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The effects of heat stress in Jersey, Hungarian Simmental and Holstein-Friesian cows

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ABSTRACT. The effects of heat stress on the concentration of 70 kDa heat shock protein (HSP70) in plasma and saliva, plasma insulin concentration and some metabolic indices (plasma glucose, free fatty acids, beta-hydroxybutyrate and urea concentrations) were assessed in Holstein-Friesian, Hungarian Simmental and Jersey cows. The study included 30 animals from a farm breeding Holstein-Friesians, 30 from a Jersey farm and 30 from a farm keeping Simmentals (10 dry cows, 10 mid-lactating and 10 lactating cows from each farm, respectively). Sampling was performed under thermoneutral (spring) and heat stress (summer) conditions. Based on plasma HSP70 and insulin concentrations, Holstein cows were determined to be most susceptible to heat stress. No characteristic breed-related changes in metabolic indices were found. The results indicated the importance of heat load-reducing strategies in the Holstein-Friesian breed.

Introduction

Heat stress causes significant intracellular and organizational changes that help animals adapt to altered conditions (Bakony et al., 2019). Due to climate change, average daily temperatures have risen significantly in recent years in the spring and summer months, and forecasts point to further increases (Solymosi et al., 2010). Thus, the number of days when only continuous and efficient cooling of stables can ensure an optimal thermal environment for high-yielding dairy cows is increasing (Fournel et al., 2017). An increase in body temperature causes changes at the cellular and systemic level that reduce the intensity of metabolic and production processes associated with heat generation in the short and long term (homeoretic adaptation). Heat shock proteins (HSP) are produced to prevent cellular damage caused by several stressors, including rising temperatures. HSP can be grouped according to their molecular weight, with the production of 70 kDa HSP (HSP70) being the most significant under heat stress conditions (Kiang and Tsokos, 1998).

There are several other metabolic and hormonal changes that help cows perform better under warm environmental conditions. Cows exposed to heat stress have increased basal insulin concentration and insulin sensitivity despite reduced dry matter intake (Itoh et al., 1998; Wheelock et al., 2010). Enhanced insulin action limits the amount of glucose available to the mammary gland by reducing fat breakdown and increasing cellular glucose uptake. Peripheral tissues utilise glucose rather than free fatty acids as a source of energy (Wheelock et al., 2010; Baumgard et al., 2011; Min et al., 2017), and in consequence, the concentration of free fatty acids decreases in blood plasma (Calamari et al., 2011). Increased breakdown of muscle proteins (glycoplastic amino acids) may also partly contribute to higher glucose formation, which can be inferred from the observed elevated plasma urea concentration under heat stress (Schwartz et al., 2009).

Earlier findings have suggested that high-producing cows are more susceptible to heat stress than low-yielding ones (Kadzere et al., 2002; Collier et al., 2006; Hansen, 2013). Breeds with higher milk yields produce more metabolic heat, which reduces the cow's ability to maintain normal temperature under heat stress conditions. Most heat stress studies used Holstein cows, and only a few works examined milk production in Jersey cows in terms of heat stress (Harris et al., 1960; Collier et al., 1981; Smith et al., 2013). Higher resistance to heat stress in Simmentals than Holsteins was demonstrated by determining the temperature-humidity index (THI) threshold for daily milk yield, fat and protein contents (Gantner et al., 2017a), as well as the analysis of daily milk yield and somatic cell count (Gantner et al., 2017b).

Based on the above findings, our study aimed to compare the expected changes in HSP70 and insulin concentrations and certain metabolic parameters in the Jersey, Holstein-Friesian and Hungarian Simmental breeds during thermoneutral and heat stress periods in Hungary under field conditions.

Material and methods

All animal handling and sampling methods were in accordance with the ethical permission of the Scientific Ethics Board for Animal Experiments, and issued by the Pest County Government Office, Budapest, Hungary (permit No.: PEI/001/3721-4/2015).

For this study, one farm breeding Holstein-Friesians (HF; Pusztaszabolcs, Hungary, 47°08'19.5"N 18°44'20.9"E), one farm breeding Hungarian Simmentals (hereafter referred to as Simmental (HS); Tevel, Hungary, 46°25'49.8"N 18°27'16.8"E) and one breeding Jersey cattle (Csengersima, Hungary, 47°51'35.4"N 22°43'25.4"E) were selected; farm data are summarized in Table 1. We selected 30 clinically healthy HF, HS or Jersey cows per herd from the selected farms. Ten pre-lactating cows/breed, maximum two weeks before the expected calving (DRY), ten cows/breed at peak lactation (PEAK), and ten low-yielding cows/breed (LOW) at the end of lactation were enrolled in the study (Table 2). Blood and saliva samplings were conducted once in the spring (March-April) and once in the summer (July–August), in the morning (10–11 am). At the time of spring and summer sampling, the temperature and humidity in the barns were measured for a week using a weather data logger (Voltcraft DL-181THP, Conrad Electronic GmbH, Hirschau, Germany; Table 3). We calculated the temperature-humidity index (THI) according to Bianca (1962) using the following formula:

$$THI = (0{,}15 \times T_{db} + 0{,}85 \times T_{wb}) \times 1{,}8 + 32,$$

where: T_{db} is dry-bulb temperature (°C), and T_{wb} is wet-bulb temperature (°C).

Blood samples were taken from caudal vessels into heparinized sampling syringes (S-Monovette, Sarstedt GmbH, Nümbrecht, Germany); saliva samples were collected into synthetic rolls (Salivette Cortisol, Sarstedt GmbH, Nümbrecht, Germany). We held the rolls with Collin tongue forceps, put

Table 1. Data of the farms selected for the study

	Breed	Number of cows	Average milk production, kg/305 day lactation	Average milk fat, %	Average milk protein, %
Farm 1	Holstein-Friesian	650	9500	3.7	3.2
Farm 2	Hungarian Simmental	400	6655	3.9	3.4
Farm 3	Jersey	380	6800	4.7	3.7

Table 2. Data of cows in the study

Period	Group	Number of lactations			Days after calving			Rectal temperature, °C		
		HF	Jersey	HS	HF	Jersey	HS	HF	Jersey	HS
Spring	DRY	4.1 ± 0.4	2.2 ± 0.3	3.3 ± 0.3	–	–	–	38.8 ± 0.1[a]	38.9 ± 0.1[a]	38.5 ± 0.1[a]
	PEAK	3.0 ± 0.4	2.1 ± 0.4	2.6 ± 0.3	134 ± 3	155 ± 3	145 ± 3	38.2 ± 0.1	38.3 ± 0.1	38.5 ± 0.1
	LOW	1.9 ± 0.3	1.7 ± 0.3	2.1 ± 0.1	308 ± 14	284 ± 6	283 ± 6	38.7 ± 0.1	38.5 ± 0.1	38.7 ± 0.1
Summer	DRY	2.3 ± 0.4	2.2 ± 0.3	3.0 ± 0.3	–	–	–	39.1 ± 0.1[b]	39.4 ± 0.1[b]	39.1 ± 0.1[b]
	PEAK	3.0 ± 0.5	2.1 ± 0.5	2.3 ± 0.2	151 ± 2	167 ± 3	132 ± 2	38.4 ± 0.1[1]	38.8 ± 0.1[2]	38.8 ± 0.1[2]
	LOW	2.7 ± 0.2	2.3 ± 0.3	2.4 ± 0.3	294 ± 5	273 ± 9	277 ± 4	38.5 ± 0.1	38.7 ± 0.1	38.9 ± 0.1

HF – Holstein Friesian, HS – Hungarian Simmental, DRY – pre-lactating cows maximum two weeks prior to the expected calving, PEAK – cows at peak lactation, LOW – low yielding cows at the end of lactation; data are presented as mean value ± SE; [ab] superscript letters indicate a significant difference between thermoneutral and heat stress periods within a breed ($P < 0.05$), [1,2] superscript numbers indicate significant differences between breeds and within groups in the heat stress period ($P < 0.05$)

them into the cow's mouth between the molars and bucca when the cows were not ruminating to avoid sample contamination. The cows were allowed to chew on the rolls for approximately 20 s. Both the Salivettes and sampling syringes were immediately placed on ice and transported to the laboratory.

At the time of blood samplings, the cows' rectal temperature was also measured using a digital thermometer (VT 1831, Microlife AG, Widnau, Switzerland).

Blood samples were centrifuged (10 min, 3000 *g*), and the plasma was subsequently separated and stored at −20 °C for further analysis. The Salivettes were centrifuged (10 min, 3000 *g*) to collect saliva from the synthetic rolls. Saliva samples were stored at −20 °C for further analysis.

Plasma and saliva samples were assayed for HSP70 concentration using ELISA (SEA873Mi, Cloud-Clone Corp., Katy, TX, USA; sensitivity: 0.56 ng/ml; intra-assay CV: < 10%; interassay CV: < 12%) according to the manufacturer's instructions. Plasma insulin levels were determined using the RIA method (RK-4000CT, Institute of Isotopes, Budapest, Hungary; sensitivity: 1.71 µIU/ml; intra-assay CV: < 5%; interassay CV: < 5%); plasma glucose, beta-hydroxybutyrate (BHB) and urea concentrations were measured using an A-25 analyzer (Biosystems S.A., Barcelona, Spain) and colourimetric assay kits (DCC430100, Diagon Ltd., Budapest, Hungary; 10121 Glucose liquicolor and 10521 Urea liqui UV by Medi-Lab Ltd., Budapest, Hungary, respectively). The concentration of non-esterified fatty acid (NEFA) in plasma samples was measured by the method of Noma et al. (1973) using a Unicam Helios Gamma photometer equipped with automatic samplers (Unicam Ltd., London, UK).

Statistical analysis

Mean values of milk yield, milk composition, heat shock protein concentration and metabolic parameters were compared between different breeds and thermal conditions (spring vs summer) at three production levels (dry period, peak lactation, low milk production). After thorough investigation of their origin, outliers (one value in plasma HSP and salivary HSP) were excluded from the analysis.

Since the experimental design allowed for repeated sampling from the same animal, the correlation of such measures was taken into account by fitting linear mixed models with individual numerical parameters as a response variable; breed, thermal condition, production and their two- and three-way interactions were applied as explanatory variables, and cow ID as a random effect, adding a random term to the intercept. Model selection was performed by removing insignificant interaction terms from the model and minimising the Bayesian information Criterion value. Comparisons between breeds and thermal conditions were performed for each production level by post-hoc multiple comparisons. *P*-values were adjusted according to the method of Bonferroni and Holm (Holm, 1979). The level of significance was set at $P < 0.05$.

The R statistical software was used for all data visualization, estimations and hypothesis testing (R Core Team, 2022). The 'lme4' (Bates et al., 2015) and 'emmeans' packages (Russell, 2022) were used for fitting linear mixed models and multiple comparisons.

Results

Regarding the microclimate data in the barns, temperature and THI were higher in summer than spring on all farms. At the same time, the THI values did not differ between the farms in spring or summer (Table 3).

Table 3. Temperature, relative humidity and temperature-humidity index (THI) at the time of samplings on different farms

Period	Average temperature, °C			Average relative humidity, RH%			Average, THI		
	HF	Jersey	HS	HF	Jersey	HS	HF	Jersey	HS
Spring	8.8	14.9	10.7	74.1	59.7	45.9	43.9	51.8	43.1
Summer	27.4	24.1	24.9	61.9	74.4	69.3	72.1	70.0	70.1

HF – Holstein-Friesian, HS – Hungarian Simmental

Table 2 shows the data of the animals enrolled in the study (lactation number, lactation days and rectal temperature). In the DRY group, rectal temperatures were higher in all breeds during heat stress. Average rectal temperatures were higher at peak lactation in Jersey and Simmental than in Holstein-Friesian.

Milk yield of Holstein-Friesian cows decreased significantly during heat stress in PEAK, while such a decrease was not observed in Jersey and Simmental cows (Table 4). Milk yield was unchanged in the LOW groups in all breeds when comparing heat stress and spring period. Fat content in milk of Simmental cows was lower in PEAK under heat

Table 4. Milk yield and milk ingredients of cows in the study

Period	Group	Milk yield, l/day			Milk fat, %			Milk protein, %		
		HF	Jersey	HS	HF	Jersey	HS	HF	Jersey	HS
Spring	DRY	–	–	–	–	–	–	–	–	–
	PEAK	36.8 ± 3.7[a]	23.5 ± 4.3	29.0 ± 3.5	4.1 ± 0.2[2]	5.7 ± 0.3[1]	4.0 ± 0.2[a2]	3.3 ± 0.1[a1]	4.0 ± 0.1[2]	3.2 ± 0.1[1]
	LOW	19.6 ± 3.2	18.8 ± 4.3	12.6 ± 3.5	4.4 ± 0.2[2]	5.5 ± 0.4[1]	4.1 ± 0.2[2]	3.6 ± 0.1[a1]	4.0 ± 0.1[2]	3.4 ± 0.1[1]
Summer	DRY	–	–	–	–	–	–	–	–	–
	PEAK	27.6 ± 3.1[b]	25.6 ± 4.6	29.3 ± 3.0	4.1 ± 0.2[2]	5.2 ± 0.2[1]	3.1 ± 0.3[b2]	3.1 ± 0.1[b1]	4.1 ± 0.1[2]	3.3 ± 0.1[1]
	LOW	19.9 ± 4.2	12.3 ± 4.6	19.0 ± 7.5	4.5 ± 0.2[2]	5.4 ± 0.2[1]	4.2 ± 0.1[2]	3.4 ± 0.1[b1]	4.0 ± 0.1[2]	3.5 ± 0.1[1]

HF – Holstein Friesian, HS – Hungarian Simmental, DRY – pre-lactating cows maximum two weeks prior to the expected calving, PEAK – cows at peak lactation, LOW – low yielding cows at the end of lactation; data are presented as mean value ± SE; [ab] superscript letters in the upper case indicate a significant difference between thermoneutral and heat stress periods within a breed in a given production group ($P < 0.05$), [1,2] superscript numbers indicate significant differences between breeds in a given production group in thermoneutral and heat stress periods ($P < 0.05$)

stress than in spring. In Jersey, milk fat was higher in both groups compared to Holstein-Friesian and Simmental, also in spring and summer. Milk protein content was lower in the case of Holstein-Friesian under heat stress also in PEAK and LOW compared to that during spring. In Jersey, milk protein content was higher in both PEAK and LOW than in the other breeds in all season.

There were no differences in plasma HSP70 concentrations between the breeds during spring. At the same time, HSP70 level was lower in Holstein-Friesian cows under heat stress both in PEAK and LOW than in the other two breeds (Table 5). There was no difference between the Jersey and Simmental breeds regarding plasma HSP70 levels during heat stress. HSP content in saliva of the Holstein-Friesian breed was lower in DRY, and tended to be lower in PEAK and LOW under heat stress conditions compared to spring. Saliva HSP concentration was lower in Holstein-Friesian and Jersey under heat stress in all groups compared to Simmental. There were no differences in saliva HSP70 concentrations between the Jersey and Holstein-Friesian breeds under heat stress conditions.

Table 5. Changes in plasma and salivary HSP70 concentrations

Item	Group	HF		Jersey		HS	
		spring	summer	spring	summer	spring	summer
Plasma HSP70, ng/ml	DRY	7.6 ± 2.0	5.1 ± 1.1[1]	15.3 ± 1.7	19.8 ± 1.9[2]	13.4 ± 1.2	16.3 ± 3.0[2]
	PEAK	10.0 ± 0.7[a]	4.1 ± 0.6[b1]	20.3 ± 2.3	19.1 ± 1.6[2]	17.4 ± 1.9	12.1 ± 1.7[2]
	LOW	14.8 ± 1.8[a]	4.1 ± 0.6[b1]	17.6 ± 2.2	15.9 ± 2.3[2]	16.5 ± 0.9	17.5 ± 4.1[2]
Saliva HSP70, ng/ml	DRY	36.3 ± 3.8[a]	8.9 ± 1.7[b1]	19.1 ± 3.7	13.4 ± 3.9[1]	47.7 ± 10.3	37.9 ± 5.8[2]
	PEAK	18.1 ± 2.8	9.6 ± 2.4[1]	18.6 ± 3.9[a]	3.5 ± 0.4[b1]	39.8 ± 10.7	54.6 ± 9.9[2]
	LOW	29.5 ± 2.8	19.7 ± 4.7[1]	13.8 ± 3.7	10.8 ± 7.2[1]	45.2 ± 10.0	58.0 ± 12.4[2]

HF – Holstein Friesian, HS – Hungarian Simmental, DRY – pre-lactating cows maximum two weeks prior to the expected calving, PEAK – cows at peak lactation, LOW – low yielding cows at the end of lactation, HSP70 – 70 kDa heat shock protein; [ab] superscript letters indicate a significant difference between thermoneutral and heat stress periods within a breed and a given production group ($P < 0.05$); [1,2] superscript numbers indicate significant differences between breeds in the heat stress period in a given production group ($P < 0.05$); data are presented as mean value ± SE

Table 6 presents selected metabolic parameters (glucose, BHB, NEFA and urea) and insulin concentration in plasma samples of cows. Under heat stress, higher insulin levels were observed both in the PEAK and LOW groups of all breeds. Plasma glucose concentration was lower in Holstein-Friesian in the DRY group under heat stress, while in PEAK, it was lower in Jersey. Plasma glucose concentration was lower in the DRY group and higher in the PEAK group under heat stress in case of Hungarian Simmental. Plasma BHB level was higher in the PEAK group under heat stress in Simmental breed. We did not find differences in plasma BHB concentrations in any other group. Plasma NEFA levels in Jersey were higher in the DRY group during heat stress, while there was no difference in plasma NEFA concentrations in other breeds. Plasma urea concentrations in Holstein-Friesian were lower in all groups under heat stress, while they were elevated in Jersey in all groups under heat stress compared to spring conditions. There were no differences in urea concentrations between the thermoneutral period and heat stress in any of the Simmental groups.

Table 6. Changes in some metabolic indices and the insulin concentrations in the blood plasma of animals in the study

Item	Group	HF		Jersey		HS	
		spring	summer	spring	summer	spring	summer
Glucose, mmol/l	DRY	4.0 ± 0.2[a]	3.3 ± 0.1[b]	4.1 ± 0.1	3.9 ± 0.2	4.0 ± 0.3[a]	3.2 ± 0.2[b]
	PEAK	3.5 ± 0.1	3.7 ± 0.1	4.2 ± 0.1[a]	3.5 ± 0.1[b]	2.3 ± 0.2[a]	3.6 ± 0.1[b]
	LOW	4.0 ± 0.1	3.7 ± 0.1	3.9 ± 0.1	3.6 ± 0.1	3.0 ± 0.3	3.4 ± 0.2
Insulin, µIU/ml	DRY	4.1 ± 1.9	5.3 ± 1.5	6.7 ± 0.9	4.2 ± 1.0	4.7 ± 0.9	3.3 ± 0.4
	PEAK	4.2 ± 0.5[a]	6.5 ± 1.9[b]	4.2 ± 0.4[a]	7.4 ± 1.4[b]	4.6 ± 1.2[a]	7.5 ± 1.7[b]
	LOW	3.7 ± 0.4[a]	5.5 ± 0.9[b]	4.3 ± 0.5[a]	8.8 ± 2.3[b]	5.6 ± 1.2[a]	6.9 ± 0.8[b]
BHB, mmol/l	DRY	0.4 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
	PEAK	0.7 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.5 ± 0.1[a]	0.9 ± 0.1[b]
	LOW	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.8 ± 0.1
NEFA, mmol/l	DRY	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01[a]	0.10 ± 0.04[b]	0.12 ± 0.04	0.08 ± 0.01
	PEAK	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
	LOW	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Urea, mmol/l	DRY	4.5 ± 0.3[a]	3.9 ± 0.4[b]	5.7 ± 0.3[a]	6.3 ± 0.4[b]	4.7 ± 0.4	4.8 ± 0.3
	PEAK	5.2 ± 0.3[a]	3.7 ± 0.3[b]	4.5 ± 0.1[a]	6.0 ± 0.2[b]	6.3 ± 0.4	6.7 ± 0.3
	LOW	4.6 ± 0.1[a]	4.2 ± 0.3[b]	3.7 ± 0.2[a]	5.0 ± 0.2[b]	5.6 ± 0.3	5.9 ± 0.4

HF – Holstein Friesian, HS – Hungarian Simmental, DRY – pre-lactating cows maximum two weeks prior to the expected calving, PEAK – cows at peak lactation, LOW – low yielding cows at the end of lactation, BHB – beta-hydroxybutyrate, NEFA – non-esterified fatty acids; [ab] superscript letters in the upper case indicate a significant difference between thermoneutral and heat stress periods within a breed in a given production group ($P < 0.05$); data are presented as mean value ± SEM

Discussion

Temperature and THI values were higher in summer than in spring, as expected. Summer samplings were performed during a long period of hot weather. The temperature, humidity and THI values in Table 1 refer to the averages of the recording period of 7 days prior to sampling. The THI values indicated no heat stress in spring but significant heat stress in summer. Reiczigel et al. (2009) showed that the formula we used to calculate THI was a reliable indicator of heat stress with a threshold of 68 in Hungary.

For rectal temperature, there are different thresholds described in the literature, depending on the degree of heat load, indicating the presence of heat stress, i.e. 38.8 °C provided by Allen et al. (2015) and 39.2 °C described by Polsky and von Keyserlingk (2017). Based on rectal temperatures, heat stress was detectable in pre-calving cows in our study. Dry and pre-calving cows are often housed in lower quality stalls compared to milk-yielding groups (the number and size of drinkers are generally inadequate, cooling is not as efficient, and the roof is not necessarily insulated). The difference in rectal temperatures highlights the importance of reducing the heat load in pre-parturition cows (Dado-Senn et al., 2020). The biological relevance of the difference in rectal temperature in the PEAK and LOW groups was questionable, mainly due to the fact that neither group showed significant uncompensated heat load.

Milk yield and milk protein content decreased in Holstein-Friesian under heat stress compared to thermoneutral conditions, as previously reported (Silanikove et al., 2009). Milk yield did not differ between summer and spring in the remaining two breeds. Other authors also showed decreased milk production in Holstein-Friesians compared to Jersey (Smith et al., 2013) or Simmental (Gantner et al., 2017b) cows during heat stress. In the study of Smith et al. (2013), milk fat content increased in Holsteins, milk protein content decreased in both Holsteins and Jerseys, although it was only 0.1%. We only found a reduced milk fat content in Simmentals during heat stress.

Despite reduced dry matter intake, basal insulin concentration and insulin sensitivity have been shown to increase in cows exposed to heat stress (Itoh et al., 1998; Wheelock et al., 2010); however, the causes of increased insulin production are not well known. Possible explanations could be high prolactin levels, adiponectin production, the action of heat shock proteins (Collier et al., 2006), and the effect of endotoxins absorbed into the bloodstream due to the leaky gut phenomenon (Rhoads et al., 2009;

Kvidera et al., 2017). Elevated insulin concentrations determine the primary, preferred source of energy for tissues. Some studies considered this to be a specific adaptation to severe heat stress, as using glucose as an energy source produces less heat (15.6 kJ/g) than fats (39.3 kJ/g) or proteins (16.7 kJ/g) during oxidation (Min et al., 2017). Since the phenomenon of increased insulin production due to heat stress has been observed in other animal species and even in lower organisms, it can be considered an ancient survival mechanism (Rhoads et al., 2013). In our study, rising plasma insulin concentrations indicated that all three breeds adapted to heat stress during milk production (Wheelock et al., 2010; Baumgard et al., 2011). Holstein-Friesians had the lowest concentrations of HSP70 for cell protection during heat stress in both plasma and saliva. We found no differences in plasma HSP70 concentrations between Jersey and Simmental cows, although saliva HSP70 levels were the highest in Simmental cows. Gaughan et al. (2013) found that inducible plasma HSP70 concentrations increased in chronic heat stress in Black Angus steers. Heat shock proteins are mainly intracellular (Kregel, 2002); however, several studies have also found extracellular HSPs under stressful conditions (Kristensen et al., 2004; Johnson et al., 2005; Aneja et al., 2006). Earlier studies demonstrated that heat stress tended to increase plasma or serum HSP levels (Gaughan et al., 2013; Shilja et al., 2016; Baek et al., 2019; Kumar et al., 2020). Decreasing plasma HSP70 levels during heat stress in Holstein-Friesian cows observed in our study is difficult to explain. The expression of HSPs has been shown to change during pregnancy (Yániz et al., 2009), after calving (Catalani et al., 2010) or with age (Kristensen et al., 2004). The cows in our study were similar in age and pregnancy status in the DRY, PEAK or LOW groups among breeds and seasons (Table 2). Gaughan et al. (2013) showed that HSP concentrations increased under heat stress, and a decrease occurred after reaching the peak concentration during a long (110 days) heat stress. Nevertheless, these decreased concentrations were higher than the baseline values.

An earlier study has shown that saliva is a possible medium for measuring HSP70 concentrations (Lamy et al., 2017). According to our results, the individual variability in saliva HSP70 levels was very high. It was weakly correlated or not correlated at all with other heat stress indices (rectal temperature or THI), indicating that salivary HSP70 was not the best indicator of heat stress. Interestingly, several other stress factors (e.g. osmolality changes, poisonings, virus infections, hypoxia, free radical load, UV light) have been demonstrated to initiate the production of heat shock proteins to protect the cells (Kregel et al., 2002; Molvarec et al., 2009). However, the production of 70 kDa HSP70 was proved to be the most significant under heat stress conditions among HSPs (Kiang and Tsokos, 1998).

When assessing metabolic parameters, a limitation of the study should be noted, namely the animals did not consume the same feed, and we were unable to control TMR quality or feed intake. Our aim was to capture possible metabolic changes reported in the literature (Bakony et al., 2019) in farm conditions. Plasma glucose concentration was lower in the DRY group in the Holstein-Friesian and Simmental breeds. The rate of decline was similar to that reported in the literature (Wheelock et al., 2010); however, it could be related to a decrease in appetite and not milk production. Rectal temperature was significantly higher in the DRY group in all three breeds. The animals were subjected to heat stress, which could reduce appetite. Blood BHB levels were higher in Simmental alone in the PEAK group and were within the physiological threshold (1.2 mmol/l) in spring and summer. The Jersey breed had the highest concentration of BHB, but this occurred in the PEAK group regardless of heat stress. In Holstein-Friesians, BHB was within the physiological range in the tested cows. Plasma NEFA concentrations were within the physiological range (0.2 mmol/l) in all animals tested. Higher NEFA levels were recorded only in the Jersey DRY group under heat stress. Nevertheless, the biological significance of this difference was questionable. Garner et al. (2017) showed elevated plasma NEFA and urea levels under heat stress, while other authors reported decreased plasma NEFA concentrations (Wheelock et al., 2010). Plasma urea levels in Holstein-Friesians were lower under heat stress in all groups in our study; they were higher in Jersey and unchanged in Simmental cows. Increased breakdown of muscle proteins (glycoplastic amino acids) could partly contribute to glucose formation, which was suggested by an increase in plasma urea concentration during heat stress (Shwartz et al., 2009). The trend that best matched the literature was detectable in the Jersey breed. The utilisation of amino acids from muscle proteins decreases their sources for the synthesis of milk protein, and thus milk protein content may be lower under heat stress (Shwartz et al., 2009), which was observed in Holstein-Friesians in our study. Since BHB levels were the highest in this breed, we assumed that elevated urea concentrations were caused by a relative protein surplus in the rumen.

Regarding metabolic parameters, we can conclude that the results from well-controlled experiments are unlikely to be reproduced under commercial farm conditions. The value of metabolic indices may depend more on the type of nutrition than on the presence of heat stress.

Other scientists have shown differences in heat tolerance of some cattle breeds (Maróti-Agóts et al., 2011). High-yielding breeds (e.g. Holstein-Friesian) are more susceptible to heat stress than lower-yielding breeds (e.g. Simmental type) (Gantner et al., 2017b), possibly due to a lower energy metabolism with lower milk production.

Conclusions

Based on our results, Holstein-Friesian seemed to be the most sensitive to heat stress among the three breeds studied. In this breed, milk production and milk protein content decreased during the summer heat, and it also had the lowest HSP70 concentration for cell protection during heat stress in both plasma and saliva. Our results highlight the possible importance of Hungarian Simmental and Jersey cows in the context of climate change and the need to intensively protect Holstein-Friesian cows from heat stress using effective cooling techniques.

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Conflict of interest

The Authors declare that there is no conflict of interest.

References

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 behavioral patterns of lactating dairy cows experiencing mild to moderate heat stress. J. Dairy Sci. 98, 118–127, https://doi.org/10.3168/jds.2013-7704

Aneja R., Odoms K., Dunsmore K., Shanley T.P., Wong H.R., 2006. Extracellular heat shock protein-70 induces endotoxin tolerance in THP-1 cells. J. Immunol. 177, 7184–7192, https://doi.org/10.4049/jimmunol.177.10.7184

Baek Y.C., Kim M., Jeong J.Y., Oh Y.K., Lee S.D., Lee Y.K., Ji S.Y., Choi H., 2019. Effects of short-term acute heat stress on physiological responses and Heat Shock Proteins of Hanwoo steer (Korean Cattle). J. Anim. Reprod. Biotechnol. 34, 173–182, https://doi.org/10.12750/JARB.34.3.173

Bakony M., Könyves L., Hejel P., Kovács L., Jurkovich V., 2019. Heat stress in dairy cows Part 1. - A review on physiological factors involved in milk yield loss (in Hungarian). Magy. Állatorv. Lapja 141, 341–350

Bates D., Maechler M., Bolker B., Walker S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67, 1–48, https://doi.org/10.18637/jss.v067.i01

Baumgard L.H., Wheelock J.B., Sanders S.R., Moore C.E., Green H.B., Waldron M.R., Rhoads R.P., 2011. Postabsorptive carbohydrate adaptations to heat stress and monensin supplementation in lactating Holstein cows. J. Dairy Sci. 94, 5620–5633, https://doi.org/10.3168/jds.2011-4462

Bianca W., 1962. Relative importance of dry- and wet-bulb temperatures in causing heat stress in cattle. Nature 195, 251–252 https://doi.org/10.1038/195251a0

Calamari L., Petrera F., Abeni F., Bertin G., 2011. Metabolic and hematological profiles in heat stressed lactating dairy cows fed diets supplemented with different selenium sources and doses. Livest. Sci. 142, 128–137, https://doi.org/10.1016/j.livsci.2011.07.005

Catalani E., Amadori M., Vitali A., Bernabucci U., Nardone N., Lacetera N., 2010. The Hsp72 response in peri-parturient dairy cows: relationships with metabolic and immunological parameters. Cell Stress Chaperones 15, 781–790, https://doi.org/10.1007/s12192-010-0186-x

Collier R.J., Dahl G.E., van Baale M.J., 2006. Major advances associated with environmental effects on dairy cattle. J. Dairy Sci. 89, 1244–1253, https://doi.org/10.3168/jds.S0022-0302(06)72193-2

Collier R.J., Eley R.M., Sharma A.K., Pereira R.M., Buffington D.E., 1981. Shade management in subtropical environment for milk yield and composition in Holstein and Jersey cows. J. Dairy Sci. 64, 844–849, https://doi.org/10.3168/jds.S0022-0302(81)82656-2

Dado-Senn B., Vega Acosta L., Torres Rivera M. et al., 2020. Pre- and postnatal heat stress abatement affects dairy calf thermoregulation and performance. J. Dairy Sci. 103, 4822–4837, https://doi.org/10.3168/jds.2019-17926

Fournel S., Ouellet V., Charbonneau É., 2017. Practices for alleviating heat stress of dairy cows in humid continental climates: a literature review. Animals 7, 37, https://doi.org/10.3390/ani7050037

Gantner V., Bobic T., Gregic M., Gantner R., Kuterovac K., Potocnik K., 2017a. The differences in heat stress resistance due to dairy cattle breed. Mljekarstvo 67, 112–122, https://doi.org/10.15567/mljekarstvo.2017.0203

Gantner V., Bobic T., Gantner R., Gregic M., Kuterovac K., Novakovic J., Potocnik K., 2017b. Differences in response to heat stress due to production level and breed of dairy cows. Int. J. Biometeorol. 61, 1675–1685, https://doi.org/10.1007/s00484-017-1348-7

Garner J.B., Douglas M.S., Williams R.O., Wales W.J., Marett L.C., DiGiacomo K., Leury B.J., Hayes B.J., 2017. Responses of dairy cows to short-term heat stress in controlled-climate chambers. Anim. Prod. Sci. 57, 1233–1241, https://doi.org/10.1071/AN16472

Gaughan J.B., Bonner S.L., Loxton I., Mader T.L., 2013. Effects of chronic heat stress on plasma concentration of secreted heat shock protein 70 in growing feedlot cattle. J. Anim. Sci. 91, 120–129, https://doi.org/10.2527/jas.2012-5294

Hansen P.J., 2013. Genetic control of heat stress in dairy cattle. Proceedings 49th Florida Dairy Production Conference. Gainesville, FA (USA)

Harris D.L., Shrode R.R., Rupel I.W., Leighton R.E., 1960. A study of solar radiation as related to physiological and production responses of lactating Holstein and Jersey cows. J. Dairy Sci. 43, 1255–1262, https://doi.org/10.3168/jds.S0022-0302(60)90312-X

Holm S., 1979. A simple sequentially rejective multiple test procedure. Scand. J. Stat. 6, 65–70, http://www.jstor.org/stable/4615733

Itoh F., Obara Y., Rose M.T., Fuse H., Hashimoto H., 1998. Insulin and glucagons secretion in lactating cows during heat exposure. J. Anim. Sci. 76, 2182–2189, https://doi.org/10.2527/1998.7682182x

Johnson J.D., Campisi J., Sharkey C.M., Kennedy S.L., Nickerson M., Fleshner M., 2005. Adrenergic receptors mediate stress-induced elevations in extracellular Hsp72. J. Appl. Physiol. 99, 1789–1795, https://doi.org/10.1152/japplphysiol.00390.2005

Kadzere C.T., Murphy M.R., Silanikove N., Maltz E., 2002. Heat stress in lactating dairy cows: a review. Livest. Prod. Sci. 77, 59–91, https://doi.org/10.1016/S0301-6226(01)00330-X

Kiang J.G., Tsokos G.C., 1998. Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology. Pharmacol. Ther. 80, 183–201, https://doi.org/10.1016/S0163-7258(98)00028-X

Kregel K.C., 2002. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J. Appl. Physiol. 5, 2177–2186, https://doi.org/10.1152/japplphysiol.01267.2001

Kristensen T.N., Løvendahl P., Berg P., Loeschcke V., 2004. Hsp72 is present in plasma from Holstein-Friesian dairy cattle, and the concentration level is repeatable across days and age classes. Cell Stress Chaperones 9, https://doi.org/10.1379/CSC-17.1

Kumar J., Yadav B., Kumar Madan A., Kumar M., Sirohi R., Vidyasagar Reddy A., 2020. Dynamics of heat-shock proteins, metabolic and endocrine responses during increasing temperature humidity index (THI) in lactating Hariana (Zebu) cattle. Biol. Rhythm Res. 51, 934–950, https://doi.org/10.1080/09291016.2019.1566986

Kvidera S.K., Dickson M.J., Abuajamieh M. et al., 2017. Intentionally induced intestinal barrier dysfunction causes inflammation, affects metabolism, and reduces productivity in lactating Holstein cows. J. Dairy Sci. 100, 4113–4127, https://doi.org/10.3168/jds.2016-12349

Lamy E., Jurkovich V., Rodrigues L. et al., 2017. Detection of 70 kDa heat shock protein in the saliva of dairy cows. J. Dairy Res. 84, 280–282, https://doi.org/10.1017/S0022029917000280

Maróti-Agóts Á., Bodó I., Jávorka L., Gyurmán A., Solymosi N., Zenke P., Skogseth M., Zöldág L., 2011. Possible genetic sign of heat stress adaptation in Hungarian *Grey Bos taurus* breed. Acta Biol. Hung. 62, 65–72, https://doi.org/10.1556/ABiol.61.2011.1.6

Min L., Zhao S., Tian H., Zhou X., Zhang Y., Li S., Yang H., Zheng N., Wang J., 2017. Metabolic responses and "omics" technologies for elucidating the effects of heat stress in dairy cows. Int. J. Biometeorol. 61, 1149–1158, https://doi.org/10.1007/s00484-016-1283-z

Molvarec A., Rigó J., Lázár L., Balogh K., Makó V., Cervenak L., Mézes M., Prohászka Z., 2009. Increased serum heat-shock protein 70 levels reflect systemic inflammation, oxidative stress and hepatocellular injury in preeclampsia. Cell Stress Chaperones 14, 151–159, https://doi.org/10.1007/s12192-008-0067-8

Noma A., Okabe H., Kita M., 1973. A new colorimetric micro-determinatoin of free fatty acids in serum. Clin. Chim. Acta 43, 317–320, https://doi.org/10.1016/0009-8981(73)90468-3

Polsky L., von Keyserling M.A.G., 2017. Effects of heat stress on dairy cattle welfare. J. Dairy Sci. 100, 8645–8657, https://doi.org/10.3168/jds.2017-12651

R Core Team, 2022. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna (Austria), https://www.R-project.org/

Reiczigel J., Solymosi N., Könyves L., Maróti-Agóts Á., Kern A., Bartyik J., 2009. Examination of heat stress caused milk production loss by the use of temperature-humidity indices (in Hungarian). Magy. Állatorv. Lapja 131, 127–144

Rhoads R.P., Sanders S.R., Cole L., Skrzypek M., Elsasser T., Duff G.C., Collier R.J., Baumgard L.H., 2009. Effects of heat stress on glucose homeostasis and metabolic response to an endotoxin challenge in Holstein steers. J. Anim. Sci. 87 (E-Suppl. 2), 78

Rhoads M.L., Kim J.W., Collier R.J., Crooker B.A., Boisclair Y.R., Baumgard L.H., Rhoads R.P., 2013. Effects of heat stress and nutrition on lactating Holstein cows: II. Aspects of hepatic growth hormone responsiveness. J. Dairy Sci. 93, 170–179, https://doi.org/10.3168/jds.2009-2469

Russell V.L., 2022. Package emmeans. Estimated marginal means, aka least-squares means. Version 1.7.2, https://CRAN.R-project.org/package=emmeans

Shilja S., Sejian V., Bagath M., Mech A., David C.G., Kurien E.K., Varma G., Bhatta R., 2016. Adaptive capability as indicated by behavioral and physiological responses, plasma HSP70 level, and PBMC HSP70 expression in Osmanabadi goats subjected to combine (heat and nutritional) stressors. Int. J. Biometeorol. 60, 1311–1323, https://doi.org/10.1007/s00484-015-1124-5

Shwartz G., Rhoads M.L., VanBaale M.J., Rhoads R.P., Baumgard L.H., 2009. Effects of a supplemental yeast culture on heat-stressed lactating Holstein cows. J. Dairy Sci. 92, 935–942, https://doi.org/10.3168/jds.2008–1496

Silanikove N., Shapiro F., Shinder D., 2009. Acute heat stress brings down milk secretion in dairy cows by up-regulating the activity of the milk-borne negative feedback regulatory system. BMC Physiol. 9, 13, https://doi.org/10.1186/1472-6793-9-13

Smith D.L., Smith T., Rude B.J., Ward S.H., 2013. Short communication: Comparison of the effects of heat stress on milk and component yields and somatic cell score in Holstein and Jersey cows. J. Dairy Sci. 96, 3028–3033, https://doi.org/10.3168/jds.2012-5737

Solymosi N., Torma C., Kern A., Maróti-Agóts Á., Barcza Z., Könyves L., Berke O., Reiczigel J., 2010. Changing climate in Hungary and trends in the annual number of heat stress days. Int. J. Biometeorol. 54, 423–431, https://doi.org/10.1007/s00484-009-0293-5

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. J. Dairy Sci. 93, 644–655, https://doi.org/10.3168/jds.2009-2295

Yániz J.L., López-Gatius F., Almería S., Carretero T., García-Ispierto I., Serrano B., Smith R.F, Dobson H., Santolaria P., 2009. Dynamics of heat shock protein 70 concentrations in peripheral blood lymphocyte lysates during pregnancy in lactating Holstein-Friesian cows. Theriogenology 72, 1041–1046, https://doi.org/10.1016/j.theriogenology.2009.06.019