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Differences in response to heat stress due to production level and breed of dairy cows

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Abstract The climatic conditions in Croatia are deteriorating which significantly increases the frequency of heat stress. This creates a need for an adequate dairy farming strategy. The impact of heat stress can be reduced in many ways, but the best long-term solution includes the genetic evaluation and selection for heat stress resistance. In order to create the basis for genetic evaluation, this research determined the variation in daily milk yield (DMY) and somatic cell count (SCC) as well as the differences in resistance to heat stress due to production level (high, low) and breed (Holstein, Simmental) of dairy cattle breed in Croatia. For statistical analysis, 1,070,554 test-day records from 70,135 Holsteins reared on 5679 farms and 1,300,683 test-day records from 86,013 Simmentals reared on 8827 farms in Croatia provided by the Croatian Agricultural Agency were used. The results of this research indicate that the high-producing cows are much more susceptible to heat stress than low-producing especially Holsteins. Also, the results of this research indicate that Simmental breed, in terms of daily milk production and somatic cell count, could be more resistant to heat stress than Holstein. The following research should determine whether Simmentals are genetically more appropriate for the challenges that are in store for the future milk production in this region. Furthermore, could an adequate production level be achieved with Simmentals by maintaining the heat resistance?

Keywords Heat stress resistance · Dairy cattle · Daily milk yield · Somatic cell count

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Introduction

The current dairy cattle production is mostly characterised by a demand for high production per cow in the environment that is rapidly changing to a less adequate one in terms of cattle breeding. Climate change is imminent according to the forecasts (IPCC 2007) and will have great impact on animal production worldwide. Battisti and Naylor (2009) stated that by year 2050, most of the world will experience median temperatures in the summer that are warmer than the warmest temperatures on record. Accordingly, Reiczigel et al. (2009), in Hungary, determined the 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 in the global warming scenarios, the heat stress of high-producing dairy cows will be an increasing concern of milk producers in Europe, while Segnalini et al. (2013) emphasise the necessity of appropriate adaptation strategies development in order to minimise the negative effects of warming in farm animals in the Mediterranean basin. Dunn et al. (2014) determined that in the future, the number of days exceeding the THI threshold value in southern parts of the UK could increase from an average 1–2 per year to over 20 per year by 2100. Regarding Europe, GIRA–Consultancy and Research Prospective and Strategie (2012), in the analysis of *Regional movements in EU Milk Production*, forecasts the movement

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from regions with intensive farming towards regions around the Atlantic with less intensive farming and more land suitable for pasture (meaning lower production costs). Hansen (2013) stated that production increase makes cows more susceptible to heat stress; therefore, even if the climate change does not occur, heat stress will become an important problem. The modern dairy cow, with its high levels of productivity, begins losing the ability to regulate its body temperature at air temperatures as low as 25–29 °C. Studies of Bohmanova (2006) and Collier et al. (2006) showed that the high-producing cows are much more susceptible to heat stress than low-producing. Kadzere et al. (2002) suggested that the thermoregulation physiology of dairy cattle has been changed due to intensive genetic selection for milk production. The cows that produce more milk have larger frames and larger gastrointestinal tracts which allow the digestion of more feed. This results in more metabolic heat and reduces cow's ability to maintain normal temperature at heat stress conditions. Kadzere et al. (2002) concluded that the increase of milk yield, feed intake and metabolic heat shifts the thermoneutrality to lower temperatures. Furthermore, Berman (2005) stated that the increase in the daily milk yield from 35 to 45 kg/day leads to a higher sensitivity to thermal stress and reduces the threshold temperature for intermediate heat stress by 5 °C. Heat stress in dairy cows reduces dry matter intake, milk production (West et al. 1999; Casa and Ravelo 2003) and reproductive performances (Bohmanova et al. 2007; Ravagnolo et al. 2000). Considering that heat stress affects feeding behaviour of dairy cattle, Collier et al. (2006) presume that predispose of dairy cows to metabolism disorders (acidosis/ketosis) could also be affected. Conversely, Sanker et al. (2013) found that heat stress did not influence the incidence of metabolism treatments. Also, heat stress is associated with changes in milk composition, somatic cell counts (SCC) and mastitis frequencies (Bouraoui et al. 2002; Collier et al. 2012; Correa-Calderon et al. 2004; Gantner et al. 2011; Ravagnolo et al. 2000; St-Pierre et al. 2003; West 2003; Hammami et al. 2013; Smith et al. 2013). Furthermore, the heat stress condition induces significant loss of profit, for example in the USA between \$897 million and \$1500 million per year (St-Pierre et al. 2003). The heat stress could be measured variously, but the most common measure in dairy cattle is the temperature-humidity index (THI). THI presents the 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 et al. (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 the highest decline during the summer period in Eastern and Mediterranean Croatia (Gantner et al. 2011). The question is how do we effectively decrease the impact of heat stress on Croatian dairy farms? There are many methods to decrease the impact of heat stress for example shading, cooling and nutrition (Valtorta et al. 1997; Kadzere et al. 2002; West 2003). Also, selection for heat stress resistance could be an effective, long-term method (Bohmanova 2006). The antagonistic relationship between cow's production and heat tolerance was determined by Ravagnolo et al. (2000). This relationship implies the deterioration effect of the selection on productivity on cow's resistance to heat stress. The unfavourable genetic relationship between THI and productive and reproductive traits was also found in other studies (Ravagnolo and Misztal 2002a, b; Freitas et al. 2006; Aguilar et al. 2009). On the other hand, the high-producing Holsteins in Israel show that selection on production could be successful in terms of heat stress (Aharoni et al. 1999). Most of the research concerning heat stress was conducted on Holsteins, only few studies compare milk production of Jersey and Holstein breeds in terms of heat stress (Harris et al. 1960; Collier et al. 1981; Smith et al. 2013). Our earlier research (Gantner et al. 2017) aiming at the determination of THI threshold value for daily milk traits (yield, fat and protein content) of dairy cattle (Holsteins and Simmentals) indicated higher resistance to heat stress in Simmentals than Holsteins.

The objectives of this research were to determine the variation in daily milk yield (DMY) and somatic cell count (SCC) as well as the differences in resistance to heat stress due to production level and breed of dairy cattle in Croatia.

Materials and methods

Data

Individual test-day records of Holstein and Simmental dairy cows collected during the regular milk recording performed by alternative milk recording method (AT4/BT4) in the period from January 2005 to December 2012 in Croatia were used for the statistical analysis. Monthly, at each recording, milk yields were measured during the evening or morning milkings. Additionally, at each recording, ambient temperature and relative humidity were recorded. Daily temperature-humidity index (THI) was calculated using the equation by Kibler (1964):

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

where Ta is the average temperature in degrees Celsius and RH is the relative humidity as a fraction of the unit. Records with lactation stage in (<6 and >500 days), age at first calving in (<21 and >36 months), missing or parity >6/7 (Holstein/Simmental), and missing or nonsense Ta and RH value were deleted from the dataset. Regarding the production level, cows were divided into two classes (*high* (≥20/15 kg) and *low* (<20/15 kg) with limit put on 20 kg of milk/day for Holsteins and 15 kg of milk/day for Simmentals. Regarding the parity, cows were divided into three groups: first, second and third+. Also, only cows with minimum three test days per parity were taken into analysis. Data, provided by the Croatian Agricultural Agency, after logical control consisted of 1,070,554 test-day records from 70,135 Holsteins reared on 5679 farms and 1,300,683 test-day records from 86,013 Simmentals reared on 8827 farms in Croatia.

Statistical analysis

The variation in daily milk yield (DMY) and somatic cell count (SCC) due to heat stress was determined by least square analyses of variance for each given THI value (from 68 to 78) with regard to the production level (high, low) and breed (Holstein, Simmental) separately for each parity class (first, second, third+) using the PROC MIXED procedure in SAS (SAS User's Guide 2000). Used statistical mixed model included following effects: days in milk as lactation curve ($i = 6$ to 500 day); calving season as fixed class effect ($j = 1/2005$ to 12/2012); age at calving class as fixed class effect ($k = 21$ to 36 month)*only for first parity; region as fixed class effect (l = Croatian counties); and THI class as fixed class effect ($m = 0$ *(normal condition—values under the given threshold)* or 1 *(heat stress condition—values equal and above the given threshold)*).

The significance of the differences between the THI classes was tested by Scheffe's method of multiple comparisons.

Results

Mean monthly values of daily ambient temperature, relative humidity and THI measured during milk recording of dairy cows in period of 2005–2012 are presented in Figs. 1 (Holsteins) and 2 (Simmentals). Mean monthly ambient temperature during milk recording of Holsteins significantly exceeded 20 °C in June, July and August, while in September, it was a bit lower. Mean monthly THI reached the value of 70 during July. The analysis of the mean monthly values of microclimate parameters showed similar conditions during the milk recording of both breed, Holstein and Simmental. The maximum values of ambient temperature, relative humidity and THI measured during milk recording of dairy cows in period from June to September in accordance to recording year are presented in Tables 1 (Holstein) and 2 (Simmental). The extremely high temperature and THI values (>35 °C; >90) indicate that both breeds were in heat stress conditions during the summer period, with slightly less unfavourable conditions measured in September.

The differences in the daily milk yield production of high and low-producing first parity Holsteins and Simmentals due to heat stress conditions are presented in the Table 3. Statistically highly significant decrease of daily milk production in amount of 0.158 (THI = 68) to 0.335 kg/day (THI = 72) was observed in high-producing first parity Holsteins. Lower drop in daily milk production was observed in low-producing first parity Holsteins with first significant drop in amount of 0.059 kg/day at the THI = 73. The high-producing first parity Simmentals experienced statistically highly significant decrease of daily milk production at all tested THI values with

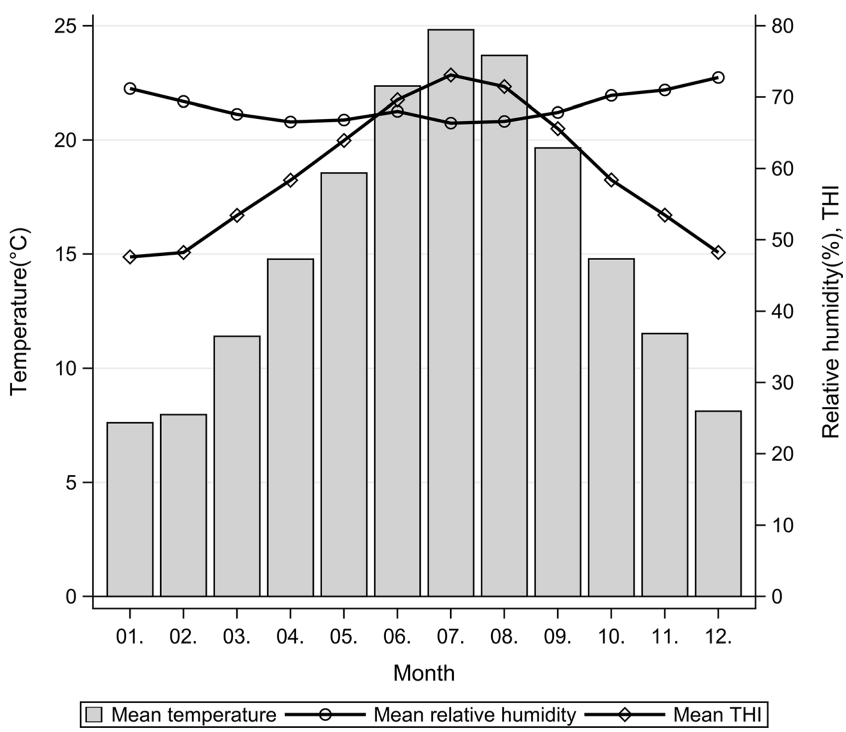

Fig. 1 Mean daily ambient temperature, relative humidity and THI measured during milk recording of Holstein cows

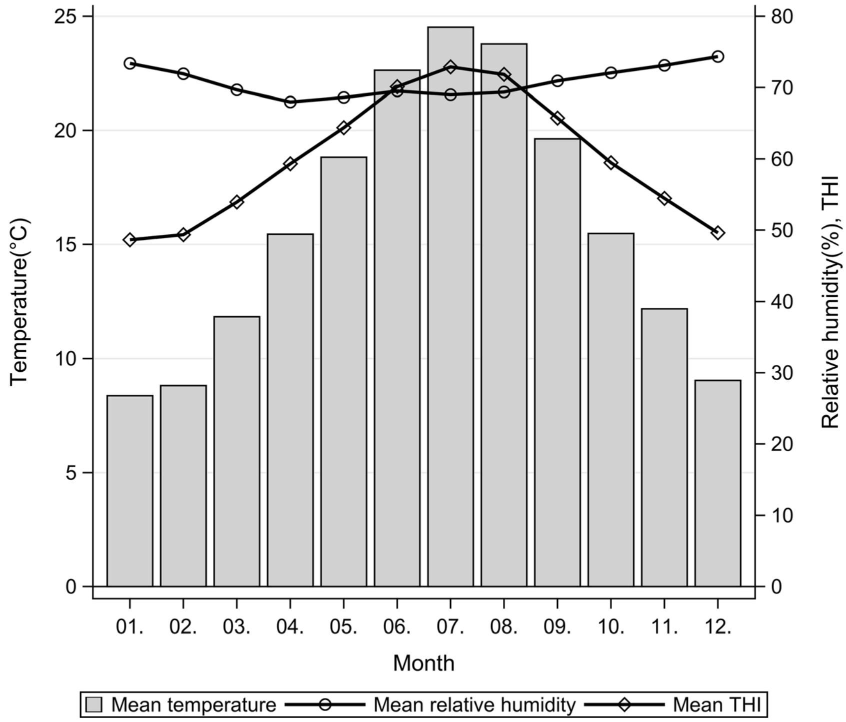

Fig. 2 Mean daily ambient temperature, relative humidity and THI measured during milk recording of Simmental cows

the highest drop when THI = 69. Although high-producing Simmentals exposed to the heat stress decreased their milk production, the drop was almost two times lower than in high-producing Holsteins. The analysis of the daily milk yield in low-producing first parity Simmentals showed small but significant increase of daily production when THI in (68, 77) and small, insignificant increase when THI = 78.

Least square means of daily milk yield (kg) regarding the given threshold value in accordance to the production level (high, low) and breed (Holstein, Simmental) for second parity cows are shown in the Table 4. A similar decreasing trend of daily milk yield due to heat stress as in first parity high-producing cows was also determined in second parity cows. In Holsteins, the drop was in interval from 0.424 to 0.768 kg/day and significantly higher than in first parity cows. A much lower drop in (0.064, 0.234) was observed in second parity Simmentals. Low-producing cows, of both breeds, experience statistically significant increase of daily milk yield at every tested THI value (68–78).

The variations in daily milk yield due to heat stress in the third+ parity cows regarding the production level and breed are presented in the Table 5. The decreasing (in high-producing) and increasing (in low-producing) trends of daily milk production were similar as in second parity cows. In high-producing Holsteins, the drop varied in (0.422, 0.763), while in Simmentals, the drop was smaller and varied in (0.090, 0.235). The increase in daily milk yield in low-producing Holsteins (0.023–0.130 kg/day) was a bit higher than in Simmentals (0.066–0.094 kg/day).

The variation in somatic cell count (SCC) due to heat stress in high and low-producing first parity cows is shown in the Table 6.

Table 1 Maximum values of ambient temperature, relative humidity and THI measured during milk recording of Holstein cows in period from June to September in accordance to recording year

Year	June			July			August			September		
	Temp	Hum	THI	Temp	Hum	THI	Temp	Hum	THI	Temp	Hum	THI
2005	35	99	90	38	99	94	35	99	91	30	99	86
2006	40	97	99	40	98	96	39	95	96	30	96	84
2007	39	96	101	40	96	99	39	96	95	30	99	83
2008	38	96	97	39	99	97	38	95	92	30	98	84
2009	35	98	90	40	96	97	38	99	95	30	96	82
2010	40	98	94	40	99	97	37	98	96	30	99	86
2011	40	99	100	40	99	101	39	98	100	30	98	84
2012	39	98	92	40	97	98	40	95	100	30	98	84

Temp ambient temperature, *Hum* relative humidity, *THI* temperature-humidity index

Table 2 Maximum values of ambient temperature, relative humidity and THI measured during milk recording of Simmental cows in period from June to September in accordance to recording year

Year	June			July			August			September		
	Temp	Hum	THI	Temp	Hum	THI	Temp	Hum	THI	Temp	Hum	THI
2005	36	99	91	40	99	97	36	99	94	30	99	86
2006	40	97	99	40	98	96	40	97	96	30	98	84
2007	39	96	101	40	96	99	39	96	95	30	99	83
2008	38	99	97	38	99	97	39	97	93	30	98	85
2009	35	98	90	40	96	97	38	96	94	30	96	83
2010	40	97	93	40	99	97	38	98	97	30	99	86
2011	40	99	100	40	99	101	40	98	100	30	98	85
2012	40	98	94	40	97	98	40	95	100	30	98	84

Temp ambient temperature, *Hum* relative humidity, *THI* temperature-humidity index

Statistically highly significant increase of SCC was determined in high-producing first parity Holsteins at all THI classes (68–78). A significant increase of SCC due to heat stress was also determined in low-producing first parity Holsteins, but the increase was lower than in high-producing cows. When Simmental first parity cows were analysed, a low decrease in

Table 3 Least square means (LSM) of daily milk yield (kg) regarding the given threshold value in accordance to the production level (high, low) and breed (Holstein, Simmental) for first parity cows

ThHo	High production (≥20 kg/15 kg)			Low production (<20 kg/15 kg)		
	LSM – 0	LSM – 1	Difference	LSM – 0	LSM – 1	Difference
Holstein						
THI68	25.62 ± 0.055	25.46 ± 0.059	0.158 ± 0.026***	14.45 ± 0.033	14.44 ± 0.035	0.014 ± 0.019n.s.
THI69	25.63 ± 0.055	25.38 ± 0.059	0.248 ± 0.027***	14.45 ± 0.033	14.44 ± 0.036	0.012 ± 0.019n.s.
THI70	25.63 ± 0.055	25.36 ± 0.060	0.267 ± 0.028***	14.45 ± 0.033	14.45 ± 0.036	−0.006 ± 0.020n.s.
THI71	25.63 ± 0.055	25.30 ± 0.060	0.334 ± 0.030***	14.45 ± 0.033	14.47 ± 0.037	−0.020 ± 0.021n.s.
THI72	25.63 ± 0.055	25.29 ± 0.061	0.335 ± 0.032***	14.45 ± 0.032	14.44 ± 0.038	0.010 ± 0.022n.s.
THI73	25.61 ± 0.055	25.37 ± 0.062	0.239 ± 0.034***	14.46 ± 0.032	14.40 ± 0.038	0.059 ± 0.024**
THI74	25.61 ± 0.055	25.34 ± 0.064	0.266 ± 0.036***	14.45 ± 0.032	14.40 ± 0.039	0.058 ± 0.025*
THI75	25.61 ± 0.055	25.33 ± 0.065	0.281 ± 0.038***	14.45 ± 0.032	14.45 ± 0.041	0.002 ± 0.027n.s.
THI76	25.60 ± 0.055	25.32 ± 0.066	0.279 ± 0.040***	14.45 ± 0.032	14.42 ± 0.042	0.026 ± 0.029n.s.
THI77	25.60 ± 0.055	25.28 ± 0.069	0.317 ± 0.044***	14.45 ± 0.032	14.37 ± 0.044	0.088 ± 0.032**
THI78	25.58 ± 0.055	25.54 ± 0.073	0.042 ± 0.050n.s.	14.46 ± 0.032	14.24 ± 0.047	0.223 ± 0.036***
Simmental						
THI68	19.41 ± 0.050	19.28 ± 0.052	0.131 ± 0.022***	11.28 ± 0.023	11.34 ± 0.025	−0.063 ± 0.014***
THI69	19.42 ± 0.050	19.27 ± 0.053	0.151 ± 0.023***	11.28 ± 0.023	11.34 ± 0.026	−0.060 ± 0.015***
THI70	19.41 ± 0.050	19.27 ± 0.053	0.140 ± 0.024***	11.28 ± 0.023	11.35 ± 0.026	−0.073 ± 0.016***
THI71	19.41 ± 0.050	19.27 ± 0.054	0.134 ± 0.025***	11.28 ± 0.023	11.36 ± 0.027	−0.078 ± 0.016***
THI72	19.40 ± 0.049	19.26 ± 0.054	0.142 ± 0.027***	11.28 ± 0.023	11.35 ± 0.027	−0.071 ± 0.017***
THI73	19.40 ± 0.049	19.27 ± 0.055	0.132 ± 0.028***	11.28 ± 0.023	11.37 ± 0.028	−0.089 ± 0.018***
THI74	19.40 ± 0.049	19.27 ± 0.056	0.127 ± 0.030***	11.28 ± 0.023	11.37 ± 0.029	−0.084 ± 0.020***
THI75	19.40 ± 0.049	19.26 ± 0.058	0.139 ± 0.032***	11.28 ± 0.023	11.36 ± 0.030	−0.079 ± 0.021***
THI76	19.39 ± 0.049	19.27 ± 0.059	0.127 ± 0.035***	11.28 ± 0.023	11.37 ± 0.031	−0.083 ± 0.023***
THI77	19.39 ± 0.049	19.27 ± 0.062	0.121 ± 0.039***	11.29 ± 0.023	11.34 ± 0.033	−0.052 ± 0.025*
THI78	9.39 ± 0.049	19.28 ± 0.064	0.109 ± 0.043***	11.29 ± 0.023	11.33 ± 0.035	−0.045 ± 0.028n.s.

ThHo given threshold value, *0* class under, *1* class above the given threshold value

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, *n.s.* $p > 0.05$

Table 4 Least square means (LSM) of daily milk yield (kg) regarding the given threshold value in accordance to the production level (high, low) and breed (Holstein, Simmental) for second parity cows

	High production (≥20 kg/15 kg)			Low production (<20 kg/15 kg)		
ThHo	LSM – 0	LSM – 1	Difference	LSM – 0	LSM – 1	Difference
Holstein						
THI68	28.34 ± 0.089	27.92 ± 0.094	424 ± 0.039***	4.26 ± 0.058	14.36 ± 0.061	−0.098 ± 0.023***
THI69	28.35 ± 0.089	27.83 ± 0.095	0.526 ± 0.040***	14.26 ± 0.058	14.36 ± 0.061	−0.091 ± 0.024***
THI70	28.36 ± 0.089	27.74 ± 0.095	0.619 ± 0.042***	14.27 ± 0.058	14.36 ± 0.062	−0.095 ± 0.025***
THI71	28.35 ± 0.089	27.70 ± 0.096	0.653 ± 0.044***	14.26 ± 0.058	14.39 ± 0.062	−0.125 ± 0.026***
THI72	28.34 ± 0.089	27.67 ± 0.098	0.667 ± 0.046***	14.27 ± 0.058	14.39 ± 0.063	−0.122 ± 0.028***
THI73	28.33 ± 0.089	27.68 ± 0.099	0.649 ± 0.049***	14.27 ± 0.058	14.38 ± 0.064	−0.116 ± 0.029***
THI74	28.32 ± 0.089	27.64 ± 0.101	0.685 ± 0.052***	14.27 ± 0.058	14.39 ± 0.065	−0.124 ± 0.031***
THI75	28.31 ± 0.089	27.70 ± 0.102	0.609 ± 0.055***	14.27 ± 0.058	14.42 ± 0.066	−0.149 ± 0.034***
THI76	28.30 ± 0.089	27.69 ± 0.105	0.602 ± 0.060***	14.27 ± 0.058	14.41 ± 0.067	−0.132 ± 0.036***
THI77	28.30 ± 0.089	27.53 ± 0.108	0.768 ± 0.066***	14.28 ± 0.058	14.38 ± 0.069	−0.099 ± 0.040*
THI78	28.28 ± 0.089	27.61 ± 0.113	0.675 ± 0.073***	14.28 ± 0.058	14.39 ± 0.072	−0.110 ± 0.045*
Simmental						
THI68	20.51 ± 0.052	20.45 ± 0.056	0.064 ± 0.027*	11.15 ± 0.035	11.25 ± 0.038	−0.101 ± 0.016***
THI69	20.51 ± 0.052	20.44 ± 0.056	0.072 ± 0.028*	11.15 ± 0.035	11.26 ± 0.038	−0.108 ± 0.017***
THI70	20.51 ± 0.052	20.44 ± 0.057	0.070 ± 0.029*	11.15 ± 0.035	11.27 ± 0.038	−0.113 ± 0.018***
THI71	20.51 ± 0.052	20.43 ± 0.058	0.077 ± 0.031*	11.16 ± 0.035	11.27 ± 0.039	−0.112 ± 0.019***
THI72	20.51 ± 0.052	20.40 ± 0.059	0.112 ± 0.033***	11.16 ± 0.035	11.26 ± 0.039	−0.102 ± 0.020***
THI73	20.51 ± 0.052	20.41 ± 0.060	0.100 ± 0.035**	11.16 ± 0.035	11.26 ± 0.040	−0.096 ± 0.021***
THI74	20.51 ± 0.052	20.37 ± 0.062	0.136 ± 0.037***	11.16 ± 0.035	11.28 ± 0.041	−0.118 ± 0.023***
THI75	20.51 ± 0.052	20.40 ± 0.063	0.103 ± 0.039**	11.16 ± 0.035	11.29 ± 0.042	−0.126 ± 0.024***
THI76	20.51 ± 0.052	20.39 ± 0.065	0.120 ± 0.043*	11.16 ± 0.035	11.30 ± 0.043	−0.133 ± 0.027***
THI77	20.51 ± 0.052	20.28 ± 0.069	0.228 ± 0.048***	11.17 ± 0.035	11.29 ± 0.045	−0.126 ± 0.029***
THI78	20.51 ± 0.052	20.27 ± 0.072	0.234 ± 0.052***	11.17 ± 0.035	11.28 ± 0.047	−0.116 ± 0.032***

ThHo given threshold value, *0* class under, *1* class above the given threshold value

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, *n.s.* $p > 0.05$

SCC was determined until THI = 77; while in THI ≥ 78, an insignificant increase was determined. In low-producing first parity Simmentals, statistically significant decrease of SCC was determined at all tested THI classes (68–78).

The response to heat stress with regard to the breed and production level of second parity cows is presented in the Table 7. A similar response as in high-producing first parity Holsteins, that is significant increase of SCC, was determined in second parity cows. However, low-producing second parity Holsteins in heat stress condition tend to decrease the SCC. In high-producing second parity Simmentals, the variation in SCC due to heat stress was insignificant while low-producing cows tend to cause statistically significant decrease of SCC.

The results for third+ parity cows are shown in the Table 8. The increase of SCC was determined only in high-producing Holsteins, while in high-producing Simmentals, the variations were mainly insignificant. In low-producing group, a significant drop was observed in Holsteins when THI in (68, 69, 70, 71), while in Simmentals, a statistically highly significant drop was determined at all tested THI values (68–78).

Discussion

The analysis of the microclimatic conditions during the milk recording of dairy cattle in Croatia indicates that mean daily temperature significantly exceeded 20 °C during the summer period with slightly less hot conditions in early autumn. The determined maximum values of daily ambient temperature (>35 °C) in combination with high maximum values of relative humidity (>90) resulted in incidence of summer days with THI > 90. Johnson (1987) estimated the optimal temperature zone for lactating dairy cows in (−0.5, 20 °C), while Berman et al. (1985) put the upper critical air temperature for dairy cows at 25–26 °C. The dependence of temperature and humidity was shown in the study of Bianca (1965) who

Table 5 Least square means (LSM) of daily milk yield (kg) regarding the given threshold value in accordance to the production level (high, low) and breed (Holstein, Simmental) for third+ parity cows

	High production (≥20 kg/15 kg)			Low production (<20 kg/15 kg)		
ThHo	LSM – 0	LSM – 1	Difference	LSM – 0	LSM – 1	Difference
Holstein						
THI68	28.68 ± 0.080	28.25 ± 0.084	0.422 ± 0.039***	14.08 ± 0.028	14.16 ± 0.032	−0.079 ± 0.021***
THI69	28.69 ± 0.079	28.13 ± 0.085	0.556 ± 0.040***	14.08 ± 0.028	14.17 ± 0.033	−0.094 ± 0.022***
THI70	28.67 ± 0.079	28.13 ± 0.086	0.549 ± 0.042***	14.08 ± 0.028	14.18 ± 0.033	−0.103 ± 0.022***
THI71	28.67 ± 0.079	28.06 ± 0.087	0.616 ± 0.044***	14.08 ± 0.028	14.21 ± 0.034	−0.130 ± 0.024***
THI72	28.68 ± 0.079	27.93 ± 0.089	0.745 ± 0.047***	14.08 ± 0.028	14.20 ± 0.035	−0.126 ± 0.025***
THI73	28.66 ± 0.079	27.98 ± 0.090	0.675 ± 0.049***	14.08 ± 0.028	14.20 ± 0.036	−0.121 ± 0.026***
THI74	28.65 ± 0.079	27.89 ± 0.092	0.763 ± 0.053***	14.09 ± 0.028	14.18 ± 0.038	−0.092 ± 0.028***
THI75	28.64 ± 0.079	27.95 ± 0.094	0.688 ± 0.056***	14.08 ± 0.028	14.21 ± 0.039	−0.130 ± 0.030***
THI76	28.62 ± 0.079	28.07 ± 0.097	0.545 ± 0.060***	14.09 ± 0.028	14.19 ± 0.041	−0.097 ± 0.033n.s.
THI77	28.61 ± 0.079	28.00 ± 0.101	0.610 ± 0.066***	14.09 ± 0.028	14.14 ± 0.044	−0.048 ± 0.036n.s.
THI78	28.60 ± 0.079	28.14 ± 0.105	0.464 ± 0.072***	14.09 ± 0.028	14.12 ± 0.047	−0.023 ± 0.040*
Simmental						
THI68	20.59 ± 0.048	20.50 ± 0.050	0.090 ± 0.019***	10.97 ± 0.030	11.05 ± 0.031	−0.085 ± 0.011***
THI69	20.58 ± 0.048	20.49 ± 0.051	0.095 ± 0.019***	10.97 ± 0.030	11.05 ± 0.031	−0.079 ± 0.011***
THI70	20.58 ± 0.048	20.48 ± 0.051	0.100 ± 0.020***	10.97 ± 0.030	11.06 ± 0.032	−0.090 ± 0.012***
THI71	20.58 ± 0.048	20.48 ± 0.051	0.101 ± 0.021***	10.97 ± 0.030	11.07 ± 0.032	−0.094 ± 0.013***
THI72	20.58 ± 0.048	20.48 ± 0.052	0.102 ± 0.022***	10.98 ± 0.030	11.05 ± 0.032	−0.077 ± 0.013***
THI73	20.58 ± 0.048	20.49 ± 0.053	0.090 ± 0.024***	10.98 ± 0.030	11.06 ± 0.033	−0.078 ± 0.014***
THI74	20.58 ± 0.048	20.46 ± 0.053	0.113 ± 0.025***	10.98 ± 0.030	11.06 ± 0.033	−0.084 ± 0.015***
THI75	20.58 ± 0.048	20.44 ± 0.054	0.141 ± 0.027***	10.98 ± 0.030	11.06 ± 0.033	−0.084 ± 0.016***
THI76	20.58 ± 0.048	20.40 ± 0.056	0.182 ± 0.029***	10.98 ± 0.030	11.06 ± 0.034	−0.079 ± 0.018***
THI77	20.58 ± 0.048	20.34 ± 0.057	0.235 ± 0.032***	10.98 ± 0.030	11.05 ± 0.035	−0.066 ± 0.019***
THI78	20.58 ± 0.048	20.34 ± 0.059	0.235 ± 0.036***	10.98 ± 0.030	11.06 ± 0.036	−0.072 ± 0.021***

ThHo given threshold value, *0* class under, *1* class above the given threshold value

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, *n.s.* $p > 0.05$

reported that at a temperature of 29 °C and 40% relative humidity, the milk yield of Holstein, Jersey and Brown Swiss cows was at 97, 93 and 98% of normal production, but when relative humidity increased to 90%, yields dropped to 69, 75 and 83% of normal production. Regarding the THI values, Vitali et al. (2009) suggested that the risk of cow's death starts to increase when THI = 80. These findings indicate that both dairy cattle breeds that were analysed were exposed to heat stress conditions during the summer and early autumn.

The analysis of the daily milk yield variations indicates that the response to heat stress highly depends on daily production level, cow's breed and parity. As for instance, high-producing cows of both breeds experienced decrease in daily milk yield in terms of heat stress in all parities (first, second and third+) with the highest decrease in the second parity. Furthermore, the Holsteins experienced higher drop in daily milk yield than Simmentals. The first significant drop of daily milk yield, in the high-producing cows, was observed at THI = 68 which could be taken as the threshold value for genetic evaluation. On the other hand, low-producing cows, with exception of first parity Holsteins, increased their daily milk production in the environment above the given THI.

The negative effect of heat stress on daily milk yield of dairy cattle was quite well researched. For instance, Casa and Ravelo (2003) concluded that heat stress in the warmer months in Argentina induces production decrease, in relation to the normal production level of 22 l/cow/day, in amount of 6% (9%) depending of the region. Bernabucci et al. (2010) and Collier et al. (2012) determined a decrease when THI = 68, while Bouraoui et al. (2002) in the Mediterranean climate observed a decrease in milk production of dairy cows when THI ≥ 69. Bouraoui et al. (2002) determined a decrease of milk yield by 0.41 kg per cow per day for each point increase in the value of THI above 69. Du Preez et al. (1990a,b) observed that dairy cows in South African conditions are affected by heat stress in case when THI values are higher than 72. The same authors determined that the amount of

Table 6 Least square means (LSM) of somatic cell count (in 000) regarding the given threshold value in accordance to the production level (high, low) and breed (Holstein, Simmental) for first parity cows

ThHo	High production (≥20 kg/15 kg)			Low production (<20 kg/15 kg)		
	LSM – 0	LSM – 1	Difference	LSM – 0	LSM – 1	Difference
Holstein						
THI68	128.40 ± 1.534	135.40 ± 1.629	−6.995 ± 0.732***	159.26 ± 1.200	161.50 ± 1.305	−2.238 ± 0.701***
THI69	128.56 ± 1.533	135.46 ± 1.642	−6.897 ± 0.758***	159.38 ± 1.199	161.25 ± 1.320	−1.863 ± 0.726*
THI70	128.88 ± 1.532	134.82 ± 1.656	−5.949 ± 0.785***	159.39 ± 1.198	161.43 ± 1.339	−2.041 ± 0.758***
THI71	128.68 ± 1.531	136.70 ± 1.677	−8.020 ± 0.825***	159.34 ± 1.196	161.93 ± 1.360	−2.582 ± 0.794***
THI72	128.87 ± 1.530	136.74 ± 1.705	−7.870 ± 0.880***	159.36 ± 1.195	162.19 ± 1.388	−2.836 ± 0.839***
THI73	129.23 ± 1.530	135.13 ± 1.732	−5.901 ± 0.932***	159.53 ± 1.194	161.50 ± 1.422	−1.971 ± 0.892*
THI74	129.25 ± 1.529	135.98 ± 1.771	−6.730 ± 1.000***	159.64 ± 1.193	160.95 ± 1.464	−1.315 ± 0.957n.s.
THI75	129.34 ± 1.529	135.84 ± 1.798	−6.499 ± 1.047***	159.50 ± 1.193	162.50 ± 1.511	−3.002 ± 1.028**
THI76	129.44 ± 1.528	135.96 ± 1.846	−6.519 ± 1.123***	159.61 ± 1.192	161.75 ± 1.573	−2.130 ± 1.114*
THI77	129.47 ± 1.528	136.75 ± 1.912	−7.278 ± 1.231***	159.68 ± 1.191	161.35 ± 1.653	−1.674 ± 1.222n.s.
THI78	129.51 ± 1.527	137.97 ± 2.025	−8.461 ± 1.403***	159.92 ± 1.191	156.75 ± 1.777	3.171 ± 1.385*
Simmental						
THI68	112.82 ± 1.695	111.50 ± 1.788	1.319 ± 0.762n.s.	134.86 ± 1.055	131.25 ± 1.182	3.617 ± 0.723***
THI69	112.86 ± 1.694	111.17 ± 1.802	1.690 ± 0.790*	134.76 ± 1.053	131.27 ± 1.200	3.481 ± 0.750***
THI70	112.83 ± 1.693	111.10 ± 1.818	1.732 ± 0.824*	134.69 ± 1.052	131.13 ± 1.224	3.557 ± 0.786***
THI71	112.77 ± 1.692	111.22 ± 1.838	1.550 ± 0.864n.s.	134.54 ± 1.050	131.39 ± 1.251	3.157 ± 0.824***
THI72	112.72 ± 1.691	111.28 ± 1.863	1.441 ± 0.912n.s.	134.37 ± 1.049	132.03 ± 1.284	2.332 ± 0.871**
THI73	112.64 ± 1.691	111.71 ± 1.891	0.928 ± 0.966n.s.	134.32 ± 1.048	132.02 ± 1.322	2.304 ± 0.926**
THI74	112.59 ± 1.690	111.98 ± 1.924	0.612 ± 1.029n.s.	134.34 ± 1.047	131.46 ± 1.366	2.880 ± 0.987**
THI75	112.59 ± 1.690	111.87 ± 1.966	0.713 ± 1.103n.s.	134.32 ± 1.046	131.25 ± 1.419	3.068 ± 1.058**
THI76	112.56 ± 1.689	112.05 ± 2.026	0.509 ± 1.205n.s.	134.29 ± 1.045	130.88 ± 1.494	3.418 ± 1.156**
THI77	112.49 ± 1.689	113.24 ± 2.110	−0.758 ± 1.341n.s.	134.23 ± 1.045	131.07 ± 1.585	3.157 ± 1.270**
THI78	112.46 ± 1.688	113.86 ± 2.197	−1.391 ± 1.473n.s.	134.18 ± 1.044	131.25 ± 1.695	.931 ± 1.405**

ThHo given threshold value, *0* class under, *1* class above the given threshold value

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, *n.s.* $p > 0.05$

milk yield for Holstein cows decreased about 10 to 40% during the summer period in comparison to the winter period (Du Preez et al. 1990b). A significant decrease of daily milk yield when THI ≥ 72 was also noticed in Croatia (Gantner et al. 2011). Research conducted in the USA determined different threshold values regarding the region, for example 72 in Georgia and 74 in Arizona (Bohmanova et al. 2007). The difference between determined threshold values could be due to better adapted cows, farm management or special housing characteristics. For instance, Lambertz et al. (2014) determined that a drop of fat-corrected milk (FCM) in Holstein cows highly depends on housing systems with the highest drop observed in the classic indoor system. They concluded that heat stress resulted in decreasing milk yield, fat and protein contents, and increasing somatic cell score (SCS).

The differences in response to heat stress due to the breed were analysed in several studies. For example, a higher drop of milk production in Holstein cows comparable to the other breeds (Jersey and Brown Swiss) was determined by Bianca (1965). Also, Collier et al. (1981) suggested that Jersey cows may be more heat tolerant than the Holsteins with respect to milk yield production. Smith et al. (2013) determined the increase of milk production of Jersey cows during heat stress. Also, in the same study, the authors determined a decrease of milk production of Holstein cows during heat stress. They concluded that Jersey cows used in their study could be more heat tolerant than Holstein cows.

Similarly, like daily milk yield, the differences in somatic cell count (SCC) due to heat stress highly depend on daily production level, breed and parity. The highest increase in the daily SCC was determined in first parity high-producing Holsteins, while statistically significant but lower increase was determined in multiparous cows. The first significant increase of SCC, in the high-producing Holsteins, was observed

Table 7 Least square means (LSM) of somatic cell count (in 000) regarding the given threshold value in accordance to the production level (high, low) and breed (Holstein, Simmental) for second parity cows

	High production (≥20 kg/15 kg)			Low production (<20 kg/15 kg)		
ThHo	LSM – 0	LSM – 1	Difference	LSM – 0	LSM – 1	Difference
Holstein						
THI68	150.52 ± 1.988	156.50 ± 2.094	−5.982 ± 0.866***	195.43 ± 3.211	191.84 ± 3.276	3.591 ± 0.895***
THI69	150.63 ± 1.987	156.67 ± 2.109	−6.039 ± 0.899***	195.41 ± 3.210	191.52 ± 3.286	3.890 ± 0.928***
THI70	150.92 ± 1.986	156.07 ± 2.128	−5.151 ± 0.938***	195.41 ± 3.209	191.00 ± 3.299	4.404 ± 0.970***
THI71	151.04 ± 1.985	155.98 ± 2.146	−4.943 ± 0.976***	195.12 ± 3.208	191.79 ± 3.316	3.331 ± 1.018***
THI72	151.16 ± 1.984	155.94 ± 2.176	−4.782 ± 1.037***	195.08 ± 3.207	191.57 ± 3.335	3.516 ± 1.079***
THI73	151.24 ± 1.983	156.02 ± 2.204	−4.776 ± 1.093***	195.00 ± 3.207	191.63 ± 3.356	3.371 ± 1.139***
THI74	151.30 ± 1.983	156.28 ± 2.243	−4.980 ± 1.167***	194.91 ± 3.206	191.83 ± 3.384	.083 ± 1.219**
THI75	151.47 ± 1.982	155.18 ± 2.278	−3.711 ± 1.232***	194.93 ± 3.206	191.13 ± 3.418	3.805 ± 1.309***
THI76	151.47 ± 1.982	156.03 ± 2.339	−4.561 ± 1.335***	194.90 ± 3.205	190.59 ± 3.465	4.308 ± 1.423***
THI77	151.67 ± 1.981	153.67 ± 2.418	−1.999 ± 1.469*	194.91 ± 3.205	189.53 ± 3.523	5.375 ± 1.559***
THI78	151.64 ± 1.981	154.92 ± 2.520	−3.285 ± 1.630*	194.93 ± 3.205	187.70 ± 3.611	7.229 ± 1.748***
Simmental						
THI68	129.11 ± 1.581	128.33 ± 1.691	0.781 ± 0.826n.s.	156.84 ± 2.458	150.23 ± 2.541	6.613 ± 0.863***
THI69	129.11 ± 1.579	128.28 ± 1.707	0.827 ± 0.856n.s.	156.69 ± 2.457	150.09 ± 2.554	6.605 ± 0.897***
THI70	129.09 ± 1.577	128.20 ± 1.730	0.890 ± 0.893n.s.	156.46 ± 2.456	150.25 ± 2.571	6.216 ± 0.939***
THI71	129.16 ± 1.575	127.67 ± 1.757	1.484 ± 0.936n.s.	156.18 ± 2.456	150.98 ± 2.589	5.204 ± 0.986***
THI72	129.01 ± 1.574	128.43 ± 1.787	0.578 ± 0.990n.s.	156.02 ± 2.455	151.29 ± 2.611	4.738 ± 1.042***
THI73	128.93 ± 1.573	128.89 ± 1.821	0.040 ± 1.047n.s.	155.91 ± 2.454	151.32 ± 2.639	4.586 ± 1.105***
THI74	128.88 ± 1.573	129.38 ± 1.861	−0.505 ± 1.114n.s.	155.86 ± 2.454	151.10 ± 2.671	4.756 ± 1.181***
THI75	128.90 ± 1.572	129.22 ± 1.909	−0.315 ± 1.191n.s.	155.76 ± 2.453	151.26 ± 2.712	4.503 ± 1.268***
THI76	128.90 ± 1.571	129.27 ± 1.979	−0.367 ± 1.298n.s.	155.63 ± 2.453	151.95 ± 2.773	3.671 ± 1.391**
THI77	128.84 ± 1.571	130.46 ± 2.078	−1.616 ± 1.443n.s.	155.59 ± 2.453	151.78 ± 2.848	3.808 ± 1.534*
THI78	128.85 ± 1.570	130.54 ± 2.178	−1.683 ± 1.582n.s.	155.53 ± 2.452	152.11 ± 2.936	3.423 ± 1.691*

ThHo given threshold value, *0* class under, *1* class above the given threshold value

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, *n.s.* $p > 0.05$

at THI = 68 which could be taken as threshold value for genetic evaluation. However, Simmentals, high and low producing, tended to decrease SCC with higher decrease in multiparous and low-producing cows.

The milk SCC presents the most important indicator of the udder health. The results indicate that in heat stress condition, Holsteins may experience higher prevalence of subclinical and clinical mastitis. The negative effects of heat stress with increasing SCC from spring to summer were also determined by Bouraoui et al. (2002). Lambertz et al. (2014) observed that increasing THI values were associated with increasing SCS in Holsteins, in four different housing systems. Smith et al. (2013) concluded, based on the analysis of heat stress effects in Holsteins and Jersey breed in terms of milk and component yields and somatic cell score, that Jersey cows appeared to be more heat tolerant than Holstein cows.

The results of this research comply with the findings of Kadzere et al. (2002), Bohmanova (2006), Collier et al. (2006) and Hansen (2013) which imply that the high-producing cows are much more susceptible to heat stress than low-producing. Also, the results of this research indicate that Simmental breed could be more resistant to heat stress than Holstein. Taking into consideration that the climate conditions are certainly going to deteriorate in Croatia, which means higher summer temperatures with more frequent prevalence of heat stress days, an adequate development strategy for dairy farming is necessary in order to ensure effective and profitable dairy farms. Further research should determine whether Simmentals are genetically more appropriate for the challenges that face the future milk production in this region. Furthermore, could an adequate production level be achieved with Simmentals by maintaining of the heat resistance?

Table 8 Least square means (LSM) of somatic cell count (in 000) regarding the given threshold value in accordance to the production level (high, low) and breed (Holstein, Simmental) for third+ parity cows

	High production (≥20 kg/15 kg)			Low production (<20 kg/15 kg)		
ThHo	LSM – 0	LSM – 1	Difference	LSM – 0	LSM – 1	Difference
Holstein						
THI68	178.02 ± 1.786	179.83 ± 1.898	−1.816 ± 0.870*	226.53 ± 1.714	224.12 ± 1.818	2.418 ± 0.819**
THI69	177.86 ± 1.784	180.78 ± 1.918	−2.917 ± 0.904**	226.52 ± 1.713	223.93 ± 1.833	2.591 ± 0.848**
THI70	178.05 ± 1.782	180.22 ± 1.937	−2.169 ± 0.938*	226.47 ± 1.711	223.80 ± 1.852	2.668 ± 0.886**
THI71	178.11 ± 1.781	180.20 ± 1.960	−2.085 ± 0.982*	226.33 ± 1.710	224.21 ± 1.874	2.118 ± 0.929*
THI72	178.00 ± 1.780	181.34 ± 1.997	−3.347 ± 1.051**	226.18 ± 1.709	224.79 ± 1.904	1.391 ± 0.986n.s
THI73	178.18 ± 1.779	180.40 ± 2.027	−2.221 ± 1.105*	226.20 ± 1.708	224.46 ± 1.935	1.739 ± 1.043n.s
THI74	178.21 ± 1.778	180.49 ± 2.074	−2.284 ± 1.187*	226.13 ± 1.708	224.74 ± 1.978	1.390 ± 1.120n.s
THI75	178.40 ± 1.778	178.79 ± 2.115	−0.394 ± 1.254n.s	226.03 ± 1.707	225.59 ± 2.028	0.432 ± 1.206n.s
THI76	178.44 ± 1.777	178.30 ± 2.178	0.144 ± 1.355n.s	225.95 ± 1.706	226.48 ± 2.098	−0.530 ± 1.317n.s
THI77	178.42 ± 1.777	178.65 ± 2.265	−0.237 ± 1.489n.s	226.01 ± 1.706	225.66 ± 2.177	0.340 ± 1.439n.s
THI78	178.43 ± 1.776	178.38 ± 2.365	0.049 ± 1.636n.s	226.03 ± 1.705	225.14 ± 2.280	0.894 ± 1.590n.s
Simmental						
THI68	155.90 ± 1.596	154.91 ± 1.659	0.993 ± 0.612n.s	195.16 ± 1.898	188.33 ± 1.951	6.828 ± 0.608***
THI69	155.89 ± 1.595	154.83 ± 1.669	1.058 ± 0.635n.s	194.94 ± 1.897	188.44 ± 1.959	6.500 ± 0.632***
THI70	155.94 ± 1.595	154.50 ± 1.680	1.440 ± 0.663*	194.81 ± 1.897	188.19 ± 1.970	6.623 ± 0.662***
THI71	155.89 ± 1.594	154.54 ± 1.693	1.351 ± 0.694*	194.69 ± 1.896	187.90 ± 1.982	6.787 ± 0.694***
THI72	155.82 ± 1.593	154.72 ± 1.712	1.097 ± 0.734n.s	194.51 ± 1.896	188.10 ± 1.996	6.410 ± 0.733***
THI73	155.76 ± 1.593	154.99 ± 1.731	0.774 ± 0.775n.s	194.36 ± 1.895	188.21 ± 2.014	6.152 ± 0.779***
THI74	155.74 ± 1.592	155.13 ± 1.755	0.609 ± 0.827n.s	194.30 ± 1.895	187.86 ± 2.035	6.440 ± 0.830***
THI75	155.77 ± 1.592	154.63 ± 1.785	1.139 ± 0.886n.s	194.19 ± 1.895	187.97 ± 2.059	6.216 ± 0.887***
THI76	155.73 ± 1.591	154.91 ± 1.829	0.822 ± 0.969n.s	194.09 ± 1.895	187.91 ± 2.096	6.175 ± 0.970***
THI77	155.72 ± 1.591	154.89 ± 1.884	0.834 ± 1.068n.s	194.01 ± 1.894	187.85 ± 2.143	6.160 ± 1.068***
THI78	155.73 ± 1.591	154.47 ± 1.947	1.265 ± 1.176n.s	193.90 ± 1.894	188.63 ± 2.202	5.265 ± 1.181***

ThHo given threshold value, *0* class under, *1* class above the given threshold value

*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, *n.s.* $p > 0.05$

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Compliance with ethical standards

Conflict of interest During the realisation of this research, there was no conflict of interest.

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