

*Poster presentation*

## FLUCTUATION OF DAILY PROTEIN AND UREA IN MILK OF DAIRY COWS IN EASTERN CROATIA DUE TO HEAT STRESS

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### Abstract

Considering the rapid climate change worldwide in order to reduce financial losses of dairy farmers and enable the sustainable farming, the necessity of implementation of breeding values for heat resistance in breeding strategies, have become more and more pronounced. First step in estimation of breeding values is determination of THI threshold value. Therefore, the objective of this paper was to determine THI threshold value for daily protein and urea content in first parity Holsteins and dairy Simmentals in Eastern Croatia. With that purpose, test-day records with data on ambient temperature and relative humidity in the barns collected in regular milk recording from January 2005 to December 2012 were analyzed. Based on analyzed data it could be concluded that  $THI \geq 66$  cause significant change in daily protein and urea content in milk of Holstein and Simmental first parity cows. Daily protein contents statistically highly significant decrease due to heat stress condition (THI in 66 – 80) in both, Holsteins and Simmentals, while daily urea content statistically highly significant increase. The  $THI = 66$ , as the lowest value at which significant decrease in analyzed traits was determined could be taken as the threshold value for first parity cows in Eastern Croatia.

*Keywords:* dairy cows, heat stress, threshold, Eastern Croatia

### Introduction

The impact of the climate change becomes is more and more expressed worldwide. In accordance to the forecasts (IPCC, 2007), in future dairy cattle will be exposed to the unfavorable climatic conditions in regions that at the time are not characterized as extreme climate. Accordingly, Reiczigel et al., (2009) in Hungary determined increase of heat stress days/year (temperature-humidity index  $THI > 68$ ) from 5 to 17 in a period of 30 years. Also, Gauly et al., (2013) stated that when the scenarios of global warming are considered, heat stress of high-yielding dairy cows is an increasing concern of milk producers in Europe. Dunn et al., (2014) concluded that the in future the number of days exceeding the

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THI threshold in southern parts of the UK could will increase from on average 1–2 per year to over 20 per year by 2100. In dairy cattle breeding in indoor housing, the optimal microclimate conditions in the barns are necessary for the realization of the productive potential of individual cows. The interrelation between ambient temperature and relative humidity is important from the aspect of animal welfare, reproduction and finally profitability of dairy farm. 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 (Bouraoui et al., 2002.; Collier and Hall, 2012; Correa-Calderon et al., 2004; Ravagnolo et al., 2000.; St-Pierre et al., 2003; West, 2003). Additionally, deteriorate effect on reproductive performances was also observed (Bohmanova et al., 2007; Ravagnolo et al., 2000). The most common measure of heat stress in dairy cows is the temperature-humidity index (THI) that present combination of ambient temperature and relative humidity and is a useful and easy way to assess the risk of heat stress (Kibler, 1964). Du Preez et al., (1990a, 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 contents) was also determined in Croatian environmental conditions with highest decline during summer period in Eastern and Mediterranean Croatia (Gantner et al., 2011). In many dairy-producing areas of the world heat stress condition represent a significant financial burden, for example in the USA between \$897 million and \$1,500 million per year (St-Pierre et al., 2003). There are many methods to decrease the impact of heat stress including the shading, cooling, nutrition (Kadzere et al., 2002; West, 2003) and selection for resistance on heat stress (Bohmanova, 2006). Ravagnolo et al., (2000) determine deteriorate effect of selection on productivity on cow's resistance to heat stress due to antagonistic relationship between cow's production and heat tolerance. The unfavorable genetic relationship between THI and productive and reproductive traits was found in few studies (Ravagnolo and Misztal, 2002a,b; Freitas et al., 2006; Aguilar et al., 2009). On the other hand, the high yielding Holstein cows in Israel is good example that selection on production could be successful in terms of heat stress (Aharoni et al., 1999). Implementation of breeding values for heat resistance in breeding strategies would certainly reduce financial losses of dairy farmers and enable sustainable farming. Estimation of breeding values requires determination of THI threshold value. Therefore, the objective of this paper was to determine THI threshold value for daily protein and urea content in milk of first parity Holsteins and Simmentals in Eastern Croatia.

### **Material and Methods**

Individual test-day records of first parity Holstein and Simmental dairy cows collected in regular milk recording performed by alternative milk recording method from January 2005 to December 2012 in Eastern Croatia were used for the analysis. Monthly, at each recording, milk yields were measured during the evening or morning milkings. Logical control of milk data was performed according to ICAR standards (2003). Additionally, at each recording, ambient temperature and relative humidity in the barns were recorded.

Daily temperature-humidity index (THI) was calculated using the equation by Kibler, (1964):

$$\text{THI} = 1.8 \times \text{Ta} - (1 - \text{RH}) \times (\text{Ta} - 14.3) + 32$$

Where Ta is average temperature in degrees Celsius and RH is relative humidity as a fraction of the unit. Records with lactation stage in (< 6 days and > 500 days), age at first calving in (< 21 and > 36 months), missing or parity > 1, and missing or nonsense Ta and RH value were deleted from the dataset. Only cows with minimum 3 test day per lactation were taken into analysis. Data, provided by the Croatian Agricultural Agency, after logical control, consisted of 205,714 test-day records from 24,307 first parity Holsteins, 188,512 test-day records from 30,013 first parity Simmentals and 322,174 records of microclimate parameters.

The THI threshold values for daily protein and urea content in milk were determined by least square analyses of variance for each given THI value (*from 66 to 80*) in regard to the breed (Holstein, Simmental) using the PROC MIXED procedure in SAS (SAS Institute Inc., 2000). Following mixed model was used:

$$y_{ijklm} = \mu + b_1(d_i / 305) + b_2(d_i / 305)^2 + b_3 \ln(305/d_i) + b_4 \ln^2(305/d_i) + S_j + A_k + R_l + T_m + e_{ijklm}$$

Where  $y_{ijklm}$  = estimated daily protein and urea content);

$\mu$  = intercept;

$b_1, b_2, b_3, b_4$  = regression coefficients;

$d_i$  = days in milk ( $i = 6$  to 500 day);

$S_j$  = fixed effect of calving season class  $j$  ( $j = 1/2005$  to 12/2012);

$A_k$  = fixed effect of age at calving class  $k$  ( $k = 21$  to 36 month),

$R_l$  = fixed effect of region  $k$  ( $l =$  Croatian counties),

$T_m$  = fixed effect of THI class ( $m = 0$  (*normal condition – values under the given threshold*) or 1 (*heat stress condition – values equal and above the given threshold*)),

$e_{ijklm}$  = residual.

The significance of the differences between the THI classes were tested by Scheffe's method of multiple comparisons. The lowest threshold value at which significant differences in analyzed traits was determined, was taken as the threshold value.

### Results and Discussion

Analysis of the microclimate parameters recorded in the barns located in the Eastern Croatia during the summer season (June, July, August) in period 2005 – 2012 are presented in Figure 1 (Holsteins) and Figure 2 (Simmentals).

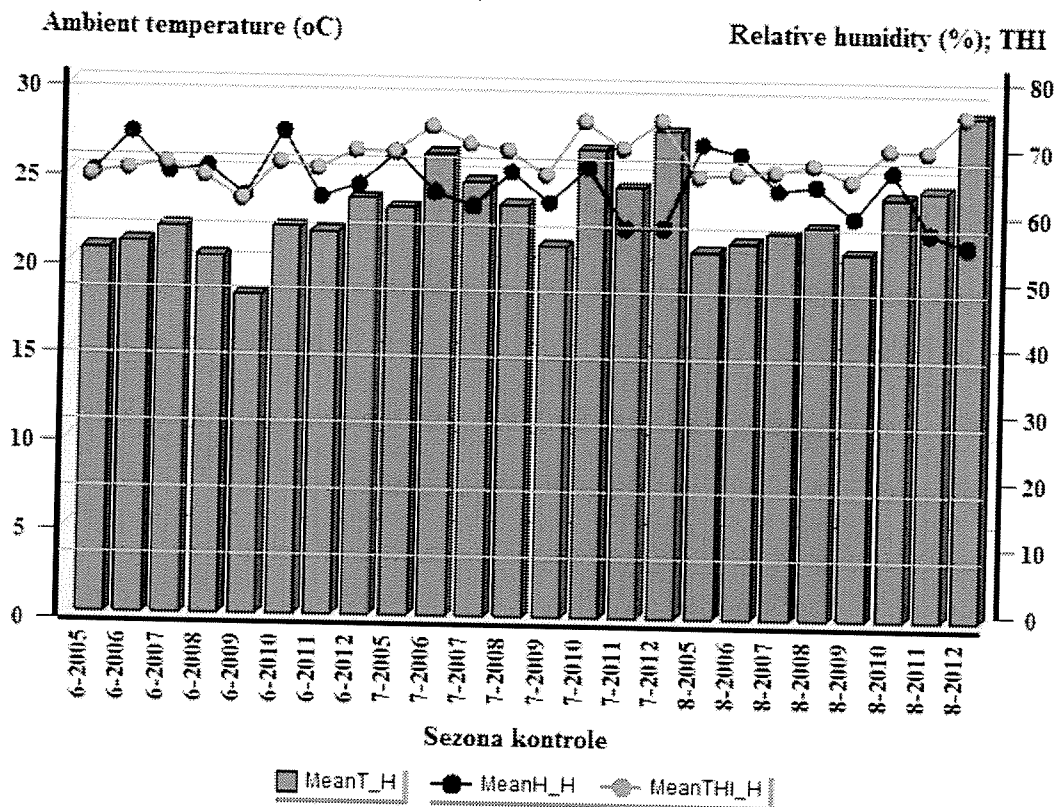


Figure 1. Mean daily ambient temperature, relative humidity and THI measured during milk recording of Holstein cows

During the June, mean ambient temperature in Holstein's barns varied in interval from 19 till 26°C, in July from 21 till 27°C, while August characterized mean ambient temperatures in interval from 21 till 29°C. Taking into account that upper limit of cow's comfort zone is 25°C (Yousef, 1985), even mean values of measured ambient temperatures in Holstein's barns indicate prevalence of heat stress conditions. Maximum measured daily ambient temperatures reached 40°C. Combination of these high temperatures with relatively high relative humidity resulted in heat stresses condition during the entire summer period.

Similar mean values of environmental parameters were also determined in the Simmental's barns, while higher prevalence of days with extreme conditions ambient temperature = 40°C, relative humidity > 96%, as well as THI > 96) was observed.

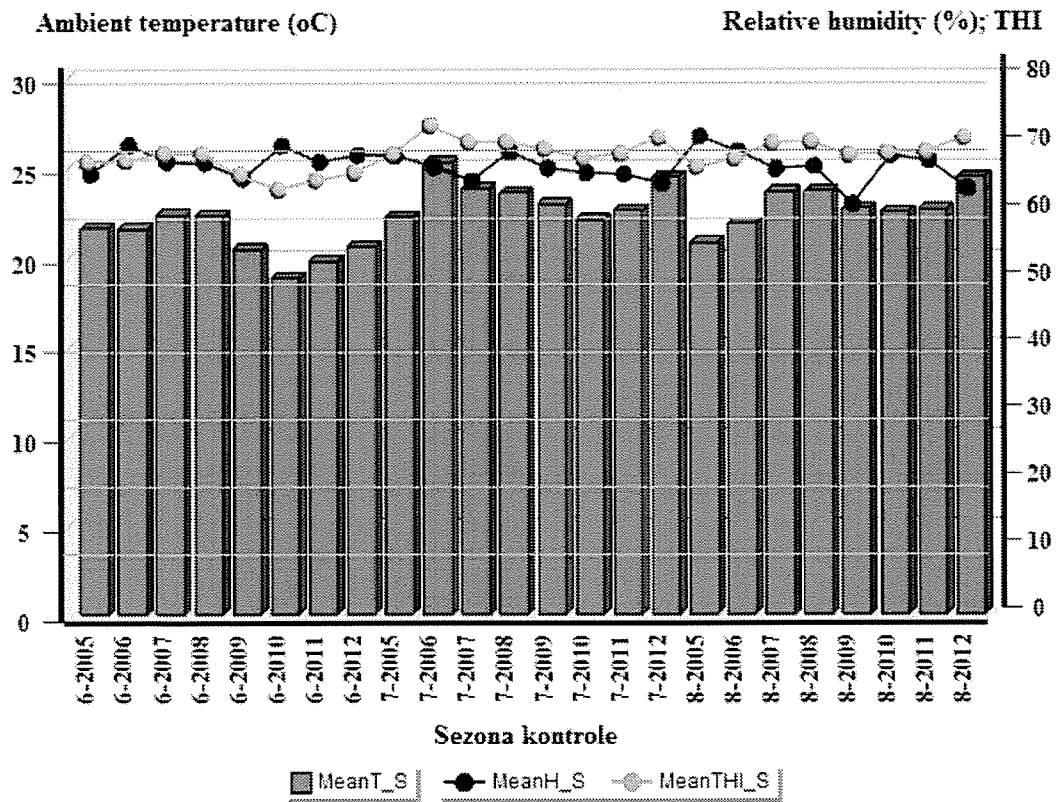


Figure 2 Mean daily ambient temperature, relative humidity and THI measured during milk recording of Simmental cows

Least square means regarding the fixed effect of THI class (0, 1) on daily protein content in accordance to the breed (Holstein, Simmental) are shown in Table 1. Environmental conditions in the barns with THI values in 66 caused statistically highly significant difference in daily protein content in milk of both analyzed breeds.

Table 1 Least square means of daily protein content in milk (%) regarding the given threshold value in accordance to the cattle breed

ThHo	Holstein			Simmental		
	Ls0	Ls1	Difference	Ls0	Ls1	Difference
THI66	3.45 ± 0.002	3.31 ± 0.002	0.135 ± 0.002***	3.47 ± 0.004	3.35 ± 0.004	0.117 ± 0.003***
THI67	3.45 ± 0.001	3.31 ± 0.002	0.134 ± 0.002***	3.47 ± 0.004	3.34 ± 0.004	0.121 ± 0.003***
THI68	3.44 ± 0.003	3.31 ± 0.004	0.130 ± 0.002***	3.46 ± 0.004	3.34 ± 0.004	0.122 ± 0.003***
THI69	3.44 ± 0.005	3.31 ± 0.005	0.132 ± 0.002***	3.46 ± 0.004	3.34 ± 0.005	0.122 ± 0.003***
THI70	3.44 ± 0.002	3.31 ± 0.003	0.131 ± 0.002***	3.46 ± 0.004	3.33 ± 0.005	0.127 ± 0.004***
THI71	3.44 ± 0.000	3.30 ± 0.001	0.135 ± 0.002***	3.46 ± 0.004	3.32 ± 0.005	0.135 ± 0.004***
THI72	3.44 ± 0.000	3.29 ± 0.000	0.143 ± 0.002***	3.46 ± 0.004	3.32 ± 0.005	0.135 ± 0.004***
THI73	3.43 ± 0.002	3.29 ± 0.003	0.147 ± 0.002***	3.45 ± 0.004	3.32 ± 0.005	0.139 ± 0.004***
THI74	3.43 ± 0.002	3.28 ± 0.003	0.152 ± 0.003***	3.45 ± 0.004	3.31 ± 0.006	0.141 ± 0.005***
THI75	3.43 ± 0.001	3.28 ± 0.003	0.154 ± 0.003***	3.45 ± 0.004	3.31 ± 0.006	0.145 ± 0.005***
THI76	3.43 ± 0.003	3.27 ± 0.004	0.153 ± 0.003***	3.45 ± 0.004	3.30 ± 0.006	0.145 ± 0.006***
THI77	3.43 ± 0.002	3.27 ± 0.004	0.158 ± 0.003***	3.45 ± 0.004	3.29 ± 0.007	0.159 ± 0.006***
THI78	3.42 ± 0.003	3.28 ± 0.004	0.147 ± 0.004***	3.45 ± 0.004	3.28 ± 0.007	0.164 ± 0.007***
THI79	3.42 ± 0.002	3.27 ± 0.005	0.148 ± 0.004***	3.45 ± 0.004	3.29 ± 0.008	0.161 ± 0.008***
THI80	3.42 ± 0.003	3.26 ± 0.006	0.163 ± 0.005***	3.45 ± 0.004	3.27 ± 0.009	0.174 ± 0.009***

Higher drop due to heat stress condition in the Eastern Croatia was determined in first parity Holsteins comparing to the Simmentals. The highest decrease in daily protein content was

determined in environmental condition characterized with THI = 80 in amount of 0.163 %/day in Holsteins and 0.174 %/day in Simmental cows.

The decrease of daily protein (2.88 vs. 2.96%) content, as well as decrease of daily protein (0.56 vs. 0.43) yields during heat stress and normal condition that is in summer in regard to spring period was also determined by Bouraoui et al., (2002). The drop in daily protein contents caused by heat stress environments were also determined by Rodriguez et al., (1985), Kadzere et al., (2002) and Lambertz et al., (2014). Knapp and Grummer, (1991) the decrease of protein with increase of maximum daily temperature explained by a decreased dry matter intake and energy intake.

Table 2. Least square means of daily urea content in milk (mg/dl) regarding the given threshold value in accordance to the cattle breed

ThHo	Holstein			Simmental		
	Ls0	Ls1	Difference	Ls0	Ls1	Difference
THI66	22.01±0.065	25.94 ±0.073	-4.161±0.054***	20.36 ±0.143	24.82 ±0.159	-4.464±0.090***
THI67	22.08±0.065	26.07 ±0.074	-4.188±0.055***	20.41 ±0.143	24.95 ±0.160	-4.544±0.093***
THI68	22.14±0.065	26.15 ±0.075	-4.251±0.057***	20.49 ±0.143	25.06 ±0.163	-4.564±0.097***
THI69	22.21±0.065	26.17 ±0.076	-4.260±0.059***	20.55 ±0.143	25.27 ±0.166	-4.720±0.102***
THI70	22.26±0.065	26.29 ±0.077	-4.121±0.063***	20.62 ±0.143	25.45 ±0.169	-4.829±0.107***
THI71	22.33±0.065	26.42 ±0.079	-4.028±0.066***	20.69 ±0.143	25.85 ±0.174	-5.153±0.114***
THI72	22.36±0.065	26.82 ±0.081	-4.097±0.070***	20.78 ±0.143	25.99 ±0.179	-5.212±0.121***
THI73	22.46±0.065	26.81 ±0.084	-4.115±0.074***	20.85 ±0.143	26.00 ±0.185	-5.152±0.129***
THI74	22.56±0.065	26.66 ±0.088	-4.146±0.080***	20.91 ±0.143	26.10 ±0.191	-5.195±0.137***
THI75	22.61±0.065	26.62 ±0.091	-4.058±0.086***	20.96 ±0.143	26.33 ±0.200	-5.365±0.149***
THI76	22.66±0.065	26.85 ±0.094	-4.212±0.094***	21.01 ±0.143	26.46 ±0.210	-5.443±0.162***
THI77	22.70±0.065	27.24 ±0.100	-4.273±0.104***	21.08 ±0.144	26.32 ±0.225	-5.239±0.181***
THI78	22.79±0.066	26.73 ±0.111	-4.362±0.115***	21.13 ±0.144	26.33 ±0.241	-5.201±0.200***
THI79	22.84±0.066	25.95 ±0.121	-4.454±0.129***	21.16 ±0.144	26.49 ±0.258	-5.328±0.221***
THI80	22.85±0.066	26.60 ±0.134	-4.648±0.146***	21.19 ±0.144	26.93 ±0.287	-5.738±0.254***

Analysis of daily urea content in milk regarding the given threshold value separately for each breed is given in the Table 2. Statistically highly significant increase of daily urea content was determined at all tested THI values (66 – 80) in both analyzed breed. Comparing to the Holstein breed, in Simmentals increase was higher at all threshold values. The increase of daily urea content amounted from 4.28 – 4.648 mg/dl in Holstein first parity cows, while in Simmentals increase ranged from 4.464 – 5.738 mg/dl.

Obtained results indicate similar trends in fluctuation of analyzed traits due to heat stress condition in both breeds. Regarding the variation in daily protein content, smaller decrease was determined in Simmentals comparing to the Holsteins, while when daily urea content was analyzed, higher differences were determined in Simmental breed.

### Conclusion

Based on analyzed data it could be concluded that  $THI \geq 66$  cause significant change in daily protein and urea content in milk of Holstein and Simmental first parity cows. Daily protein contents statistically highly significant decrease due to heat stress condition (THI in 66 – 80) in both, Holsteins and Simmentals, while daily urea content statistically highly significant increase. The  $THI = 66$ , as the lowest value at which significant decrease in analyzed traits was determined could taken as the threshold value for first parity cows in Eastern Croatia.

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