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INFLUENCE OF HEAT STRESS ON MILK PRODUCTION IN FRATERNAL TWINS

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Abstract

The aim of this study was the evaluation of heat stress in milk production in dairy cows (daily milk yield; milk production in 1st lactation). The comparison was performed between fraternal twins (full sisters), produced on the same farm and same parity. One of the twins produced in a hot period whereas her sister produced in a cooler period. Also, there can be differences in milk production between fraternal twins. Therefore, a second group of fraternal twins was also created that produced without heat stress. Databases of fraternal twins were analysed using PROC GLM analysis of Statistica® with selected effects. In conclusion, a negative effect of heat stress on milk production was found. There was an obvious tendency (in some cases insignificant) for worse milk production during heat stress in both the daily milk yield during hot days and in 1st lactation started in the hot season. Fat content (in %) was also lower in the hot period but differences were insignificant. On the other hand, there was found very low differences in milk production (and milk components) between fraternal twins produced in season without heat stress.

Keywords: heat stress, milk production

INTRODUCTION

The issue of heat stress is currently a very important topic and especially from the perspective of global warming, which could be connected to the heat stress of animals. In this case, Schüller *et al.* (2014) stated that heat stress in cows is also related to areas with a mild (subtropical) climate, where there are fewer days with high temperatures. Heat stress is related to many problems and reflected in the production or reproduction traits in many cows.

In general, the negative relationship of heat stress has been demonstrated in the reproduction of cows (Bezdíček *et al.*, 2019, 2020; Tučková and Filipčík, 2019; Klementová *et al.*, 2017), in milk production including somatic cell count (Rhoads *et al.*, 2009; Gantner *et al.*, 2016; Herbut *et al.*, 2018; Hammami *et al.*, 2013), in the lower value of casein fractions in milk (Bernabucci *et al.,* 2015) as well as immune function and the health of cows (Rakib *et al.*, 2020; He *et al.*, 2020). In this article, we focus on heat stress in the milk production (daily milk yield and milk production in the 1st lactation) by fraternal twins.

Heat stress is a one of the important factors affecting milk production in cows. These findings are reported, for example, by André *et al.* (2011), who found loss of milk production in the duration of the heat stress season. Also, Bernabucci *et al.* (2002) reported that daily production of milk (in kg) and protein percentages was significantly lower in summer than in spring. In his later study, the authors present lower values of milk components (fat, protein, total solids, and solids-not-fat), the lowest values of casein fractions (exception is γ-CN) and poorer milk coagulation properties but greater content of IgG and serum albumin during heat stress (Bernabucci *et al.*, 2015). Also, Broucek *et al.* (2009) noticed that environmental factors play an important role in milk production and proved lower milk production in cows during heat stress. The authors also present the necessity to study methods leading to the reduction of temperature in open barns. Wheelock *et al.* (2010) found a decrease of milk production during heat stress and presented that heat stress reduced dry matter intake in Holstein cows by approximately 30%. Similar conclusions are also reported by West *et al.* (2003), which present both lower milk production and lower dry matter intake during increased air temperature. From the point of view of heat stress, milk components were also studied. Cowley *et al.* (2015) presented a change in protein concentration and protein composition (α_{S1}-casein increased; α_{S2}-casein decreased) during heat stress; the authors also found an increase of urea milk concentration in this period but did not find any influence of heat stress on lactose and milk fat content. In conclusion the authors proposed that stressed cows (during heat stress or restricted dry matter intake) may mobilize different endogenous sources of energy (Cowley *et al.*, 2015).

From the above presented overview, it is obvious that most of the authors agree on the negative impact of heat stress on milk production. At the same time many authors (Müschner-Siemens *et al.*, 2020; Bouček *et al.*, 2009 etc.) presented that high-yielding cows and cows of higher parities (later lactation) may be more affected from the heat stress than young and low-yielding cows. The breed and its resistance to heat stress also play an important role (Gantner *et al.*, 2017; Mariasegaram *et al.*, 2007; Dikmen *et al.*, 2008).

In general, most of the observations were based on the evaluation of differences between cows in temperature-optimal conditions vs. animals under heat stress. This evaluation requires an adjustment of many factors, such as genetics (father; mother), parity, breed, environment, etc., which affects the overall expression of trait.

The aim of this study was to evaluate the effect of heat stress on milk production (daily milk yield; milk production in 1st lactation). Because it is very important to take into account the above presented factors (age; parity; genetic, etc.), this study compared the production of full siblings (fraternal twins), stabled on the same farm. One of the twins had milk production during the heat (stress) period, while the sister (fraternal twins) produced in temperature-optimal conditions.

MATERIALS AND METHODS

The evaluation of quantitative traits (including milk production) is very significantly affected by both environmental and genetic factors (e.g., the influence of father and mother; effect of farm). These effects can be eliminated in a number of ways. In this work, we eliminate some factors by comparing the milk production between fraternal twins, which had the same father and mother, the same parity and produced on the same farm.

Animals in the Experiment

Two groups of cows (fraternal twins) were created.

- The first group compares fraternal twins in the HOT vs. COOL period (one of the twins had milk production in the hot period and her sister produced in the cooler period). The aim was to find out the differences in milk production between fraternal twins in the hot and cold periods (HOT vs. COOL period).
- The second group compares fraternal twins in the COOL vs. COOL period. This comparison is based on the fact that also in optimal temperature conditions there can be differences in milk production between fraternal twins. Therefore, this second group of fraternal twins was created; the aim was to find out the differences in milk production between fraternal twins produced without heat stress (COOL1 vs. COOL2 period).

Evaluated Traits

The daily milk yield (in kg) and milk production in first lactation (in kg) were analysed in this study. The first milk control of the cows took place during the period from the 6th to the 30th day after calving. The number of days after calving was also considered as a factor in the statistical evaluation by the GLM method. Hot or cool conditions were defined two days before the daily milk control.

Another evaluated trait was the milk production in the first lactation. The length of the first lactation is generally determined at 305 days. Hot or cool conditions were based on the day of calving (and thus the beginning of lactation).

I: *Studied groups and months*

Production in kg	Season in general	Months	
daily milk yield	HOT vs. COOL	first daily milk control:	Jun–September vs. October–May
	COOL1 vs. COOL2	first daily milk control:	September–December vs. January–March
1. lactation	HOT vs. COOL	calving in:	Jun and July vs. October–May
	COOL1 vs. COOL2	calving in:	September–December vs. January–March

Statistical Processing

The data of the full sisters (fraternal twins) were analysed in this study using PROC GLM of Statistica®. Different effects were used for GLM analysis (separately for: HOT vs. COOL and COOL1 vs. COOL2 period) and t-test (dependent samples) for basic statistical analysis. A test for homogeneity (first step of calculation) was done using the Cochran-Hartley-Bartlett test and residuals. These results presented the suitability for the next analysis and the GLM method. Selected effects were used in the final calculation: the season effect (HOT vs. COOL; COOL1 vs. COOL2 season); effect of days after calving; effect of breed (Holstein, Czech Fleckvieh) and effect of breeding value of sire.

Statistical processing was performed separately for daily milk yield (in kg) and for first lactations (in kg) and separately for the period: HOT vs. COOL and COOL1 vs. COOL2. The calculation was performed according to the following model equations:

Model for daily milk yield (in kg):

$$Y(HOT\ vs.\ COOL)_{ijkl} = \mu + Season1_i + Days1_j + Breed1_k + RBV1_l + e_{i,j,k,l} \quad (1)$$

$$Y(COOL1\ vs.\ COOL2)_{ijkl} = \mu + Season2_i + Days2_j + Breed2_k + RBV2_l + e_{i,j,k,l} \quad (2)$$

where:

Y_{ijkl} ....corrected value (dependent variable) = daily milk production (in kg),

μ ....mean value,

$Season1_i$....HOT/COOL season = two days before first milk control in hot or cool season (in generally: HOT season = Jun–September, n = 32; COOL season = October–May, n = 32),

$Season2_i$....COOL1/COOL2 season = two days before first milk control in cool or cool season (in generally: COOL1 season = September–December, n = 39; COOL2 season = January–March, n = 39),

$Days1_j$........days after calving (1 = to 25 days, n = 22; 26 and more, n = 42),

$Days2_j$........days after calving (1 = to 25 days, n = 33; 26 and more, n = 45),

$Breed1_k$ .....breed of cow (1 = Holstein, n = 40; 2 = Czech Fleckvieh, n = 24),

$Breed2_k$ .....breed of cow (1 = Holstein, n = 54; 2 = Czech Fleckvieh, n = 24),

$RBV1_l$.........relative breeding value of sire (1 = to 110, n = 32; 2 = 111 and more, n = 32),

$RBV2_l$.........relative breeding value of sire (1 = to 110, n = 66; 2 = 111 and more, n = 12),

e_{ijkl} ....residual error.

Model pro milk production in 1st lactation (in kg):

$$Y(HOT\ vs.\ COOL)_{ijk} = \mu + Season1_i + Breed1_j + RBV1_k + e_{i,j,k} \quad (3)$$

$$Y(COOL1\ vs.\ COOL2)_{ijk} = \mu + Season2_i + Breed2_j + RBV2_k + e_{i,j,k} \quad (4)$$

where:

Y_{ijkl} ....corrected value (dependent variable) = milk production in 1. lactation (in kg),

μ ....mean value,

$Season1_i$....HOT/COOLl season = calving in hot vs. cool season (HOT season = Jun and July, n = 44; COOL1 season = calving in October–May, n = 44),

$Season2_i$....COOL1/COOL2 season = calving in cool vs. cool season (COOL1 season = September–December, n = 53; COOL2 season = January–March, n = 53),

$Breed1_k$ .....breed of cow (1 = Holstein, n = 66; 2 = Czech Fleckvieh, n = 22),

$Breed2_k$ .....breed of cow (1 = Holstein, n = 68; 2 = Czech Fleckvieh, n = 38),

$RBV1_l$.........relative breeding value of sire (1 = to 110, n = 50; 2 = 111 and more, n = 38),

$RBV2_l$.........relative breeding value of sire (1 = to 110, n = 66; 2 = 111 and more, n = 40),

e_{ijkl} ....residual error.

THI Index and Time-point of Heat Stress

It is advantageous for the evaluation of heat stress to use Temperature Humidity Index (THI) which includes the air temperature and relative humidity. The THI was calculated according to the following equation:

$$\text{Temperature-humidity index } (THI) = 0.8 \times \textit{ambient temperature} + [(\%\ \textit{relative humidity} \div 100) \times (\textit{ambient temperature}-14.4)] + 46.4 \quad (5)$$

(Buffington *et al.*, 1981).

The maximum day temperature and maximum day humidity were obtained from the Czech Hydro Meteorological Institute in Brno.

In this study the limit of heat stress was based at 71 and more, which is based on the conclusions of other authors. The THI index was used for the evaluation of the level of heat stress and for the creation groups of animals: under and without heat stress.

In the case of the daily milk yield, the THI index was determined two days before the milk control (similar in reproduction; cows are sensitive to heat stress a few days before insemination (Al-Katanani

II: *THI index in different temperature periods*

	THI		
	mean	min.	max.
HOT	76.46	70.88	86.14
COOL	50.96	15.36	70.57
COOL1	46.42	27.74	68.03
COOL2	43.94	26.15	70.65

et al., 1999; Schüller *et al.*, 2014; Morton *et al.*, 2007)). In the case of milk production in first lactation, the THI index was determined on the day of calving (and thus the beginning of lactation).

RESULTS

Daily Milk Yield

Tab. III presents the basic statistical comparison of the daily milk yield between the fraternal twins in the hot vs. cool period (HOT vs. COOL). The results showed a significantly lower milk yield in the hot season than in the cold season (24.83 vs. 26.53 kg); the milk components tended to have a lower fat percentage content during the hot period (insignificant).

However, in the case without heat stress there can also be differences in milk production between the full sisters (COOL1 vs. COOL2). This comparison is presented in Tab. IV. In the case of the twins who were not affected by heat stress (COOL1 vs. COOL2), the differences in milk production were very low (26.23 vs. 25.81).

The evaluation of the daily milk yield was also performed by the GLM method, taking into account selected factors (days after calving; breed; RBV). The results are shown in Tabs. V and VI. Although the results were not statistically significant, there is a lower milk yield in the hot period than in the cold season (HOT vs. COOL; 23.48 vs. 25.5 kg); on the other hand, the comparison between the full sisters in the cold vs. cold period presented very low differences (COOL1 vs. COOL2; 24.87 vs. 24.72 kg). Results of fat content also tended to have a lower value during the hot period (insignificant).

The breed of the cows shows to be a very important factor as well. Significant differences were found in the daily milk yield between Holstein vs. Czech Fleckvieh in both studied groups: HOT vs. COOL; COOL1 vs. COOL2 (27.62 vs. 21.36; 26.76 vs. 22.85 kg). In general, a lower protein content (in %) was also found in Holstein cows.

III: *Basic statistical evaluation of first daily milk yield control (in kg) in HOT vs. COOL period*

Trait	Season	Mean	± SD	N	p
Milk (kg)	HOT	24.83	6.40	32	0.0293
	COOL	26.53	5.34	32	
Fat (%)	HOT	3.86	0.71	32	0.1495
	COOL	4.11	0.68	32	
Protein (%)	HOT	3.12	0.44	32	0.2903
	COOL	3.06	0.33	32	

first daily milk control in:
HOT season = Jun–September
COOL season = October–May

IV: *Basic statistical evaluation of first daily milk yield control (in kg) in COOL1 vs. COOL2 period*

Trait	Season	Mean	± SD	N	p
Milk (kg)	COOL1	26.23	4.47	39	0.5966
	COOL2	25.81	5.80	39	
Fat (%)	COOL1	3.97	0.64	39	0.0367
	COOL2	4.22	0.66	39	
Protein (%)	COOL1	3.15	0.36	39	0.4382
	COOL2	3.10	0.37	39	

first daily milk control in:
COOL1 season = September–December
COOL2 season = January–March

V: *GLM analysis of daily milk yield (in kg), fat (in %) and protein (%) in HOT vs. COOL period*

Trait	Category	N	Milk (kg)		Fat (%)		Protein (%)	
			LSM	± SD	LSM	± SD	LSM	± SD
Season	HOT	32	23.48	0.98	3.92	0.13	3.22	0.07
	COOL	32	25.50	0.93	4.14	0.12	3.13	0.06
Days in milk	1	22	23.17	1.15	4.18	0.15	3.28	0.08
	2	42	25.81	0.79	3.88	0.11	3.07	0.05
Breed	1	40	27.62A	0.82	4.03	0.11	2.97A	0.06
	2	24	21.36A	1.14	4.04	0.15	3.37A	0.08
RBV	1	32	24.91	0.99	3.87	0.13	3.22	0.07
	2	32	24.07	0.94	4.19	0.12	3.12	0.06
p			A = 0.0001				A = 0.001	
r^2			0.3063		0.124		0.2522	

RBV – Relative breeding value
1st daily milk control in: HOT season = Jun–September; COOL season = October–May

VI: *GLM analysis of daily milk yield (in kg), fat (in %) and protein (%) in COOL1 vs. COOL2 period*

Trait	Category	N	Milk (kg)		Fat (%)		Protein (%)	
			LSM	± SD	LSM	± SD	LSM	± SD
Season	COOL1	39	24.87	0.99	3.95	0.13	3.13	0.07
	COOL2	39	24.72	0.97	4.15	0.13	3.08	0.07
Days in milk	1	33	24.04	1.05	4.20A	0.14	3.13	0.07
	2	45	25.56	0.92	3.90A	0.12	3.07	0.06
Breed	1	54	26.76A	0.86	4.00	0.11	2.97	0.06
	2	24	22.85A	1.13	4.11	0.15	3.23	0.08
RBV	1	66	25.32	0.65	4.18	0.09	3.21	0.05
	2	12	24.27	1.43	3.93	0.19	3.00	0.10
p			A = 0.0024		A=0.0251			
r^2			0.1454		0.1098		0.1586	

RBV – Relative breeding value
1st daily milk control in: COOL1 season = September–December; COOL2 season = January–March

VII: *Basic statistical analysis of differences in milk production (1st lactation) between fraternal twins in HOT vs. COOL period*

	Season	Mean	± SD	N	p
Milk (kg)	HOT	6945.27	3902	44	n.s.
	COOL	7221.48	4278	44	
Fat (%)	HOT	4.01	3.08	44	n.s.
	COOL	4.06	3.00	44	
Protein (%)	HOT	3.40	2.90	44	n.s.
	COOL	3.42	2.91	44	

n.s. non significant
first lactation started in:
HOT season = Jun and July
COOL season = October–May

VIII: *Basic statistical analysis of differences in milk production (1st lactation) between fraternal twins in COOL1 vs. COOL2 period*

	Season	Mean	± SD	N	p
Milk (kg)	COOL1	6817.27	4375	52	n.s.
	COOL2	6870.77	3687	52	
Fat (%)	COOL1	3.87	2.90	52	n.s.
	COOL2	3.95	2.85	52	
Protein (%)	COOL1	3.35	2.81	52	n.s.
	COOL2	3.39	2.85	52	

n.s. non significant
first lactation started in:
COOL1 season = September–December
COOL2 season = January–March

Milk Production in 1st Lactation

Milk production (in first lactation; 305 days in milk) is extensively affected mainly at the beginning of lactation, when there is an extensive increase in milk production. This period is crucial for overall lactation and heat stress can be an important factor.

The results of the basic statistical analysis are shown in Tabs. VII and VIII. Although statistically significant differences were not found, lower milk production is evident in cows that started lactation during periods of higher temperatures (HOT vs. COOL; 6945.27 vs. 7221.48 kg – Tab. VII). Milk components in first lactation (fat, protein) were not affected by heat stress. By comparison, the fraternal twins without heat stress at the beginning of lactation (Tab. VIII), the differences between the full sisters in milk production per 1st lactation were very low (COOL1 vs. COOL2; 6817.27 vs. 6870.77 kg).

Subsequently, an evaluation of milk production in 1st lactation using the GLM analysis was performed, considering the following factors: season; breed; relative breeding value of sire (RBV) – Tabs. IX and X. Although statistically significant differences were not found between the fraternal twins, lower milk production was obvious when lactation started during the hot season (HOT vs. COOL; 6540.10 vs. 6816.31 kg – Tab. IX). On the other hand, the difference between fraternal twins produced without heat stress (COOL1 vs. COOL2) was very low (6522.11 vs. 6612.11 kg – Tab. X). Negative effects were not found on the milk components during the hot summer period. Significant the influence of the breed proved to be evident. Holstein cattle (compared to Czech Fleckvieh) presented a significantly lower milk production (7613.27 vs. 5743.14 kg) and content of fat (3.94 vs. 4.28%) and protein (3.36 vs. 3.57%) as well. The same tendency we can see in fraternal twins produced in optimal season. In the case of Relative breeding value (RBV), there is a tendency for higher milk production from fathers with a higher breeding value. The results of the GLM analysis in 1st lactation are shown in Tabs. IX and X.

IX: *GLM analysis of milk production in 1st lactation in HOT vs. COOL period*

Trait	Category	N	Milk (kg)		Fat (%)		Protein (%)	
			LSM	± SD	LSM	± SD	LSM	± SD
Season	HOT	44	6540.10	250.18	4.08	0.09	3.45	0.05
	COOL	44	6816.31	250.17	4.13	0.09	3.47	0.05
Breed	1	66	7613.27A	194.73	3.94A	0.07	3.36A	0.04
	2	22	5743.14A	330.83	4.28A	0.12	3.57A	0.06
RBV	1	50	6220.90	254.45	4.19	0.09	3.50	0.05
	2	38	7135.51	254.45	4.02	0.09	3.43	0.05
p			A = 0.001		A = 0.0397		A = 0.0084	
r^2			0.2384		0.0695		0.0918	

RBV – Relative breeding value
1st lactation started in: HOT season = Jun and July; COOL season = October–May

X: *GLM analysis of milk production in 1st lactation in COOL1 vs. COOL2 period*

Trait	Category	N	Milk (kg)		Fat (%)		Protein (%)	
			LSM	± SD	LSM	± SD	LSM	± SD
Season	COOL1	53	6522.11	185.91	3.92	0.06	3.39	0.04
	COOL2	53	6612.11	185.91	3.99	0.06	3.42	0.04
Breed	1	68	7461.63A	176.96	3.85A	0.06	3.28A	0.03
	2	38	5672.96A	217.10	4.06A	0.07	3.52A	0.04
RBV	1	66	6454.46	182.46	3.88B	0.06	3.43	0.03
	2	40	6680.14	210.38	4.03B	0.07	3.38	0.04
p			A = 0.001		A = 0.0035 B = 0.0114		A = 0.001	
r^2			0.2881		0.1057		0.1057	

RBV – Relative breeding value
1st lactation started in: COOL1 season = September–December; COOL2 season = January–March

DISCUSSION

Other authors have also shown a negative effect of heat stress on milk production, not only in the form of daily milk yield but also in milk production for the whole lactation. Brouček *et al.* (2009) presented a significant decrease of milk production in the hot months (in August). Specifically, in June, a daily milk yield of 35.95 kg was found in contrast to the hot period (in August) with a decrease of milk production to 32.26 kg. Similarly, the evaluation for lactation showed a negative effect of heat stress when lactation started in the hot months. In this case a significantly lower milk production (8954.4 ± 1526.9 kg) was found compared to the other two groups of cows, which had in the warmest months 51–120 vs. 121–200 days in milk (milk production per lactation 9614.1; 9254.3 kg). Also, Wheelock *et al.* (2010) found a decrease of milk production during heat stress in Holstein cows; specifically, during pair-feeding a decrease of 13.9% (4.8 kg) was found and during heat stress there was a decrease of 27.6% (9.6 kg). Losses in milk production during heat stress were also demonstrated by André *et al.* (2011), who presented loss of milk production 31.4 ± 12.2 kg of milk/cow per year. Bernabucci *et al.* (2002) reported that daily production of milk (in kg) and protein percentages were significantly lower in summer by 10% vs. 9.9% than in spring (specifically milk 26.7 vs. 29.5 l/day: protein 3.01 vs. 3.31%). Also in the following research, Bernabucci *et al.* (2015) presented the lowest values of milk components (fat; protein) in summer than in the winter period. Specifically, the authors found the content of fat in the summer vs. winter period (3.20 vs. 3.80%) and protein content 3.29 vs. 3.50%). A negative impact of heat stress in milk production was also found by West *et al.* (2003). The authors reported a reduction in milk yield (Holstein and Jersey cows) during the hot period with 0.88 and 0.84 kg/d per unit increase in THI. On the other hand, Cowley *et al.* (2015) did not find any influence of heat stress on lactose and milk fat content but found influence of heat stress in protein concentration and protein composition.

In accordance with our findings, most authors agree on the negative impact of temperature stress on milk production, presented in both daily milk production and in production per lactation. Our results in the milk fat content are also in agreement with the conclusions of other authors. The effect of heat stress in the protein content was not found in our study, although some authors have shown a negative effect of heat stress in protein concentration or in protein composition.

Therefore, it is very important to pay attention to heat stress. The solution is e.g., using a water shower, ventilation or providing shade (Kendal *et al.*, 2007; Herbut *et al.*, 2015; Legrand *et al.*, 2011). The correct timing of cooling and correct management is also very important (Fabris *et al.*, 2019).

CONCLUSION

In conclusion, this study has shown a negative effect of heat stress on milk production (in kg) in both daily milk production during hot days and in production per 1st lactation started in the hot season. Also fat percentage tended to be lower during the hot period (insignificant). On the other hand, the differences in milk production between the fraternal twins were very low in the case without heat stress.

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