Temperature humidity Indices as Indicators of Milk Production Losses due to Heat Stress. *Journal of Dairy Science* 90 (4), 1947-1956.
<https://doi.org/10.3168/jds.2006-513>
10. Bouraoui, R., Lahmar, M., Majdoub, A., Djemali, M., Belyea, R (2002): The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate. *Animal Research* 51 (6), 479-491.
<https://doi.org/10.1051/animres:2002036>
11. Bruügemann, K., Gernand, E., von Borstel, U.K., König, S. (2012): Defining and evaluating heat stress thresholds in different dairy cow production systems. *Archives Animal Breeding* 55 (1), 13-24.
<https://doi.org/10.5194/aab-55-13-2012>
12. Carabaño, M.J., Logar, B., Bormann, J., Minet, J., Vanrobays, M.-L., Díaz, C., Tychon, B., Gengler, N., Hammami, H. (2016): Modeling heat stress under different environmental conditions. *Journal of Dairy Science* 99 (5), 3798-3814.
<https://doi.org/10.3168/jds.2015-10212>
13. Carabaño, M.J., Ramon, M., Menéndez-Buxadera, A., Molina, A., Díaz, C. (2019): Selecting for heat tolerance. *Animal Frontiers* 9 (1), 62-68.
<https://doi.org/10.1093/af/vfy033>
14. Cardoso, C.S., von Keyserlingk, M.A.G., Filho, L.C.P.M., Hötzel, M.J. (2021): Dairy heifer motivation for access to a shaded area. *Animals* 11 (9), 2507.
<https://doi.org/10.3390/ani11092507>
15. Casa, A.C., Ravelo, A.C. (2003): Assessing temperature and humidity conditions for dairy cattle in Córdoba, Argentina. *International Journal of Biometeorology* 48 (1), 6-9.
<https://doi.org/10.1007/s00484-003-0179-x>

16. Chavez, M.I., García, J.E., Véliz, F.G., Gaytán, L.R., de Santiago, A., Mellado, M. (2020): Effects of in utero heat stress on subsequent reproduction performance of first-calf Holstein heifers. *Spanish Journal of Agricultural Research* 18 (2), e0404.
<https://doi.org/10.5424/sjar/2020182-15721>
17. Cincović, M., Belić, B. (2011): Metabolička adaptacija na peripartalni i toplotni stress kod mlečnih krava. *Veterinarski žurnal Republike Srpske* 11, 155-159.
18. Collier, R.J., Gebremedhin, K.G. (2015): Thermal biology of domestic animals. *Annual Review Animal Biosciences* 3 (1) 513-532.
<https://doi.org/10.1146/annurev-animal-022114-110659>
19. Collier, R.J., Hall, L.W., Rungruang, S., Zimbleman, R.B. (2012): Quantifying heat stress and its impact on metabolism and performance. *Department of Animal Sciences University of Arizona* 68 (1), 1-11
<https://animal.ifas.ufl.edu/apps/dairymedia/rms/2012/6CollierRNS2012a.pdf>
20. Collier, R., Renquist, B., Xiao, Y. (2017): A 100-Year Review: Stress physiology including heat stress. *Journal of Dairy Science* 100 (12), 10367-10380.
<https://doi.org/10.3168/jds.2017-13676>
21. Collier, R.J., Baumgard, L.H., Zimbleman, R.B., Xiao, Y. (2019): Heat stress: physiology of acclimation and adaptation. *Animal Frontiers* 9 (1), 12-19.
<https://doi.org/10.1093/af/vfy031>
22. Cowley, F.C., Barber, D.G., Houlihan, A.V., Poppi, D.P. (2015): Immediate and residual effects of heat stress and restricted intake on milk protein and casein composition and energy metabolism. *Journal of Dairy Science* 98 (4), 2356-2368.
<https://doi.org/10.3168/jds.2014-8442>
23. Das, R., Sailo, L., Verma, N., Bharti, P., Saikia, J., Imtiwati, Kumar, R. (2016): Impact of heat stress on health and performance of dairy animals: A review. *Veterinary World* 9 (3), 260-268.
<https://doi.org/10.14202/vetworld.2016.260-268>
24. Dikmen, S., Hansen, P.J. (2009): Is the temperature-humidity index the best indicator of heat stress in lactating dairy cows in a subtropical environment? *Journal of Dairy Science* 92 (1), 109-116.
<https://doi.org/10.3168/jds.2008-1370>
25. Dunn R.J.H., Mead N.E., Willett K.M., Parker D.E. (2014): Analysis of heat stress in UK dairy cattle and impact on milk yields. *Environmental Research Letters* 9 (6), 064006.
<https://doi.org/10.1088/1748-9326/9/6/064006>
26. Ekine-Dzivenu, C.C., Mrode, R., Oyieng, E., Komwihangilo, D., Lyatuu, E., Msuta, G., Ojango, J.M.K., Okeyo, A.M. (2020): Evaluating the impact of heat stress as measured by temperature-humidity index (THI) on test-day milk yield of small holder dairy cattle in a sub-Saharan African climate. *Livestock Science* 242, 104314.
<https://doi.org/10.1016/j.livsci.2020.104314>
27. Fiore, G., Natale, F., Hoffherr, J., Mainetti, S., Ruotolo, E. (2009): Study on temperatures during animal transport, final report. *JRC Scientific and Technical Reports. Luxembourg*, 1-6.
28. Freitas, M., Misztal, I., Bohmanova, J., Torres, R. (2006): Regional differences in heat stress in US Holsteins. Proceedings of the 8th World Congress on Genetics Applied to Livestock Production 01-11. 13-18 august 2006. Instituto Prociencia, Belo Horizonte, Brazil. ISBN 8560088016, RN 20063169278
29. Gaafar, H.M.A., Gendy, M.E., Bassiouni, M.I., Shamiah, S.M., Halawa, A.A., Hamd, M.A. (2011): Effect of heat stress on performance of dairy Friesian cow's milk production and composition. *Researcher* 3(5), 85-93.
30. Gantner, V., Mijić, P., Kuterovac, K., Solić, D., Gantner, R. (2011): Temperature-humidity index values and their significance on the daily production of dairy cattle. *Mljekarstvo* 61 (1), 56-63.
31. Gantner, V., Bobić, T., Gantner, R., Gregić, M., Kuterovac, K., Novaković, J., Potocnik, K. (2017): Differences in response to heat stress due to production level and breed of dairy cows. *International journal of biometeorology* 61 (9) 1675-1685.
<https://doi.org/10.1007/s00484-017-1348-7>
32. Garner, J.B., Douglas, M.L., Williams, S.R., Wales, W.J., Marett, L.C., Nguyen, T.T.T., Reich C.M., Hayes B.J. (2017): Genomic selection improves heat tolerance in dairy cattle. *Scientific Reports* 6 and 7.
<https://doi.org/10.1038/srep39896>

33. Hall, L.W., Dunshea, F.R., Allen, J.D., Rungruang, S., Collier, J.L., Long, N.M., Collier, R.J. (2016): Evaluation of dietary betaine in lactating Holstein cows subjected to heat stress. *Journal of Dairy Science* 99 (12) 9745-9753.
<https://doi.org/10.3168/jds.2015-10514>
34. Hall, L.W., Villar, F., Chapman, J.D., McLean, D.J., Long, N.M., Xiao, Y., Collier, J.L., Collier, R.J. (2018): An evaluation of an immunomodulatory feed ingredient in heat-stressed lactating Holstein cows: Effects on hormonal, physiological, and production responses. *Journal of Dairy Science* 101, 7095-7105.
<https://doi.org/10.3168/jds.2017-14210>
35. Hammami, H., Bormann, J., M'Hamdi, N., Montaldo, H.H., Gengler, N. (2013): Evaluation of heat stress effects on production traits and somatic cell score of Holsteins in a temperate environment. *Journal of Dairy Science* 96 (3) 1844-1855.
<https://doi.org/10.3168/jds.2012-5947>
36. Hammami, H., Vandenplas, J., Vanrobays, M.-L., Rekik, B., Bastin, C., Gengler, N. (2015): Genetic analysis of heat stress effects on yield traits, udder health, and fatty acids of Walloon Holstein cows. *Journal of Dairy Science* 98 (7), 4956-4968.
<https://doi.org/10.3168/jds.2014-9148>
37. Herbut, P., Angrecka, S. (2012): Forming of temperature-humidity index (THI) and milk production of cows in the free-stall barn during the period of summer heat. *Animal Science Papers and Reports* 30 (4), 363-372.
38. Joksimović-Todorović, M., Davidović, V., Hristov, S., Stanković, B. (2011): Effect of heat stress on milk production in dairy cows. *Biotechnology in Animal Husbandry* 27 (3), 1017-1023.
39. Kadzere, C.T., Murphy, M.R., Silanikove, N., Maltz, E. (2002): Heat stress in lactating dairy cows: a review. *Livestock Production Science* 77 (1), 59-91.
[https://doi.org/10.1016/S0301-6226\(01\)00330-X](https://doi.org/10.1016/S0301-6226(01)00330-X)
40. Kibler, H.H. (1964): Thermal effects of various temperature-humidity combinations on Holstein cattle as measured by eight physiological responses. Environmental physiology and shelter engineering. *Agricultural Experiment Station, Missouri*, pp. 1-42
41. Knob, D.A., Neto, A.T., Schweizer, H., Weigand, A., Kappes, R., Scholz, A.M. (2021): Energy balance indicators during the transition period and early lactation of purebred Holstein and Simmental cows and their crosses. *Animals* 11 (2), 309.
<https://doi.org/10.3390/ani11020309>
42. Lambertz, C., Sanker, C., Gauly, M. (2014): Climatic effects on milk production traits and somatic cell score in lactating Holstein-Friesian cows in different housing systems. *Journal of Dairy Science* 97 (1) 319-329.
<https://doi.org/10.3168/jds.2013-7217>
43. Luo, H., Brito, L.F., Li, X., Su, G., Dou, J., Xu, W., Yan, X., Zhang, H., Guo, G., Liu, L., Wang, Y. (2021): Genetic parameters for rectal temperature, respiration rate, and drooling score in Holstein cattle and their relationships with various fertility, production, body conformation, and health traits. *Journal of Dairy Science* 104 (4) 4390-4403,
<https://doi.org/10.3168/jds.2020-19192>
44. Maggiolino, A., Dahl, G., Bartolomeo, N., Bernabucci, U., Vitali, A., Serio, G., Cassandro, M., Centoducati, G., Santus, E., De Palo, P. (2020): Estimation of maximum thermo-hygrometric index thresholds affecting milk production in Italian Brown Swiss cattle. *Journal of Dairy Science* 103 (9), 8541-8553.
<https://doi.org/10.3168/jds.2020-18622>
45. McDowell, R.E., Hooven, N.W., Camoens, J.K. (1976): Effect of Climate on Performance of Holsteins in First Lactation. *Journal of Dairy Science* 59 (5), 965-971.
[https://doi.org/10.3168/jds.S0022-0302\(76\)84305-6](https://doi.org/10.3168/jds.S0022-0302(76)84305-6)
46. Mylostyyvi, R., Lesnovskay, O., Karlova, L., Khmeleva, O., Kalinichenko, O., Orishchuk, O., Tsap, S., Begma, N., Cherniy, N., Gutyj, B., Izhboldina, O. (2021): Brown Swiss cows are more heat resistant than Holstein cows under hot summer conditions of the continental climate of Ukraine. *Journal of Animal Behaviour and Biometeorology* 9 (4).
<https://doi.org/10.31893/jabb.21034>
47. Nardone, A., Ronchi, B., Lacetera, N., Ranieri, M.S., Bernabucci, U. (2010): Effects of climate changes on animal production and sustainability of livestock systems. *Livestock Science* 130 (1-3), 57-69.
<https://doi.org/10.1016/j.livsci.2010.02.011>

48. National Research Council (1971): A Guide to Environmental Research on Animals. *National Academia of Science*. Washington, DC
49. Nguyen, T.T.T., Hayes, B.J., Pryce, J.E. (2017): A practical future-scenarios selection tool to breed for heat tolerance in Australian dairy cattle. *Animal Production Science* 57 (7), 1488-1493. <https://doi.org/10.1071/an16449>
50. Nordlund, K.V., Strassburg, P., Bennett, T.B., Oetzel, G.R., Cook, N.B. (2019): Thermodynamics of standing and lying behavior in lactating dairy cows in freestall and parlor holding pens during conditions of heat stress. *Journal of Dairy Science* 102 (7), 6495-6507. <https://doi.org/10.3168/jds.2018-15891>
51. Ouellet, V., Bellavance, A.L., Fournel, S., Charbonneau, E. (2019): Short communication: Summer on-farm environmental condition assessments in Québec tiestall farms and adaptation of temperature-humidity index calculated with local meteorological data. *Journal of Dairy Science* 102 (8), 7503-7508. <https://doi.org/10.3168/jds.2018-16159>
52. Petrović, M.D., Bogdanović, V., Petrović, M.M., Bogosavljević-Bošković, S., Đoković, R., Đedović, R., Rakonjac, S. (2015): Effect of non-genetic factors on standard lactation milk performance traits in simmental cows. *Annals of Animal science* 15 (1) 211-220. <https://doi.org/10.2478/aoas-2014-0073>
53. Polsky, L., von Keyserlingk, M.A.G. (2017): Invited review: Effects of heat stress on dairy cattle welfare. *Journal of Dairy Science* 100 (11), 8645-8657. <https://doi.org/10.3168/jds.2017-12651>
54. Popovac, M., Miletić, A., Raguž, N., Beskorovajni, R., Stanojević, D., Radivojević, M., Mičić, N., Djurić, N. (2020): Phenotypic and genetic parameters of milk yield traits in first-calf heifers of Holstein-Friesian breed. *Mljekarstvo* 70 (2) 93-102. <https://doi.org/10.15567/mljekarstvo.2020.0203>
55. Ramón-Moragues, A., Carulla, P., Mínguez, C., Villagrà, A., Estellés, F. (2021): Dairy cows activity under heat stress: A case study in Spain. *Animals* 11 (8), 2305. <https://doi.org/10.3390/ani11082305>
56. Rauba, J., Heins, B., Chester-Jones, H., Diaz, H., Ziegler, D., Linn, J., Broadwater, N. (2019): Relationships between protein and energy consumed from milk replacer and starter and calf growth and first-lactation production of Holstein dairy cows. *Journal of Dairy Science* 102 (1), 301-310. <https://doi.org/10.3168/jds.2018-15074>
57. Ravagnolo, O., Misztal, I. (2000): Genetic component of heat stress in dairy cattle, parameter estimation *Journal of Dairy Science* 83 (9), 2126-2130. [https://doi.org/10.3168/jds.s0022-0302\(00\)75095-8](https://doi.org/10.3168/jds.s0022-0302(00)75095-8)
58. Ravagnolo, O., Misztal, I., Hoogenboom, G. (2000): Genetic component of heat stress in dairy cattle, development of heat index function. *Journal of Dairy Science* 83 (9), 2120-2125. [https://doi.org/10.3168/jds.s0022-0302\(00\)75094-6](https://doi.org/10.3168/jds.s0022-0302(00)75094-6)
59. Renna, M., Lussiana, C., Malfatto, V., Mimosi, A., Battagliani, L.M. (2010): Effect of exposure to heat stress conditions on milk yield and quality of dairy cows grazing on Alpine pasture. In *Proceedings of 9th European IFSA Symposium (pp. 1338-1348)*. Vienna, Austria
60. Sahin, E., Ugurlu, N. (2017): Effects of heat stress on dairy cattle. *Eurasian Journal of Agricultural Research* 1 (1), 37-43. <https://dergipark.org.tr/en/pub/ejar/issue/37324/431530>
61. Shock, D.A., LeBlanc, S.J., Leslie, K.E., Hand, K., Godkin, M.A., Coe, J.B., Kelton, D.F. (2016): Studying the relationship between on-farm environmental conditions and local meteorological station data during the summer. *Journal of Dairy Science* 99 (3), 2169-2179. <https://doi.org/10.3168/jds.2015-9795>
62. Shu, H., Wang, W., Guo, L., Bindelle, J. (2021): Recent advances on early detection of heat strain in dairy cows using animal-based indicators: A review. *Animals* 11 (4), 980. <https://doi.org/10.3390/ani11040980>
63. Smith, D.L., Smith, T., Rude, B.J., Ward, S.H. (2013): Comparison of the effects of heat stress on milk and component yields and somatic cell score in Holstein and Jersey cows. *Journal of Dairy Science* 96 (5) 3028-3033. <https://doi.org/10.3168/jds.2012-5737>

64. Soyeurt, H., Dehareng, F., Gengler, N., McParland, S., Wall, E.P.B.D., Berry, D.P., Coffey, M., Dardenne, P. (2011): Mid-infrared prediction of bovine milk fatty acids across multiple breeds, production systems, and countries. *Journal of Dairy Science* 94 (4), 1657-1667, <https://doi.org/10.3168/jds.2010-3408>
65. Stoop, W.M., Bovenhuis, H. Heck, J.M.L., Van Arendonk, J.A.M. (2009): Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. *Journal of Dairy Science* 92 (4), 1469-1478. <https://doi.org/10.3168/jds.2008-1468>
66. Strączek, I., Młynek, K., Danielewicz, A. (2021): The capacity of holstein-friesian and simmental cows to correct a negative energy balance in relation to their performance parameters, course of lactation, and selected milk components. *Animals* 11 (6), 1674. <https://doi.org/10.3390/ani11061674>
67. Summer, A., Lora, I., Formaggioni, P., Gottardo, F. (2019): Impact of heat stress on milk and meat production. *Animal Frontiers* 9 (1), 39-46. <https://doi.org/10.1093/af/vfy026>
68. Tamami, F.Z., Hafezian, H., Rahimi-Mianji, G., Abdollahpour, R., Gholizadeh, M. (2018): Effect of the temperature-humidity index and lactation stage on milk production traits and somatic cell score of dairy cows in Iran. *Songklanakarinn Journal of Science Technol.* 40 (2), 379-383. <https://doi.org/10.14456/sjst-psu.2018.36>
69. Thom, E.C. (1958): Cooling degree days. Air Conditioning, Heating and Ventilating. US Department of commerce 55, 65-69.
70. Vučković, G., Bobić, T., Mijić, P., Gavran, M., Gregić, M., Potočnik, K., Bogdanović, V., Gantner, V. (2020) Genetic parameters and breeding values for daily milk production of Holstein cows in terms of heat stress. *Mljekarstvo* 70 (3), 201-209. <https://doi.org/10.15567/mljekarstvo.2020.0306>.
71. Vujanac I. (2010): Ispitivanje funkcionalnog stanja endokrinog pankresa kod visoko mlečnih krava u različitim uslovima spoljašnje temperature. *Doktorska disertacija*. Fakultet veterinarske medicine, Beograd.
72. Wang, X., Bjerg, B.S., Choi, C.Y., Zong, C., Zang, G. (2018): A review and quantitative assessment of cattle-related thermal indices. *Journal of Thermal Biology* 77, 24-37. <https://doi.org/10.1016/j.jtherbio.2018.08.005>
73. Wang, J., Li, J., Wang, F., Xiao, J., Wang, Y., Yang, H., Li, S., Cao, Z. (2020): Heat stress on calves and heifers: a review. *Journal of Animal Science and Biotechnology* 11 (1), 1-8. <https://doi.org/10.1186/s40104-020-00485-8>
74. Wheelock, J.B., Rhoads R.P., VanBaale M.J., Sanders S.R., Baumgard L.H. (2010): Effects of heat stress on energetic metabolism in lactating Holstein cows. *Journal of Dairy Science* 93 (2), 644-655. <https://doi.org/10.3168/jds.2009-2295>
75. Wildridge, A.M., Thomson, P.C., Garcia, S.C., John, A.J., Jongman, E.C., Clark, C.E.F., Kerrisk, K.L. (2018): Short communication: The effect of temperature-humidity index on milk yield and milking frequency of dairy cows in pasture-based automatic milking systems. *Journal of Dairy Science*, 101 (5), 4479-4482. <https://doi.org/10.3168/jds.2017-13867>
76. Woolpert, M.E., Dann, H. M., Cotanch, K.W., Melilli, C., Chase, L.E., Grant, R.J., Barbano, D.M. (2016): Management, nutrition, and lactation performance are related to bulk tank milk de novo fatty acid concentration on northeastern US dairy farms. *Journal of Dairy Science* 99 (10), 8486-8497. <https://doi.org/10.3168/jds.2016-10998>
77. Xiong, Q., Chai, J., Xiong, H., Li, W., Huang, T., Liu, Y., Suo, X., Zhang, N., Li, X., Jiang, S., Chen, M. (2013): Association analysis of HSP70A1A haplotypes with heat tolerance in Chinese Holstein cattle. *Cell Stress Chaperones* 18 (6), 711-718. <https://doi.org/10.1007/s12192-013-0421-3>
78. Yan, G., Li, H., Shi, Z. (2021): Evaluation of thermal indices as the indicators of heat stress in dairy cows in a temperate climate. *Animals* 11 (8), 2459, <https://doi.org/10.3390/ani11082459>
79. Zimbelman, R.B., Baumgard, L.H., Collier, R.J. (2010): Effects of encapsulated niacin on evaporative heat loss and body temperature in moderately heat-stressed lactating Holstein cows. *Journal of Dairy Science* 93 (6), 2387-2394. <https://doi.org/10.3168/jds.2009-2557>.