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EFFECT OF HEAT STRESS ON METABOLIC DISORDERS PREVALENCE RISK AND MILK PRODUCTION IN HOLSTEIN COWS IN CROATIA

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Abstract

The objectives were to evaluate the effect of heat stress on daily milk traits (yield, fat and protein content, F/P ratio) as well as to determine the differences in metabolic disorders (acidosis, ketosis) prevalence risk regarding the heat stress conditions. For statistical analysis 1,187,781 test-day records of milk, fat, and protein from 89,030 Holsteins reared on 6,388 farms provided by the Croatian Agricultural Agency, were used. Based on the results it could be concluded that heat stress condition causes decline of daily milk yield and components as well as increase of acidosis risk regardless of the lactation stage and increase of ketosis risk during mid-lactation. The research results point out that the test-day records and environmental measurements collected in regular milk recording could be used as a tool for dairy herd monitoring enabling the early detection of unfavourable environmental conditions and the subclinical disorders. Since environmental conditions significantly affect daily milk yield and components, and consequently F/P ratio, further research with the purpose of detailed formulation of metabolic disease risk in relation to the environmental conditions is needed.

Key words: acidosis, ketosis, temperature-humidity index, test-day records, prevalence risk

In dairy cattle breeding, in order to achieve better production results, optimal environmental conditions in the barns must be ensured. The interrelation between ambient temperature and relative humidity is important from the aspect of animal welfare, reproduction and finally profitability of dairy farm. Any extreme combination is potentially harmful. In environments with low temperature and high humidity, cows increase heat production and consume more feed in order to compensate body energy losses. When the animal is overheated, high humidity could cause respiratory

or udder infections (Vermunt and Tranter, 2011). On the other hand, high temperature and low relative humidity may dehydrate mucous membranes, thus increasing vulnerability to viruses and bacteria (Romaniuk and Overby, 2005). The combination of high temperature and high relative humidity has the most detrimental effect through inducing heat stress in cows. Under heat stress conditions, lactating cows tend to reduce their dry matter intake (DMI) and milk production (West et al., 1999). Moreover, besides milk production heat stress is associated with changes in milk composition, somatic cell counts (SCC) and mastitis frequencies (Rodriguez et al., 1985; Nickerson, 1987; Ravagnolo et al., 2000; Bouraoui et al., 2002; St-Pierre et al., 2003; West, 2003; Correa-Calderon et al., 2004; Collier and Hall, 2012).

The most appropriate measure of heat stress in dairy cows is the temperature-humidity index (THI) that presents a combination of ambient temperature and relative humidity and is a useful and easy way to assess the risk of heat stress (Kibler, 1964). Hill and Wall (2015) observed that in cattle kept indoors, increasing THI values were associated with an overall decrease in milk yield, while when cattle were outside, milk yield increased with THI up to THI threshold value, followed by a decrease as THI continued to increase. Lambertz et al. (2013) found out that dairy cows were exposed to heat stress not only during summer months but also conditions under temperate climate. The high producing cows are much more susceptible to heat stress than low producing cows (Kadzere et al., 2002; Tapki and Sahin, 2006). Du Preez et al. (1990 a, b) determined that milk production and feed intake is affected by heat stress when THI values are higher than 72. Bouraoui et al. (2002) put the threshold on 69, while Bernabucci et al. (2010) as well as Collier and Hall (2012) on 68. Vitali et al. (2009) suggested that the risk of cow's death starts to increase when THI reaches 80. The significant decrease of daily milk traits (yield and content) was also determined in Croatian environmental conditions with highest decline during summer period in Eastern Croatia (Gantner et al., 2011). In many dairy-producing areas of the world heat stress condition represents a significant financial burden, for example in the USA around $900 million/year. The significant economic losses of the dairy farms could also be caused by metabolic disorders prevalence.

Ketosis is a metabolic disorder that can occur in both clinical and subclinical forms where subclinical ketosis is defined as a preclinical stage of ketosis (Shaw, 1956). Clinical ketosis most frequently occurs in high producing cows between the 2nd and 7th week after calving as a consequence of inadequate nutrition and management (Gillund et al., 2001). Prevalence of ketosis could be influenced by breed, parity, season and herd-related factors. Rajala-Schultz and Gröhn (1999) as well as Østergaard and Gröhn (1999) quoted that clinical ketosis induces economic losses to the dairy farmer through treatment costs, decreased milk production, impaired reproduction efficiency, and increased involuntary culling. Subclinical ketosis can be revealed by determining levels of plasma glucose, plasma non-esterified fatty acids, milk or urine ketone body concentration (Andersson, 1988).

Another metabolic disorder, subacute ruminal acidosis (SARA) has become an increasing problem in well-managed, high yielding dairy herds. SARA occurs in indoor dairy cows to the extent of 19% during early lactation and 26% in mid-lactation

while in grazing dairy cows the prevalence was determined between 10% and 15% (Bramley et al., 2005; O'Grady et al., 2008). Dirksen et al. (1985) stated that the early lactation cows are at higher risk due to reduced absorptive capacity of the rumen, poorly adapted rumen microflora, and rapid introduction to high-energy dense diets. Oetzel (2005) observed that cows at peak DM intake are at increased risk due to the greater amount of acids produced in the rumen. Regarding SARA diagnosis Enemark (2008) stated that recent developments in technology led to the use of indwelling rumen pH probes, as well as to the use of rumen valerate and urinary net acid base excretion.

Considering that heat stress affects feeding behaviour of dairy cattle (decreases feed intake, induces selective consumption of concentrates and minimal intake of forages) it could be assumed that predisposition of dairy cows to metabolic diseases could also be affected (Collier et al., 2006). Conversely, Sanker et al. (2013) found that heat stress did not influence the incidence of metabolic treatments.

For monitoring the herd health, test-day records (TDR) represent an alternative which is much more cost effective and non-invasive when compared to above mentioned specific diagnostic methods (Duffiled et al., 1997; Duffiled, 2004; Eicher, 2004). TDR includes daily milk, fat and protein production, and fat to protein ratio (F/P ratio). Beening (1993) and Gravert (1991) indicated that the ideal range for F/P ratio is 1–1.25, while Duffield et al. (1997) sets 1.33 as upper margin. Haas and Hofirek (2004) reported that the F/P ratio higher than 1.4 indicates energy deficit and, if ketone bodies are present, subclinical ketosis is indicated. Duffield (2004) and Richardt (2004) defined a 1.5 value of F/P ratio as risk level for subclinical ketosis, while Eicher (2004) beside F/P ratio, for indication of metabolic disorders (acidosis, ketosis) also took into account daily milk production.

The objective of this study was to evaluate the effect of heat stress on daily milk traits (yield, fat and protein content, F/P ratio) as well as to determine the differences in metabolic disorders (acidosis, ketosis) prevalence risk regarding the heat stress using monthly test-day records.

Material and methods

Individual test-day records of Holstein cows collected in regular milk recording performed by alternative milk recording method (AT4/BT4) from January 2009 to December 2013 were used for analysis. Monthly, at each recording, milk yields were measured during the evening or morning milkings, alternately. Also, one milk sample was taken from each cow during the recorded milking. Additionally, at each recording, temperature (Ta) and relative humidity (RH) in the barns were measured with digital data-logger PCE-HT71. Daily milk yield and fat content was projected from partial ones according to correction factors by DeLorenzo and Wiggans (1986). Logical control of data was performed according to ICAR standards (2003). Variability of daily milk yield, fat and protein content as well as fat/protein ratio according to parity is reported in Table 1.

Table 1. Descriptive statistics for milk traits according to parity (n = 1,187,781)

Trait*	DMY (kg)			DFC (%)			DPC (%)			F / P		
Statistic	mean	SD	CV	mean	SD	CV	mean	SD	CV	mean	SD	CV
Parity 1	19.95	7.3	36.5	4.16	0.9	22.2	3.41	0.5	13.4	1.23	0.3	21.6
Parity 2	22.06	9.7	43.5	4.20	0.9	23.0	3.45	0.5	13.9	1.23	0.3	22.3
Parity 3	22.30	9.7	43.5	4.19	1.0	23.2	3.41	0.5	14.0	1.24	0.3	23.2
Parity 4	21.77	9.5	43.9	4.17	1.0	23.2	3.38	0.5	13.9	1.25	0.3	23.4
Parity 5+	20.18	8.9	44.1	4.12	0.9	23.0	3.34	0.5	13.9	1.24	0.3	23.2

*Trait: DMY – daily milk yield; DFC – daily fat content; DPC – daily protein content; F/P – fat to protein ratio.

Records with lactation stage in day 0 < and > 500 days, missing or nonsense parity, Ta and RH value were deleted from dataset. Data, provided by the Croatian Agricultural Agency, after logical control consisted of 1,187,781 test-day records of milk, fat, and protein from 89,030 cows reared on 6,388 farms in Croatia. Regarding the stage of lactation (in days), cows were divided into seven groups: L1 (< 30), L2 (30–60), L3 (61–90), L4 (91–120), L5 (121–150), L6 (151–180), L7 (>180). According to parity, five classes were formed: P1, P2, P3, P4 and P5+ that included cows in 5th and higher lactations. Daily THI was calculated using the equation by Kibler (1964):

$$THI = 1.8 \times Ta – (1 – RH) \times (Ta – 14.3) + 32$$

Variability of Ta, RH and THI per recording season is presented in Table 2.

Table 2. Environmental conditions in the barns during the recording seasons (n = 149,709)

Season*	Temperature, Ta (°C)				Relative humidity, RH (%)				THI			
	mean	SD	min	max	mean	SD	min	max	mean	SD	min	max
S1	15.1	5.9	1.0	30.0	68.7	10.8	30.0	99.0	58.7	8.8	34.7	84.1
S2	23.4	5.4	10.0	40.0	68.6	11.1	30.0	99.0	71.2	8.0	50.2	101.2
S3	15.7	5.8	1.0	30.0	71.4	10.3	30.0	99.0	59.8	8.8	34.4	85.8
S4	8.9	4.9	−9.0	20.0	72.9	10.2	30.0	99.0	49.5	7.6	16.0	67.6

*Season: S1 – Spring (March, April and May); S2 – Summer (June, July and August); S3 – Autumn (September, October and November); S4 – Winter (December, January and February).

According to the daily THI value, two classes of heat stress status were defined: HS when THI ≥ 72 meaning **stressful environment**, HN when THI < 72 meaning normal environmental conditions.

The effect of heat stress on milk traits was determined using the following mixed model separately by parity:

$$y_{ijklm} = \mu + b_1(d_i/305) + b_2(di/305)^2 + b_3\ln(305/d_i) + b_4\ln^2(305/d_i) + S_j + R_k + H_l + e_{ijklm}$$

where:

y_{ijklm} – estimated daily milk yield, fat content, protein content or F/P ratio,
μ – intercept,
b_1, b_2, b_3, b_4 – regression coefficients,
d_i – days in milk,
S_j – fixed effect of season j (j = S1; S2; S3; S4),
R_k – fixed effect of region k (Croatian counties, k =1, 2,.....20),
H_l – fixed effect of heat stress status l (l = HN, HS),
e_{ijklm} – residual.

The significance of the differences between the classes of heat stress status was tested by Scheffe's method of multiple comparisons using the MIXED procedure of SAS (SAS Institute Inc., 2000).

The risk of metabolic disorders prevalence was indicated by the F/P ratio where (Eicher, 2004): K when F/P ≥ 1.5 meaning **ketosis risk**, N when F/P 1.0–1.5 meaning normal conditions, A when F/P < 1.0 meaning **acidosis risk**.

The metabolic disorders prevalence was defined as incidence risk and was calculated as frequency of cows indicated with risk of metabolic disorder in total number of cows in regard to heat stress and lactation stage classes separately for each parity.

Results

A significant variation of daily milk traits (yield, fat and protein content) due to heat stress conditions (THI ≥ 72) was determined in this research (Table 3). Statistically highly significant (P<0.01) decline caused by heat stress was determined in daily milk yield and daily fat and protein content in all parity classes. Higher values of F/P ratio in stress condition were determined in 1st, 2nd and 4th lactations.

Table 3. Estimated LSMs of heat stress status on daily milk traits by parity (n = 1,187,781)

Trait*	DMY (kg)		DFC (%)		DPC (%)		F / P	
HS status	HN	HS	HN	HS	HN	HS	HN	HS
Parity 1	18.67 A	18.16 B	4.11 A	4.10 B	3.42 A	3.39 A	1.21 A	1.22 A
Parity 2	20.74 A	20.51 B	4.06 A	4.03 A	3.41 A	3.38 B	1.20 A	1.21 A
Parity 3	20.53 A	20.42 B	4.13 A	4.10 A	3.39 A	3.35 B	1.23A	1.23 A
Parity 4	20.23 A	20.09 B	4.10 A	4.07 A	3.36 A	3.32 B	1.23 A	1.24 A
Parity 5+	19.34 A	19.09 B	4.05 A	4.01 A	3.31 A	3.28 B	1.23 A	1.23 A

*Trait: DMY – daily milk yield; DFC – daily fat content; DPC – daily protein content; F/P – fat to protein ratio; HS status: HN – normal environment; HS – stressful environment; LSMs marked with different letter (A, B) differ statistically highly significant (P<0.01).

Regarding the acidosis prevalence risk, increased risk in heat stressed condition compared to normal environment was determined in almost all classes of lactation stage and parity with exceptions marked in Table 4. In terms of heat stress, acidosis prevalence risk increases from 0.3 to 4.1%, in regard to the lactation stage (seven groups: L1 (< 30), L2 (30–60), L3 (61–90), L4 (91–120), L5 (121–150), L6 (151–180), L7 (>180)) and parity (five groups: P1, P2, P3, P4 and P5[+]).

Regarding the ketosis prevalence risk, the trend was not so simple. Analysing the heat stress effect on ketosis risk in the first 60 days of lactation, lower risks were determined in stressed than normal conditions in all lactations. In the first parity, from 61st to 150th days of lactation increased ketosis risk in stressed condition was found, after which a slight decline was observed. In the second parity from 61st day of lactation ketosis risk increased due to heat stress. Heat stressed cows in the third parity had lower ketosis risk during almost all lactation (exception was L5 class) comparable to non-stressed animals. Higher ketosis risk due to high temperature and high humidity in barns was in cows in the fourth lactation after 90th day of lactation.

Table 4. Risk of metabolic disorder prevalence (%) regarding the heat stress and lactation stage classes by parity (n = 1,187,781)

Disorder / Stress status	Lactation stage classes													
	L1		L2		L3		L4		L5		L6		L7	
	HN	HS	HN	HS	HN	HS	HN	HS	HN	HS	HN	HS	HN	HS
Parity = P1														
A	17.1	19.4	17.7	19.2	19.3	20.9	19.9	21.5	20.3	21.8	19.7	20.7	17.7	18.3
K	16.2	12.9	22.0	19.6	14.0	14.3	10.4	11.3	8.6	8.8	7.6	6.8	5.6	5.2
N	66.8	67.7	60.3	61.3	66.7	64.8	69.7	67.3	71.2	69.4	72.7	72.6	76.8	76.4
Parity = P2														
A	18.4	21.4	18.5	21.5	20.9	22.2	20.9	22.0	20.2	20.1	19.4	21.1	19.3	20.7
K	18.4	17.1	22.6	21.3	15.8	16.1	12.4	13.9	10.9	12.1	9.8	9.7	5.7	5.8
N	63.2	61.5	58.8	57.2	63.2	61.7	66.6	64.1	68.9	67.8	70.8	69.1	75.0	73.5
Parity = P3														
A	18.4	20.8	17.3	17.0	19.1	19.4	19.5	20.6	20.0	17.9	18.6	21.3	19.5	20.4
K	22.1	19.0	26.4	23.5	18.3	19.1	14.8	13.7	11.9	13.8	11.2	10.8	6.7	6.5
N	59.5	60.2	56.3	59.5	62.6	61.5	65.7	65.7	67.9	68.3	70.2	67.9	73.8	73.1
Parity = P4														
A	17.7	20.7	15.0	18.5	17.2	17.9	17.9	18.3	18.3	17.2	18.5	19.2	18.9	19.6
K	23.9	20.8	28.2	23.7	20.4	17.5	15.4	16.5	12.5	14.2	11.0	10.6	7.2	6.5
N	58.3	58.4	56.7	57.8	62.4	64.6	66.6	65.2	69.1	68.6	70.5	70.1	73.8	73.8
Parity = P5+														
A	17.6	21.7	13.9	18.0	16.2	19.7	16.4	17.7	16.7	19.8	17.3	19.6	19.3	20.7
K	23.3	18.8	28.3	25.8	20.1	17.8	15.4	13.8	11.9	14.2	11.0	11.4	6.9	6.9
N	59.1	59.5	57.8	56.2	63.7	62.5	68.2	68.6	71.3	66.0	71.7	69.1	73.9	72.4

* HS status: HN – normal environment; HS – stressful environment; Disorder: A – acidosis risk; K – ketosis risk; N – normal.

Cows in the fifth and higher lactations had lower ketosis risk during all lactation (with exception of L5 class) in heat stressed than normal conditions. Regardless of the parity, lower ketosis risks were determined in stressed than normal conditions during the early lactation. In the mid-lactation ketosis risk was higher in stressed animals, while in late lactation the prevalence of ketosis risk was low and similar regardless of the environmental conditions. The highest ketosis risk was determined in the first 60 days of lactation (with higher values in normal compared to stressed conditions), while the lowest frequency was observed after the 180th day.

Discussion

Similar results to those determined in this study were obtained by Du Preez et al. (1990 a, b) who determined milk yield decreases during summer compared to winter in about 10%–40% cows. Bouraoui et al. (2002) reported that milk production in dairy cows begins to decline when THI reaches the value of 69 by 0.41 kg per cow per day for each point increase above threshold, while Collier and Hall (2012) observed that daily THI of 68 results in a milk loss of 2.2 kg/day for each cow. Lambertz et al. (2013) concluded that heat stress condition resulted in decreasing daily milk yield with lowest value in THI class ≤ 45 and THI class 55–60. Hill and Wall (2015) determined that relation between milk production and THI value differs regarding the housing. Accordingly, when cows were outdoors, milk yield increased with increasing THI to 24.0 kg at 54.9 THI units, after which it decreased as THI continued to increase. When cattle were indoors, increasing THI values were associated with an overall decrease in milk yield.

Similarly to this research, Bouraoui et al. (2002) determined decrease of daily fat (3.24 vs. 3.58%) and protein (2.88 vs. 2.96%) content, as well as decrease of daily fat (0.68 vs. 0.48) and protein (0.56 vs. 0.43) yields during heat stress and normal condition that is in summer in regard to spring period. The depressions in milk yield, fat and protein percentages associated with heat stress environments were also determined by Rodrigez et al. (1985). Decrease of fat, and protein percentages, and increase of SCS in heat stress condition were also determined by Lambertz et al. (2013). Hill and Wall (2015) determined an overall decrease of fat content with increase of THI for cows kept outdoors, while for cows indoors, milk fat increased to a maximum of 3.8% at 50.2 THI units, and then decreased with THI increase. In the same research, protein content decreased as THI increased for both cows kept outdoors and indoors, while the decrease was greater when animals were outside than inside. Conversely, Knapp and Grummer (1991) found no significant decrease in fat content for cows under heat stress as well as decrease of protein with increase of maximum daily temperature. The reduction in milk protein was probably caused by a decreased dry matter intake and energy intake.

High milk production leads to the formation and release of large amounts of metabolic heat, so when temperature increases above the optimum interval value (4–25°C; Roenfeldt, 1998), cows lose the ability for the adequate cooling and enter-

ing the heat stress. Consequently, heat stress induces increase of body temperature. When the body temperature is significantly elevated, feed intake, metabolism, body weight and milk yields decrease to help alleviate the heat imbalance (West et al., 1999). Accurately identifying heat-stressed cows and understanding the biological mechanisms by which thermal stress reduces milk synthesis and reproductive indices is critical for developing effective approaches (i.e. genetic, managerial and nutritional) to maintain production or minimize the reduction in dairy cow productivity during stressful summer months.

Regarding the acidosis prevalence risk, Oetzel (2005) stated that early lactation cows and cows at peak DM intake are most at risk from SARA, while in this study highest risk was determined during the mid-lactation. Similar trend of ketosis incidence risk was obtained by Rajala-Schultz et al. (1999). Dohoo and Martin (1984) determined prevalence peak of positive ketosis test results in the 21–25 day period postpartum, and 56% of the positive results were found in the interval between 11 and 35 days of lactation. They also determined losses of 1.0 and 1.4 kg milk/day associated with positive reactions (+1 and +2) on subclinical ketosis test. The prevalence peak in the first month of lactation and significant negative effect of subclinical ketosis on daily milk yield was also determined in research of Gantner et al. (2009). The reduction of milk yield within 14 days before the diagnosis of clinical ketosis was found by Rajala-Schultz et al. (1999). Same authors determined that milk yield started to decrease 2 to 4 weeks before the diagnosis of clinical ketosis and continued to decline for a varying time period afterwards. They determined greatest loss within the 2 weeks after the diagnosis in the interval from 3.0 to 5.3 kg/day in accordance to parity. Østergaard and Gröhn (1999) determined high milk yield as a risk factor for ketosis and enteritis. They also stated that metabolic diseases had a more significant detrimental effect on body weight loss than did reproductive disorders and mastitis. Toni et al. (2011) determined that cows with a very high F/P ratio in milk in early lactation suffer from both increased disease and culling incidence, while in cows in second and higher lactations, an increase in F/P ratio was also associated with decreased milk production, particularly in early lactation.

During the late dry period and early lactation, an insufficient DMI in combination with a high energy need caused by milk production initiation leads to a negative energy balance (NEBAL). Because milk fat concentration tends to increase and milk protein concentration tends to decrease during the postpartum negative energy balance, the early postpartum F/P ratio in milk could be used as a potential indicator of a lack of energy supply through feed. The F/P ratio in milk with a value outside the range from 1 to 1.5 is a strong predictor of cow health events, early milk production performance, and survival in the current lactation.

Conclusion

Heat stress condition in the barns caused the decline in daily milk yield and content of fat and protein, while F/P ratio increased in terms of heat stress during the 1st, 2nd and 4th lactations. The increased acidosis risk due to heat stressed condition was determined in almost all classes of lactation stage and parity with few exceptions. The highest ketosis risk was determined in early lactation (till 60th day), while the

lowest frequency was after the 180th day. Lower ketosis risks were determined in stressed than normal conditions during the early lactation. In the mid-lactation ketosis risk was higher in stressed animals, while in late lactation ketosis risk was low and similar regardless of the environmental condition. The research results point out that heat stress condition causes decline of daily milk yield and components as well as increase of acidosis risk regardless of the lactation stage and increase of ketosis risk in mid-lactation. The test-day records and environmental measurements collected in regular milk recording could be used as a tool for dairy herd monitoring. By early detection and treatment of the heat stressed condition and the subclinical disorders of animals, farmer's economic losses could be decreased or completely avoided. Since environmental conditions significantly affect daily milk yield and components, and consequently F/P ratio further research with the purpose of detailed formulation of metabolic disease risk and environmental conditions relation is needed.

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