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Effects of thermal stress on physiological state and hormone concentrations in Holstein cows under arid climatic conditions

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Abstract

This study was conducted to investigate the effect of heat stress on the physiology of dairy cows and to detect the relationship between rectal temperature (RT) and respiration rate (RR), heart rate (HR), and plasma concentrations of cortisol, thyroxine, and prolactin. During the experiment, 44 Holstein cows were allocated to two groups for each season. The average temperature-humidity index (THI) values were 55 ± 2.31 in winter and 78 ± 1.9 in summer. As the THI values increased from 55 to 78, RR rose by 35 inspirations per minute, HR by 3 beats per minute, and RT by 1.2 °C. In addition, the average concentration of cortisol increased from 19.30 to 21.04 nmol/L, and prolactin from 58.52 to 129.79 ngm/L, whereas free thyroxine decreased from 15.43 to 14.01 pmol/L. Plasma sodium and potassium concentrations were similar in the two seasons. These results confirmed that RT is an indicator of the response in dairy cows to hot environmental temperatures. However, they also showed signs of stress, which were reflected in higher levels of cortisol and in certain physiological responses.

Keywords: dairy cows, heat stress, physiology, rectal temperature, temperature-humidity index
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Introduction

Heat stress is defined as an event that affects an animal's homeostasis and health owing to a physiologically harmful heat load (Gaughan *et al.*, 2012). The welfare and comfort of dairy cows have increasingly been seen as moral and practical concerns, especially in developed countries (Silanikove, 2000). Heat stress may prompt physiological dysfunction, which affects an animal's production and reproduction capacity negatively, and causes economic losses that are estimated to be billions of dollars (Rosenkrans *et al.*, 2010). Heat stress also influences a cow's health negatively by altering the normal physiological functions of the cow, which results in a higher incidence of udder health problems during summertime (Turk *et al.*, 2015). Animal discomfort because of heat stress has been found to be a primary cause of production losses in the global dairy industry, especially for high-producing cows (Thatcher *et al.*, 2010; Dunshea *et al.*, 2019). The usual on-farm mitigation technique to combat heat stress for dairy cows is to control the animal's thermal environment (Mader *et al.*, 2007).

The temperature-humidity index (THI) is the most commonly used measure of environmental heat stress that is used to assess the physical impact of the environment on the dairy cow (Polsky & Von Keyserlingk, 2017). Radiation can also contribute to heat gain by direct and indirect means (Krishnan *et al.*, 2017). However, owing to distance, significant differences can occur between THI, which is recorded daily in the cow shed, and data from the nearest weather station (Shock *et al.*, 2016). Thus, other physiological parameters such as RT, RR, panting, sweating, feed intake, standing time, and activity are also used to indicate heat stress (West, 2003; Schutz *et al.*, 2008; Hansen, 2013)

Therefore, it is essential to understand the mechanisms through which heat stress affects dairy cows adversely to develop abatement strategies. The objectives of the present study were to evaluate the effects of heat stress on the physiology of dairy cows and to detect the relationship between RT and several physiological and biochemical parameters in Holstein dairy cows raised in south-eastern Tunisia.

Materials and Methods

The guidelines for animal experiments were outlined by the Official Animal Care and Use Committee of the National Institute of Agronomy of Tunisia (protocol No 05/15), which approved this study.

This study was carried out in two seasons, namely winter (P1) (from 1 to 30 January; mean daily THI value 55 ± 2.31, no heat stress) and summer (P2) (from 1 to 30 August; mean daily THI value 78 ± 1.9, stress conditions) at El Hajeb Dairy Farm, Sfax (south-eastern Tunisia), situated at 34°44′26″ N and 10°45′37″ E. Daily mean, temperature, and humidity were recorded in the feeding area using a thermos hygrometer (HI 91610C, Hanna Instruments, Portugal). The THI was calculated according to the formula described by Kibler (1964):

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

where: Ta is the average ambient temperature in °C and
RH is the average relative humidity expressed as a fraction of the unit.
Daily averages were used.

The study was conducted on 44 mid-lactating Holstein cows. The cows were divided into two groups of 22 animals. The cows were 150 to 160 days post partum and varied in level of milk production. The cows that were used in winter had calved from mid August to early September and those that were used in the summer had calved in February or March. The cows were maintained in loose housing under a group management system that facilitated free movement and provided abundant air circulation. The nutrient requirements of the animals were met by feeding green fodder ad libitum and limited concentrate. The diets were based on a forage ratio of 59% and 51% and concentrate of 41% and 49% for P1 and P2, respectively. The concentrate consisted of corn, barley, soya bean meal, and mineral and vitamin supplement. Four equal meals of concentrate were fed daily (8 kg/cow/day) (Table 1). Water was available ad libitum. During the experiment, routine health management of the farm was followed to ensure that cows did not have health disorders.

Table 1 Composition of diets provided to Holstein dairy cows being monitored for the effects of heat stress

Nutritional profile, %	Concentrate	Oat hay	
		Winter	Summer
Dry matter	90.52	86.71	90.93
Crude protein	20.9	5.84	5.96
Crude fibre	6.55	31.26	32.39
Calcium	0.97	0.48	0.53
Phosphorus	0.7	0.11	0.12

Rectal temperature, HR, and RR were recorded daily before milking. Rectal temperature was measured by inserting a veterinary digital thermometer 60 mm into the rectum for 1 minute and the temperatures were recorded to one decimal point. HR was determined with a stethoscope for one minute. Respiratory rate was measured by counting the flank movements of individual cows for 1 minute of uninterrupted breathing and reported as the number of inspirations per minute. Blood samples were collected from caudal vein puncture at 07h00 into vacuum tubes (10 ml) before the cows had been milked and fed. A solution of EDTA (anticoagulant substance) was placed In each tube before sterilization. The samples were kept in an ice bath for a few hours until centrifugation (3000 rpm at 4 °C) to recover plasma. Blood plasma was analysed for sodium, potassium and free thyroxine (T4) using a radioimmunoassay kit (Immunotech, ref. 1363). The total cortisol concentration was determined with the 125I RIA kit (DiaSorin, CA-1529, CA-1549). For prolactin, the blood samples were taken 5 minutes after milking, and the samples were moved directly to the laboratory for analysis.

All data were analysed by repeated-measures one-way ANOVA and hypothesis testing was done at a 5% significance level using SAS (SAS Institute Inc., Cary, North Carolina, USA). The model was:

$$Y_{ijkl} = \mu + S_i + P_j + L_k + SL_{ij} + C_l + e_{ijkl}$$

Where: Y_{ijkl} = observations for physiological parameters;
μ = overall mean;
S_i = fixed effect of the season (i = winter, summer);
P_j= fixed effect of parity (multiparous or primiparous);
L_k = fixed effect of production level (high or low);
SL_{ij} = interaction of season with production level;
C_l = effect of the cow, and
e_{ijkl}= random error.

Estimates of Pearson correlations among the physiological measures were calculated and means were compared with Duncan's multiple range test.

Results and Discussion

The temperature-humidity index is used to assess heat stress in dairy production (Sammad *et al*., 2020; Ammer *et al*., 2018; Wildridge *et al*., 2018). According to Pinto *et al.* (2020), when THI exceeds a threshold value of 72, heat stress begins for dairy cattle. However, Heinicke *et al.* (2018) indicated a lower THI value of 67 as the threshold for heat stress. A THI above 72 results in hyperthermia-derived discomfort, altered physiology and a decline in milk yield and composition (Herbut *et al*., 2018; (Kadzere *et al*., 2002). The degree to which the cows are stressed also increases with the THI (Ray *et al.,* 2004, Ammer *et al*., 2018). But thermal stress may manifest differently for physiological measures, with RR being affected at a lower THI and RT increasing at a higher THI (Pinto *et al*., 2020). In this study, higher values of THI were recorded in summer (78 ± 1.9) compared with those observed in winter (55 ± 2.31). The average THI for the winter and summer periods of this study are shown in Figure 1. Thus, the cows were not subjected to thermal stress in winter, but were clearly subjected to thermal stress in summer.

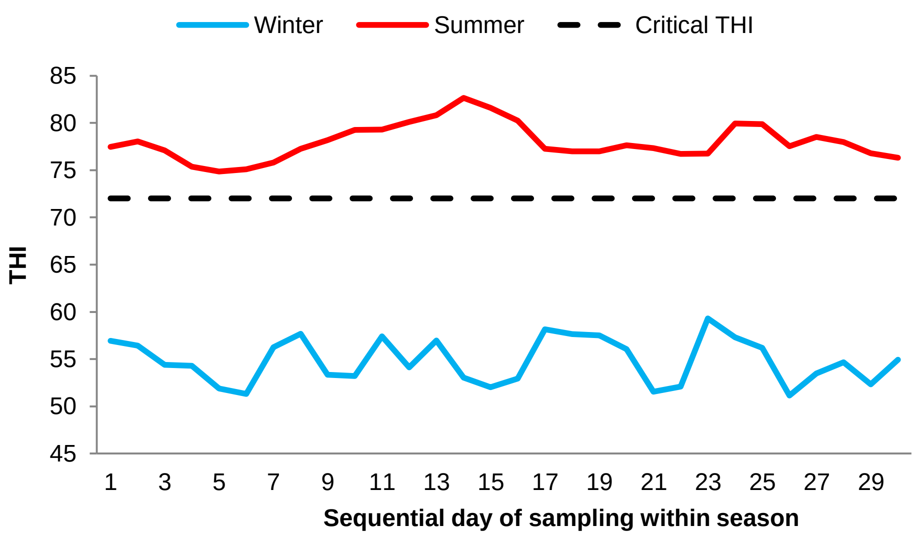

Figure 1 Daily variation of mean temperature-humidity index during winter and summer
THI: temperature-humidity index

Research indicated that RT, RR, and changes in some hormonal concentrations may be used as indicators of climatic stress (Li *et al*., 2020; Pinto *et al*., 2020; Amamou *et al*., 2019). In the present study, heat stress, as indicated by THI, altered (*P* <0.001) RT, respiratory, and heart rates (Table 2). Parity and production levels affected RT and RR significantly, as did the interaction between season and production level (*P* <0.001). On the other hand, parity did not affect the HR (*P* >0.05). A daily increase of 1.2 °C was observed for RT when the THI value increased from 55 to 78. Likewise, HR and RR increased by 3 beats and 35 inspirations per min, respectively. Such responses were adaptive mechanisms initiated by the cow in an attempt to restore its thermal balance. The current results agree with those reported in many studies (Valente *et al.*, 2015, Shilja *et al.*, 2016, Cardoso *et al.*, 2015) For example, Gonzalez Pereyra *et al. (*2010) reported that high-producing cows became heat stressed when the THI threshold for dairy cattle was above 78. However, Thatcher *et al. (*2010) reported that lactating cows were considered unstressed when the THI was less than 72. Various studies under field conditions demonstrated that body temperature increased with environmental temperatures above 21 °C in European breeds (McDowell, 1958).

Table 2 Effects of season, parity, and production level on respiration rate, heart rate, rectal temperature, cortisol, and plasma-free thyroxine and prolactin concentrations, potassium and sodium

	Mean	SE	Season	Parity	Level	Season x Level
RR per day, Insp/min	46.31	0.91	P <0.001	P >0.05	P <0.001	P <0.001
HR per day, beat/min	70.48	0.04	P <0.001	P >0.05	P <0.001	P <0.001
RT per day, °C	38.65	0.32	P <0.001	P <0.001	P <0.001	P <0.001
Cortisol, nmol L^{-1}	20.17	0.51	P <0.001	P >0.05	P <0.001	P >0.05
Thyroxin, pmol•L-1	14.67	0.43	P <0.001	P >0.05	P >0.05	P >0.05
Prolactin, ng mL^{-1}	94.15	0.94	P <0.001	P <0.001	P >0.05	P >0.05
Sodium, mmol L^{-1}	136.56	0.03	P >0.05	P >0.05	P >0.05	P >0.05
Potassium. mmol L^{-1}	4.36	0.09	P >0.05	P >0.05	P >0.05	P >0.05

RR: respiration rate, HR: heart rate, RT: rectal temperature

An elevated RT was evident at ambient temperatures greater than 25 °C, which was suggested to be the upper limit of temperature at which Holstein cows may maintain a thermal balance (Berman *et al.*, 1985). Data from the current study indicated that RT increased significantly (P <0.001) from winter to summer (Table 3). These findings agree with those of many studies that reported increased RT when lactating cows were subject to temperatures above their thermoneutral zone (Li *et al.*, 2020, Sejian *et al.*, 2017, Cardoso *et al.*, 2015). Cows are generally considered stressed by high temperature when their RT is above 39.2 ˚C (Thatcher *et al.*, 2010; Wenz *et al.*, 2011). Under Tunisian conditions, RT increased from 38.5 °C to 39.6 ˚C, indicating that the cows were under heat stress. Respiration rate increased from 53.75 inspirations per minute in winter to 80.6 inspirations per minute in summer, and HR increased from 63 beats/min to 77.3 beats/min with the seasonal change from winter to summer (Rejeb *et al.*, 2016). The overall averages for HR and RR were both significantly higher in summer from 07h00 to 22h00, with peaking at midday. This suggested that the cows were subject to heat stress mainly during the daylight hours. Similar results were observed for Friesian cows in South Africa (Muller and Botha, 2018; Williams *et al.*, 2016), by Bouraoui *et al.* (2002) in central Tunisia, and by Coppock *et al.* (1982), Ben Younes *et al.* (2011), and Rejeb *et al.* (2016).

Plasma sodium and potassium concentrations of cows were similar in both seasons and THI, parity, and production level did not affect these minerals (Table 4). Hormonal changes that occurred in response to heat stress may have played a role in the decline of milk yield as THI increased. The THI level and parity affected significantly (P <0.001) the circulating concentrations of the hormones that were studied. On the other hand, the production level of the cows and the interaction between THI and production level did not affect these hormones (P >0.05). Serum metabolites could be another indication of changes owing to heat stress (Yue *et al.*, 2020). Also, heat stress has resulted in hypofunction of the thyroid and affected metabolism patterns of mammals to reduce metabolic heat production (Helal *et al.,* 2010). As THI increased from 55 to 78, the free thyroxine concentration decreased from 15.43 to 14.01 pmol/L and thus Thompson *et al.* (1963) concluded that adaptation to high temperatures resulted in increased body temperature and decreased thyroid activity. The concentration of thyroxine (T4) is positively correlated with the metabolic rate in mammalian species (Kahl *et al.*, 2015). A lower T4 level limits the production of metabolic heat and helps the animal to cope with stress (Bernabucci *et al.*, 2010). The results of this study agree with those of Bouraoui *et al. (*2002), who found that in response to heat stress free T4 concentration decreased from 15.5 to 14.5 pmol/L, whereas the average cortisol concentration went from 21.75 to 23.5 nmol/L. In the present study, the average cortisol concentration went from 19.30 to 21.04 nmol/L whereas the average prolactin concentration increased from 58.52 to 129.79 (ng mL^{-1}). Koprowski and Tucker (1973) studied the effect of the season on prolactin in dairy cows and found prolactin was elevated significantly in summer compared with autumn and spring. In that study the maximum value of prolactin was recorded in July, whereas the minimum value was recorded in January.

Table 3 Heat stress effects on rectal temperature, respiration rate and heart rate of Holstein cows

	Winter (THI 59)	Summer (THI 80)	Difference (winter-summer)
RR per day, inspiration/minute	28 ±3.16^a	63± 9.96^b	+35
RR at 7:00 h, inspiration/minute	28 ± 3.29^a	68± 6.86^b	+40
RR at13:00 h, inspiration/minute	29± 2.89^a	65± 6.72^b	+36
RR at 22:00 h, inspiration/minute	27 ± 3.11^a	61± 6.71^b	+34
HR per day, beats/minute	69 ± 4.57^a	72± 14.88^b	+3
HR at 7:00 h, beats/minute	71 ± 3.7^a	73± 24.67^b	+2
HR at 13:00 h, beats/minute	70 ± 4.61^a	74± 3.56^b	+4
HR at 22:00 h, beats/minute	64 ± 4.49^a	67± 9.47^b	+3
RT per day, °C	38.7 ± 0.62^a	39.27± 1.13^b	+1.20
TR at 7:00 h, °C	37.88± 0.64^a	39.13± 1.58^b	+1,25
TR at 13:00 h, °C	38.1± 0.59^a	39.58± 0.79^b	+1.47
TR at 22:00 h, °C	38.17± 0.59^a	39.09 ± 0.76^b	+0.92

Within a row, values with a common superscript were not different with probability P =0.05
THI: temperature-humidity index, RR: respiration rate, HR: heart rate, RT: rectal temperature

Table 4 Heat stress effects on cortisol, plasma-free thyroxine, prolactin, potassium and sodium concentrations in Holstein cows

	Winter (THI 59)	Summer (THI 80)	Difference (winter-summer)
Cortisol, nmol/L	19.30± 1.56^a	21.04 ± 1.51^b	+1.74
Thyroxin, pmol/L	15.43 ± 1.23^a	14.01± 0.74^b	-1.42
Prolactin, ng/mL	58.52± 4.14^a	129.79± 13.46^b	+71.27
Sodium, mmol/L	136.86± 2.21^a	136.27± 1.48^a	+122.41
Potassium, mmol/L	4.43± 0.42^a	4.3± 0.23^a	-0.13

THI: temperature-humidity index

The RT, RR, HR, and cortisol, thyroxine, and prolactin concentrations were found to be functionally related to RT and THI (Table 5). The THI had lower coefficients of determination (R^2) with the responses to the thermal environment compared with RT. These values were assumed to indicate the sensitivity of RT as an indicator of responsiveness to the environment. Respiration rate and HR were positively correlated to RT (P <0.01). Similar results were reported by Gaughan *et al.* (2012) and Rejeb *et al.* (2016). Cortisol and prolactin were also positively correlated to RT (P <0.01), whereas thyroxine was negatively correlated with RT (P <0.01). Thus, these results suggest that RT was an indicator of thermal equilibrium and could be used to assess the adversity of the thermal environment that affects dairy cows.

An increase in serum cortisol level has been shown to be closely related to abnormal behaviour of animals such as anxiety and sensitivity (Möstl & Palme, 2002; Bristow & Holmes, 2007). Reduced animal productivity (Thun *et al.*, 1998; Cooke *et al.*, 2012) has been used as an indicator in measuring stress levels (Bova *et al.*, 2014). Elevated heart rate has also been associated with increased peripheral blood pressure (Reule & Drawz, 2012).

Table 5 Regression equations relating to rectal temperature, respiration rate, heart rate, and cortisol, thyroxine and prolactin concentrations to the rectal temperature of Holstein cows and the temperature-humidity index they experienced in summer

	Independent variable			
Dependent variables	Rectal temperature, °C		Temperature humidity index	
Rectal temperature, ˚C			= 0.0632×THI + 64.11	R^2 = 0.61***
Respiration rate, inspirations/minute	= 5.0506×RT -133.86	R^2 = 0.87***	= 1.5259×THI - 60.57	R^2 = 0.61***
Heart rate, beats/minute	= 1.52×RT + 15.01	R^2 = 0.54**	= 0.889×THI - 14.238	R^2 = 0.32**
Cortisol, nmol/L	= 0.51×RT + 41.61	R^2 = 0.61***	= 0.09×THI + 13.45	R^2 = 0.43**
Thyroxine, pmol/L	= -0.86×RT + 20.14	R^2 = 0.84***	= -0.038×THI + 17.12	R^2 = 0.81***
Prolactin, ng/mL	= 5.21×RT - 126.5	R^2 = 0.90***	= 2.5719×THI - 18.2	R^2 = 0.84***

RT: rectal temperature, THI: temperature-humidity index

Conclusion

Heat stress influenced the physiological status and productive functions of Holstein cattle. Rectal temperature was an important indicator of homeostatic regulation of body temperature. The relationship between cortisol levels and RT provided an indication of the relationship between heat stress and metabolic function. Therefore, the concentrations of serum cortisol, thyroxine, and prolactin could be used to quantify the level of heat stress that is being experienced by Holstein cattle in Tunisia.

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Authors' Contributions

HD designed and performed the experiments, interpreted the results and led the writing of the manuscript. NMH analysed the data, contributed to interpretation of the results and aided in writing the manuscript. RB contributed to the data analysis and interpretation of the results. TN provided research direction. All authors discussed the results and contributed to the final manuscript.

Conflicts of Interest Declaration

The authors declare there are no conflicts of interest. This research did not receive any specific funding.

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