



## Thermotolerance indicators related to production and physiological responses to heat stress of holstein cows

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### ABSTRACT

Heat stress (HS) adversely influences dairy cattle welfare and productivity. This study aimed to investigate the effects of HS on production and physiological parameters of Holstein cows. Two experiments each lasted 6 weeks were conducted in four Tunisian farms, firstly during summer under HS ( $n = 80$ ,  $\text{THI} = 77$ ) and later during autumn under thermo-neutral ( $n = 80$ ,  $\text{THI} = 54$ ) conditions. Respiration rate (RR), skin temperature (ST), rectal temperature (RT) and milk yield were measured, and milk samples were collected on 2 days every week during each experimental period. Temperature and relative humidity were measured inside the barn to calculate the temperature-humidity index (THI). Mixed models were used to evaluate the effects of period and the relationships between THI and physiological and production traits. Reaction norm models were applied to quantify the individual responses of cows across the trajectory of THI during the HS period. A clustering methodology was developed to identify tolerant and sensitive cows to HS based on their slope for response of physiological and production traits during HS period. In summer, RR (61 breaths/min) and ST ( $37.7^\circ\text{C}$ ) were 2.3- and 1.3-fold higher, whereas milk yield per milking was 24% lower compared with thermo-neutral conditions. Linear relationship between THI and RR, ST and RT was observed and showed increases by 2 breaths/min,  $0.5^\circ\text{C}$  and  $0.04^\circ\text{C}$  per increase in one THI unit, respectively. Inversely, milk, fat and protein yields showed a drop of 0.13 kg, 0.4 g and 0.3 g per milking per increase in one THI unit, respectively. Cows qualified to be heat tolerant by our work tended to have higher RR, ST, and RT and lower to almost no decay in milk yield compared to cows qualified to be heat sensitive. Specifically, RR could be used as a reliable indicator for thermotolerance. The results of this study deepen our understanding of different aspects of HS resilience.

### 1. Introduction

In Tunisia, the dairy cattle sector is an important component of agriculture and the national economy. Breeding policies have oriented to promote dairy systems by the import of pure high-yielding breeds and the substitution of local and cross breeds (GIVLait, 2018). Exotic High-yielding breeds, originated from temperate regions, may not tolerate heat stress (HS) and low herd management, and their ability to cope with the environment disturbances is therefore hampered (Hammami et al., 2009). The climate in Tunisia is hot enough to cause HS in cattle, characterised by hot and dry summers. Dairy cows are experiencing more than 5 months of warmer temperatures above the thermo-neutral zone (Bouraoui et al., 2002). The increased interest in HS effects on livestock productivity is a direct consequence of the changes in the global climate. Annual average temperatures are

expected to increase by  $1.1^\circ\text{C}$ , along with a higher frequency of extreme weather events and a decrease in annual precipitation and land areas suitable for agriculture, with a decline in water resources in Tunisia by 2030 (GIZ, 2011).

The thermal environment is one of the major factors that negatively affect milk production in dairy cows, especially in animals of high genetic merit (Nardone et al., 2010), and dairy herds are the livestock group most affected by HS in terms of economic losses (St-Pierre et al., 2003). The expected climate change scenarios, particularly regarding HS, will strongly affect dairy systems, animal welfare and production performance (Kanca, 2017; Tao et al., 2018; West, 2003), necessitating an increased emphasis on the adaptation capacity of dairy systems to deal with HS (Amamou et al., 2018; Dedieu and Ingrand, 2010; Koltes et al., 2018). The interest in using feeding and environmental conditioning as strategies to alleviate the effects of HS has declined, as costs

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have increased (Collier et al., 2006). The selection of animals that are adapted and productive without the need for managerial interventions is therefore the best method to mitigate the effects of HS and to improve animal welfare and productivity (Scholtz et al., 2011). In this sense, a long-term and cost-effective option to mitigate the effects of HS is breeding for heat-tolerant dairy cows (Nguyen et al., 2017), although it is still unclear how adaptation is accurately measured (Dalcin et al., 2016). Many studies have pointed out the complexity of adaptation phenomena and especially the problems of phenotyping of dairy cow tolerance to HS (Baumgard et al., 2012; Carabano et al., 2016; Santana et al., 2016). Several factors such as physiological mechanisms, including increases in body temperature, respiration and panting rates, and sweating may be evolved for dealing with HS (Blackshaw and Blackshaw, 1994; Gaughan et al., 2000). Moreover, the antagonistic relationship between high production efficiency and heat tolerance has been highlighted in Holstein cows by numerous previous studies (Aguilar et al., 2010; Bernabucci et al., 2014; Carabaño et al., 2014). Selection for high milk production without considering this fact will increase the susceptibility of cows to HS, thereby decreasing the ability to cope with HS and reducing reproductive and production efficiency (Vasconcelos and Demetrio, 2011). Although the effect of HS on livestock production has been evaluated in numerous studies, and heat mitigation techniques have been improved in recent years, many efforts still need to be made to elucidate the physiological mechanisms involved in the thermoregulation of bovine species (de Andrade Ferrazza et al., 2017). Thus, understanding how dairy cows respond to HS by studying production and physiological parameters and by identifying the adaptive responses by which Holstein cows mitigate the impact of HS appears important to facilitate the development of robust and cost-effective strategies for HS resilience.

In this context, the objectives of this study were (1) to evaluate heat stress effects on physiological (i.e. RR, ST, and RT) and production (i.e. milk, fat and protein yields) parameters of Holstein cows and (2) to identify indicators of heat tolerance which could improve resilience to HS without adversely affecting productivity.

## 2. Materials and methods

### 2.1. Data

The study was carried out in four commercial dairy farms, located in the north and the centre of Tunisia, characterised by a Mediterranean climate with mild winters and hot dry summers. The experiments were carried out over two different periods, firstly during 6 weeks in the summer season (July–August 2017) supposed to induce HS conditions and secondly over a similar duration in the autumn season (November–December 2017) supposed to induce thermo-neutral conditions. Twenty multiparous mid-lactating Holstein cows were selected in each dairy farm and each experimental period, based on similar lactation numbers and stages, so that all groups of cows were similar at the beginning of the experiments (Table 1). The selected cows were housed with the remaining herd and managed similarly to the rest of cows in the herd. Holstein cows were raised in open buildings and free

**Table 1**  
Descriptive statistics for cow characteristic at the beginning of the experiments.

	Summer				Autumn			
	N	Herd	Mean	SD	N	Herd	Mean	SD
Lactation number	80	4	1.8	0.8	80	4	2.0	0.9
Lactation stage (Days in milk)	80	4	146	66	80	4	140	43
Age at calving (month)	80	4	57	21	80	4	62	20
Milk yield <sup>a</sup> (kg)	80	4	6.5	1.7	80	4	8.4	2.2

N = number of cows; SD = standard deviation.

<sup>a</sup> Yield only measured at the noon milking.

stall barns with straw bedding and metal roofs covering the stalls. There was no specific equipment employed to control and modify the ambient conditions (e.g. cooling). Cows were milked three times per day. In summer, feeding was based on oat silage and concentrate. Oat silage was fed *ad libitum*, and the concentrate was fed in three equal meals daily according to the milk production level. Forage and concentrate were separately provided to the cows. During the autumn period, green alfalfa forage was additionally provided. Drinking water was provided *ad libitum*.

Data were collected on 2 days (Monday and Thursday) every week during each experimental period. Prior to the experiment, blank experiments were performed to explain the protocol to the operator and to ensure its proper functioning and implementation. The technical persons were trained to always perform the measurement under similar conditions. Physiological parameters included respiration rate (RR), skin temperature (ST) and rectal temperature (RT). For each cow, RR and ST were recorded three times daily (at 0900, 1100 and 1400 h), and RT was only recorded at 1400 h. Respiration rate (RR) was determined by visually counting flank movements of uninterrupted breathing during a time period of 30 s with a stopwatch and then converted to the number of breaths per minute. Skin temperatures at shoulder and rump were measured on a shaved area approximately 5 by 5 cm with an infrared temperature gun (model MiniTemp MT6; Raytek Corp., Santa Cruz, CA). Values from the two skin sites were averaged to calculate mean skin temperature (ST). The general recommendations of the gun manufacturer were followed, a distance of 50 cm between the infrared thermometer gun and the animal body were kept. The typical distance to target (spot) is up to 2 m (6 ft) and measuring a target area of approximately 50 mm in diameter (covered area of the gun) from a distance of 500 mm away (D:S = 10:1). Rectal temperature was measured prior to the noon milking on all test days, using a digital thermometer (Digital Thermometer; PIC Vedodigit II; Pic Solution Co., Como, Italy; with 0.1 °C accuracy of measurement) inserted in the rectum wall of the animal for approximately 60 s until the audible end measurement. In addition to physiological parameters, milk yield was recorded for each cow at 1400 h on all test days, and an individual milk sample was collected. Milk samples were analysed at the laboratory of the Directorate of Genetic Improvement, Office of Livestock and Pasture (Sidi Thabet, Tunisia), using a Bentley Instrument (FTS/FCM).

Daily ambient temperature (T, °C) and relative humidity (RH, %) were continuously recorded inside the barn at 10-min intervals during the experimental periods, using a data logger (BL30 Climate datalogger; Trotec, Heinsberg, Germany), installed at the level of the cow head. The temperature humidity index (THI) was calculated by applying the formula of the National Research Council (NRC, 1971), considering the temperature (T °C) and relative humidity (RH %) as follows:

$$THI = (1.8 * T^{\circ}C + 32) - [(0.55 - 0.0055 * RH\%)] * (1.8 * T^{\circ}C - 26)$$

### 2.2. Statistical analyses

#### 2.2.1. Responses of physiological and production parameters during summer and autumn: population level

The whole data set collected during summer and autumn periods was used in this part of the study. Two models were fitted; the first aimed to determine the period effect on physiological (i.e. RR, ST, and RT) and production (i.e. milk, fat and protein yields) parameters, while the second model aimed at depicting patterns of THI effect on physiological and production parameters recorded during both experimental periods. Data were analysed by the PROC MIXED function of SAS version 9.4 (SAS Institute Inc., Cary, NC). The main mixed model used for both analyses included the fixed effects of the herd nested within the day of measurement, days in milk classes, age at calving classes, type of barn, type of feeding, operator and period (model 1) or THI classes (model 2); the random effect of the animal; and the residual error. Data of production parameters (i.e. milk, fat, and protein yields) and RT were

analysed on a daily basis. For physiological parameters (RR and ST) measured at 0900, 1100 and 1400 h, both models additionally included the fixed effect of measurement hour. Least squares estimates of period effect (summer vs. autumn; model 1) and THI effects (40 classes; model 2) at population level were obtained.

Principal components analysis (PCA) was carried out to evaluate how the studied parameters were associated among each other. Relationships between thermal indices (average and maximal THI), production parameters (milk, protein and fat yields) and physiological parameters (RR, ST and RT) were analysed with PCA, using the PRINCOMP procedure (SAS, version 9.4).

### 2.2.2. Individual responses of physiological and production parameters to heat stress

The sensitivity of individuals to HS was modelled using only the data obtained during the summer period. In this analysis, the model fitted a continuous function to describe the HS effect on physiological and production traits and accounted for the fact that response to increasing THI may vary across individuals. Between-individual variability was accounted for by the introduction of two individual specific random effects: i) the intercept, standing for the general component of the parameter independent of the THI effect and ii) the slope, reflecting the response of the parameter to a unit increase in THI. In summary, the model was a mixed regression model including the fixed effects of the herd nested within the day of measurement, parity classes, days in milk, age at calving, type of barn, type of feeding and operator; the individual variability (intercept and slope) as random effects; and the residual error. The average 3-day lag maximal THI value was used as a covariate for production parameters, whereas the hourly THI was assigned for physiological parameters. This choice was made after applying and comparing several models based on different criteria such as Bayesian information criterion (BIC) values and percentage of squared bias (PSB) (Ali and Schaeffer, 1987). The covariance structure for random effects was obtained, and solutions for intercept and slope for each cow and for each physiological and production parameter were estimated.

After standardisation of individual slopes, a hierarchical cluster analysis, based on Ward's method, was applied. The procedure CLUSTER (SAS, version 9.4) was used to group cows in accordance with their degree of similarity in terms of response to THI. The clustering was based on estimated slopes for each cow for production (i.e. milk, fat and protein yields) and physiological parameters (i.e. RR, ST, and RT). Division into similar groups would allow us to identify cows with similar response to HS based on certain parameters, thus reducing response heterogeneity while increasing intra-group response homogeneity.

## 3. Results and discussion

In this study, we determined the responses for physiological and production traits of Holstein cows to weather fluctuations in Tunisian commercial farms. The average daily THI was  $54 \pm 4.3$  and  $77 \pm 2.5$  during the first (November–December) and second experiment (July–August), respectively. The THI values in the summer season were the highest, indicating that cows were exposed to continuous HS during the trial summer period (Fig. 1). In a previous study by Bouraoui et al. (2002), conducted in a Central Tunisia dairy farm, average THI values were 68 and 78 during their spring and summer experimental design, respectively. Moreover, the overall mean THI value observed during our summer experiment was higher than the overall mean values (70–79) calculated using a 10-year period with meteorological data provided from the different weather stations in Tunisia (Salem and Bouraoui, 2009). The recording of meteorological data in close distance to the cow inside the barn, and at exactly the same time point where physiological and production parameters were measured, was imperative for our study addressing dairy cow responses to HS. The significant difference ( $P < 0.05$ ) between autumn and summer in terms of

least squares estimates of period effect on production and physiological parameters, reported in Table 2, reveals a sensitivity of Holstein cows to HS. Increases in physiological parameters and reductions in milk, fat and protein yields during the summer period were in accordance with results reported by Bouraoui et al. (2002), studying the impacts of HS on Holstein cows. The physiological parameters showed the highest values in summer and the lowest in the autumn period (Table 2). During the summer period, RR and ST averages were 2.3- and 1.3-fold higher compared with the autumn period conditions, respectively. Collier et al. (2012) identified a threshold for milk yield losses by matching THI with RR and RT. They found that milk yield losses become significant when RR and RT exceed 60 breaths per min and  $38.5^\circ\text{C}$ , respectively. In our study, 57% of RR and 76% of RT records were higher than these latter values. Inversely, the production parameters were lower during summer compared to values estimated during the autumn period. Interestingly, these results are reflected by those generated from the principal components analysis illustrated in Fig. 2. The representation of the studied traits and thermal indices (average THI and maximal THI) on axis 1 and 2 showed that the physiological traits (RR, ST, and RT) and the thermal indices are situated on the same side of the axis 1 (Fig. 2), indicating that they are highly and positively correlated and that an increase in THI leads to increases in these physiological traits. Contrariwise, traits related to milk, fat and protein yields are on the other side of the axis and therefore negatively correlated to thermal indices. The patterns of THI effect on physiological and production parameters are shown in Fig. 3. The trends of response to THI trajectory for physiological and production parameters were different. Physiological traits (RR, ST and RT) showed similar trends marked with an increase as THI increased (Fig. 3a, b and 3c). In general, the physiological parameters, specifically RR and ST, responded nearly linearly to THI. Only a slight flat response to increased THI was observed for values below 72 (diamond symbols). Subsequently, a consistent increase was observed as THI values moved away from this point (dot symbols). According to Dangi et al. (2014), animals enhance RR in order to dissipate additional body heat via vaporisation in the surrounding environment. The loss of water from the respiratory tract by panting accounts for about 60% of the total heat loss, whereas the remaining body heat dissipates from the skin surface by sweating (Cwynar et al., 2014). In this study, for each point increase in the THI value, there was an increase in RR by 2 breaths per minute (Fig. 3a). Previous studies indicated that RR is positively correlated with THI and may be a valuable indicator of HS in cattle (Johnson et al., 2012; Kadzere et al., 2002; Zimbelman et al., 2010). In the latter study, HS was defined as an increase in RR by 2 breaths per min per increase in one THI unit. Nevertheless, this increase varied from 2.8 to 3.3 breaths per min per  $1^\circ\text{C}$  increase in ambient temperature in the study by Gaughan et al. (2000). According to Scharf et al. (2012), RR is a valuable and sensitive HS indicator and can be easily measured and monitored without the need for any costly equipment or causing further stress to the animals. Skin temperature was a highly sensitive trait in response to THI values, showing a linear association as THI increased ( $R^2 = 0.96$ ). McDowell (1958) has reported that in European cattle breeds, body temperature increases with increasing environmental temperatures above  $21^\circ\text{C}$ . In fact, skin is an important pathway for heat exchange, and ST is the result of the regulation of this exchange between the skin and body core by the blood flow. For RT, a high coefficient of determination ( $R^2 = 0.79$ ) and an increase by  $0.04^\circ\text{C}$  per increase in one THI unit were detected (Fig. 3c). Also, a strong relationship between HS and increasing RT has been identified by Bouraoui et al. (2002), especially during the hot summer period in Tunisia. According to Suthar et al. (2012), RT is a sensitive indicator for heat stress identification. An increase in RT by  $1^\circ\text{C}$ , as a response of HS, is sufficient to reduce dairy cow performance (Maurya et al., 2015). Since elevation of RT strongly indicates hyperthermia, this parameter may reflect the adaptability degree of livestock to a particular environment. Milk, fat and protein yields were negatively correlated with

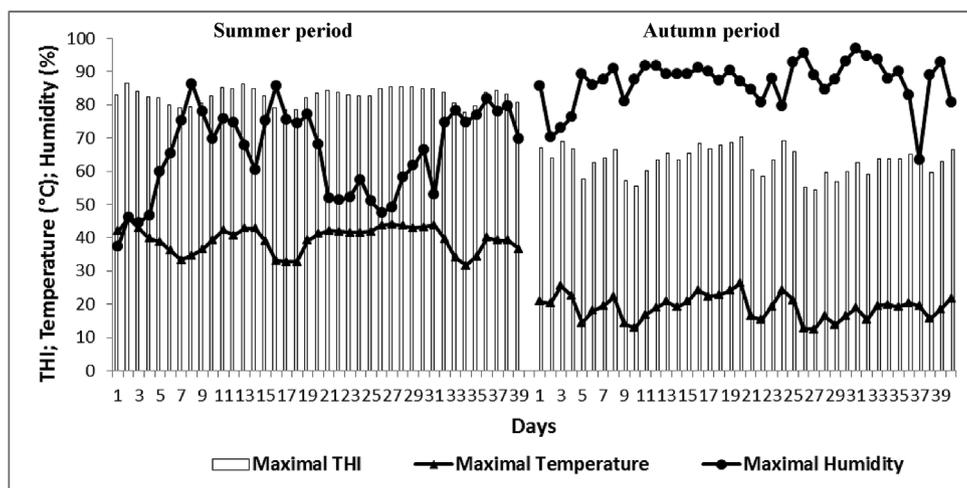


Fig. 1. Maximal temperature, humidity and temperature-humidity index (THI) variation during the two experimental periods.

Table 2

Least squares estimates of period effects on production and physiological parameters for Holstein cows under heat stress (summer) and thermo-neutral (autumn) conditions.

Parameters	Farm 1		Farm 2		Farm 3		Farm 4		N	Mean	SE
	Mean	SE	Mean	SE	Mean	SE	Mean	SE			
<b>Heat stress</b>											
Respiration rate (bpm)	60.8	2.9	62.7	3.0	60.2	4.1	60.1	4.3	2880	60.8 <sup>a</sup>	3.8
Skin temperature (°C)	38.2	1.5	38.4	0.9	37.1	1.7	37.2	1.9	2880	37.7 <sup>a</sup>	1.7
Rectal temperature (°C)	39.3	0.3	39.3	0.2	39.1	0.3	39.1	0.4	960	39.2 <sup>a</sup>	0.3
Milk yield* (kg)	6.5	0.3	6.7	0.3	6.7	0.4	6.3	0.3	960	6.5 <sup>b</sup>	0.4
Fat yield* (g)	180.2	20.2	180.4	10.9	180.3	20.3	160.6	20.1	960	170.8 <sup>b</sup>	20.3
Protein yield* (g)	190.6	10.7	200.2	10.4	190.9	10.2	180.6	10.5	960	190.5 <sup>b</sup>	10.6
<b>Thermo-neutral</b>											
Respiration rate (bpm)	26.8	3.2	26.8	2.9	24.4	2.5	27.5	6.0	2880	26.4 <sup>b</sup>	4.1
Skin temperature (°C)	28.9	1.8	28.7	2.1	27.4	1.7	29.1	2.3	2880	28.5 <sup>b</sup>	2.1
Rectal temperature (°C)	38.3	0.1	38.2	0.1	38.2	0.1	38.3	0.2	960	38.2 <sup>b</sup>	0.1
Milk yield* (kg)	8.5	0.5	8.8	0.5	8.2	0.5	8.0	0.7	960	8.4 <sup>a</sup>	0.6
Fat yield* (g)	270.2	30.2	300.9	30.5	280.6	20.9	340.5	40.9	960	300.3 <sup>a</sup>	40.6
Protein yield* (g)	280.1	20.3	290.6	20.4	260.3	20.7	250.4	20.8	960	270.4 <sup>a</sup>	30.0

SE = standard error; bpm = breaths per min; N = number of observations.

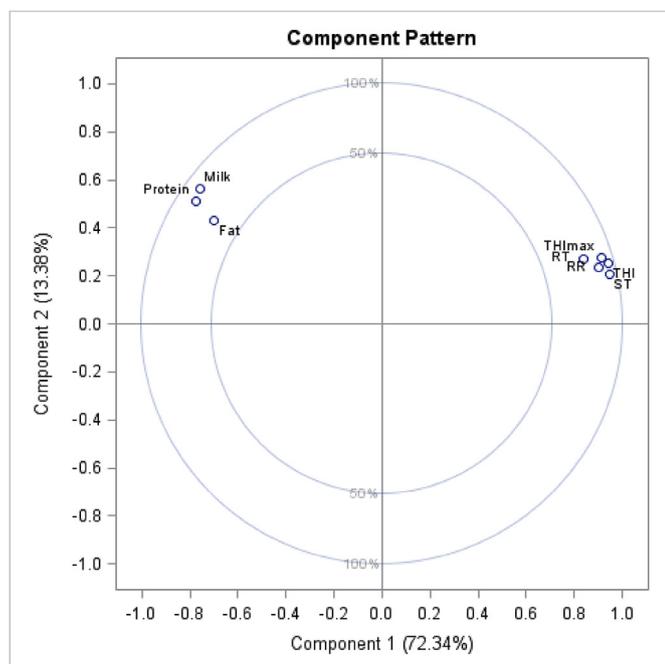
Means of a parameter followed by different letters are significantly different between the 2 periods ( $P < 0.0001$ ).

\* Yield only measured at the noon milking.

THI and showed a continuous decline across THI values (Fig. 3d, e and 3f). Several authors have documented the adverse effects of high summer temperatures and relative humidity on milk production of cows (Du et al., 1990; Kanca, 2017; Polsky and von Keyserlingk, 2017). Milk yield showed a drop of 0.13 kg per cow per milking per increase in one THI unit (Fig. 3d), with a decrease in milk yield by 24% during HS conditions. A study by Bouraoui et al. (2002) in Tunisia showed, at THI levels between 68 and 78, a decrease for milk production by 21%, with a drop of 0.41 kg of milk per cow per day for each THI increase unit above 69. The latter study in Tunisia observed a drop in fat (3.24 vs. 3.58%) and protein (2.88 vs. 2.96%) contents of cows calving in summer compared to cows calving in winter. In this context, many studies reported that when a lactating Holstein cow faced HS conditions, the milk protein and fat contents decreased (Liu et al., 2017; McDowell et al., 1976; Nasr and El-Tarabany, 2017; Ravagnolo et al., 2000). However, other studies found no difference in milk protein and fat contents between warm and cool weather (Knapp and Grummer, 1991; West, 1999; Wheelock et al., 2010). Generally, most of cited studies have been realised in climatic chambers or were based on climate data provided from weather stations which are relatively remote from the precise barn locations.

We still lack knowledge about the mechanisms by which hyperthermia negatively affects milk synthesis because environmental

conditions impact animals in multifactorial ways combining physiological, productive and behavioural aspects (Wheelock et al., 2010). Cow performance and feed intake are negatively affected by HS, resulting in potential discomfort for animals, but also in decreased feed efficiency as a minor share of the energy in feed is converted into milk when HS becomes severe (Hill and Wall, 2017). Such complex aspects of dynamic interactions among the components of animal production systems should be considered, as well as the ability to predict the effects of HS on Holstein cows, to ensure animal welfare and performance and to increase profitability (de Andrade Ferrazza et al., 2017). The THI has been widely studied as an environmental descriptor to assess HS levels for different animal species. This index was usually classified into different classes to define the level of HS. However, the definitions of those levels vary among index and authors (Wang et al., 2018a, 2018b). Armstrong (1994) classified HS according to the variation of THI into mild (72–78), moderate (79–89) and severe (> 89). Phenotypic correlations between cow effects at a THI of 79 (minimum value observed during the summer experiment) and cow effects at the remaining THI points (during the summer period) for production and physiological traits are shown in Fig. 4. The minimum THI value of 79 represents the mild HS level in Tunisia, as determined in study by Bouraoui et al. (2002). For all parameters, correlations were consistently high (> 0.90) up to a THI of 83 and then started to decrease as THI increased. For



**Fig. 2.** Plots of components pattern describing the relationship between thermal indices (average and maximal temperature-humidity index (THI and THI<sub>max</sub>)), production traits (milk, protein and fat yields) and physiological traits (respiration rate (RR), skin temperature (ST) and rectal temperature (RT)) derived from principal components analysis.

milk, fat and protein yields, and for RT, correlations between mild (THI 79) and acute to severe HS level (THI 87) remained somewhere high, with 0.87, 0.88, 0.87 and 0.85, respectively (Fig. 4). These high correlations between cow effects at acute and severe HS levels indicate that similar variability in individual cow response to increasing heat loads is observed. In other words, cows ranked similarly in terms of production and RT response to THI during the acute and the severe HS levels. In contrast, lower correlations between cow effects at THI of 79 and cow effects at extremely high THI of 87 were observed for ST and RR (0.54 and 0.59, respectively), indicating that physiological response of cows to acute and severe HS levels are quite different. This would imply that cows in the range of ST and RR at acute HS level will be re-ranked in other range at more high loads and extreme HS levels. In other words, cows that are responding physiologically modestly during the acute level may respond highly during extreme HS level and vice versa. Genetic analysis of HS effects on production traits used a correlation threshold of 0.80 as cut-off point to investigate the magnitude of genotypes by environments interactions (Bohlouli et al., 2013; Carabaño et al., 2014; Hammami et al., 2015). Hammami et al. (2015) reported that somatic cell score, fat yield and oleic fatty acid are the most sensitive to HS among 23 studied traits as they have lower genetic correlations (< 0.67). Then, ST and RR might be used as early physiological predictors of the thermal status, as the adaptation to disturbances usually takes some time. Spiers et al. (2004) indicated that ST will increase first, with a shift in THI, representing the initial stimulus for animal responses leading to the activation of thermoregulatory effectors. A study by Dalcin et al. (2016) confirms that among the different physiological parameters (RR, RT, heart rate and panting score), RR was most suited to indicate HS in dairy cattle.

Commonly, a plethora of bio-climatic indices, even developed with empirical formulas, is used to evaluate the climatic impacts on traits of interests. Many studies applied a threshold approach (Aguilar et al., 2010; Bernabucci et al., 2014; Bohmanova et al., 2007); the authors used a population tipping point and estimated slope values representing the individual decay after this point. However, thresholds of THI differ

among studies, reflecting geographic, production and management system specificities (Bohmanova et al., 2007; Carabano et al., 2016). Other studies (Brügemann et al., 2011; Carabaño et al., 2014; Hammami et al., 2015) applied a random regression model to estimate the individual response for milk yield and components across a trajectory of THI scales without fixing any tipping point in prior. Generally, both approaches were mostly interested in modelling the genetic components of the individual response to increasing heat loads exclusively for production traits and somatic cell scores, using data provided from the milk recording along with weather information from weather stations. In our study, data was provided from daily measurements of production and physiological parameters, on-farm weather information and cows in uniform physiological conditions over a period of 6 weeks during the summer and autumn of the same year. In our study, estimation of the individual responses of production and physiological parameters to HS revealed the existence of an individual variability for all studied traits. Analysis of the pattern of individual responses across the THI scale within each trait shows differences between cows in terms of sensitivity to heat loads. For each trait, there are cows who respond negatively (negative slope with respect to the mean population response), whereas others respond positively (slope > 0) or indifferently (slope ≈ 0) to the increase in THI. Fig. 5 shows the individual responses to HS for production and physiological traits for 10 representative sampled cows (for better illustration of the trends). Generally, cows exhibit similar reaction norms for all traits, for example, cows with positive slope for milk yield (Fig. 5d) have also similar positive slopes for physiological traits (especially RR) (Fig. 5a, b and 5c). Consequently, we can stipulate that the maintenance of the milk yield level associated with higher RR might be one of the major components of heat invulnerability in Holstein dairy cattle. Interestingly, Fig. 5 shows also that other cows exhibit different reaction norms across the traits (e.g. positive slopes for physiological parameters and in opposite negative slopes for production traits or vice versa). Therefore, attention should be paid to both physiological and productive responses of cows to HS and to their slopes intensity compared to the mean population response when defining sensitive and tolerant cows. A study of Yano et al. (2014) on Holstein cattle in Japan showed that individual cows respond differently to heat, some cows tend to be particularly sensitive to heat whereas most remained robust. Santana et al. (2016) indicate that animals become increasingly productive and positive plastics when trends for the level and slope of reaction norms of the cows are positive and higher for this level. The identification of heat-tolerant animals may be useful only if these animals are able to maintain high productivity and survivability when exposed to heat stress conditions (Bernabucci et al., 2014; Gaughan et al., 2009). According to previous studies, the physiological responses of cows at high temperatures could be considered as adaptation or acclimatisation processes (Al-Kanaan, 2016; Kadzere et al., 2002). Adaptation is a very general term describing the ability of an animal to cope with the constraints of the environment. Forsman (2015) defined phenotypic plasticity or environmental sensitivity as the ability of a genotype to exhibit a range of different phenotypes in response to variations in the environment. According to Lenis Sanin et al. (2016), adaptation might be classified into two types: physiological adaptation and genetic adaptation. The first implies modifications in physiological processes that are independent of changes in gene expression in order to maintain homeostasis; other authors call it acclimatisation (Horowitz, 2002). The second implies changes in the animal genetic material and requires the passage of hundreds and thousands of years (Horowitz, 2002; Maróti-Agóts et al., 2011). For this reason, scientific studies addressing dairy cattle's HS sensitivity and evaluating the levels of plasticity among individuals and populations are of increasing importance (Baumgard et al., 2012; Carabano et al., 2016; Hammami et al., 2015; Santana et al., 2016). All these studies point out the adaptation phenomena complexity and especially the problems of phenotyping and characterisation of heat tolerance. In this context, it appears important to decrease the

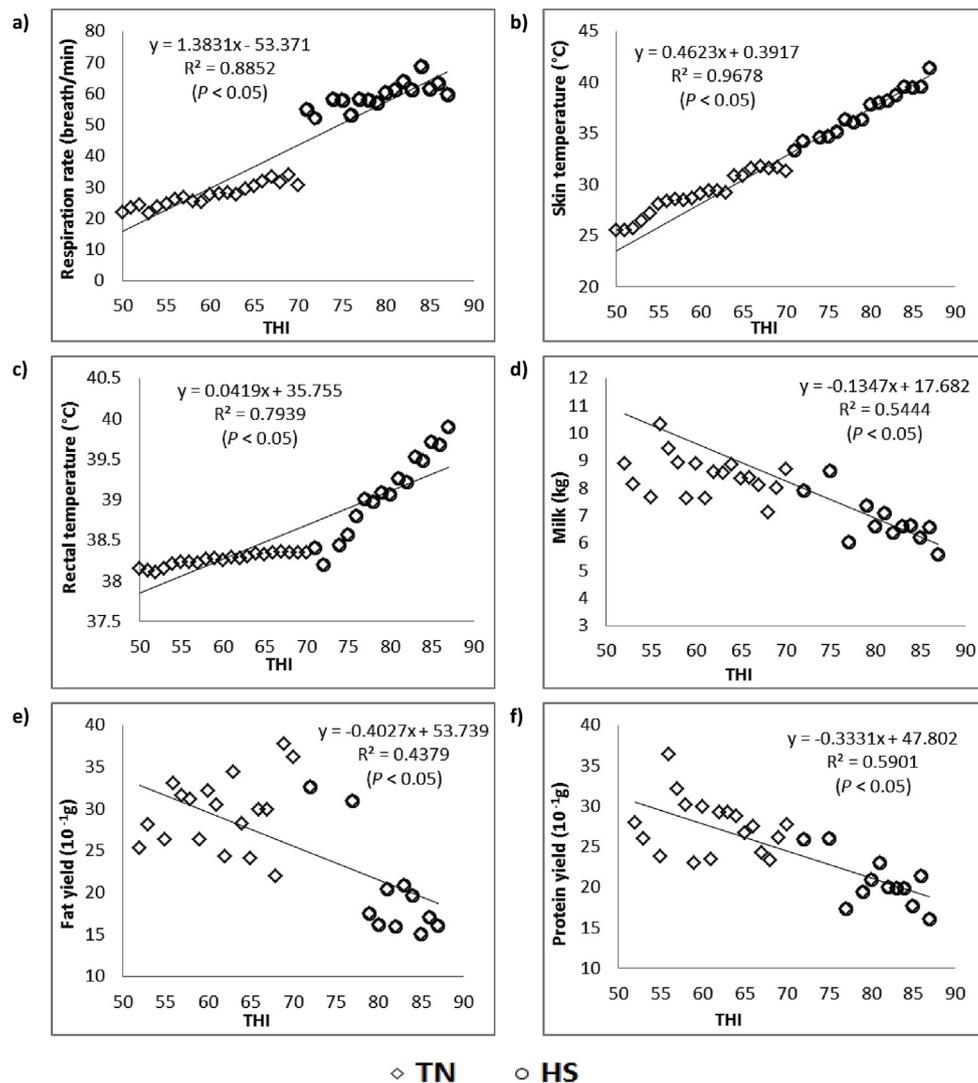


Fig. 3. Least squares estimates of the effects of the temperature-humidity index (THI) on (a) respiration rate, (b) skin temperature, (c) rectal temperature, (d) milk yield, (e) fat yield and (f) protein yield during thermo-neutral (TN; diamond symbols) and heat stress (HS; dot symbols) conditions.

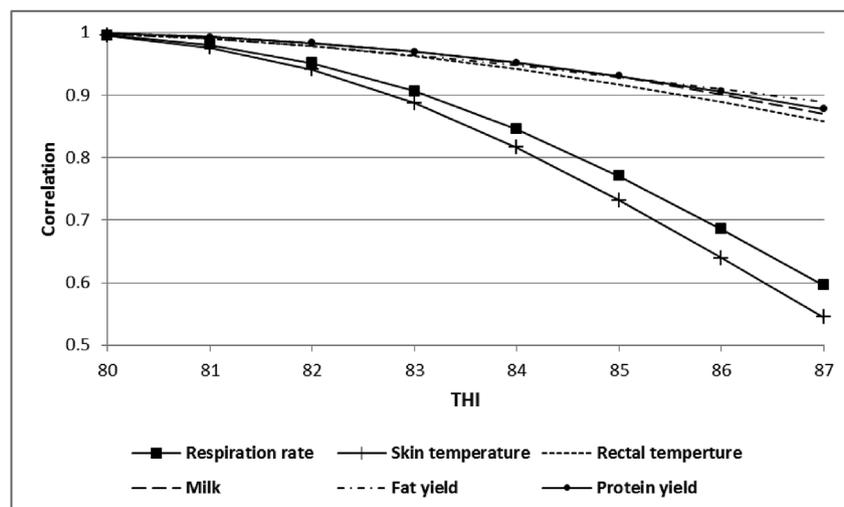


Fig. 4. Phenotypic correlations between cow effects at a temperature-humidity index (THI) of 79 and cow effects at the other THI values during the summer period for production traits (milk, protein and fat yields) and physiological traits (respiration rate, skin temperature and rectal temperature).

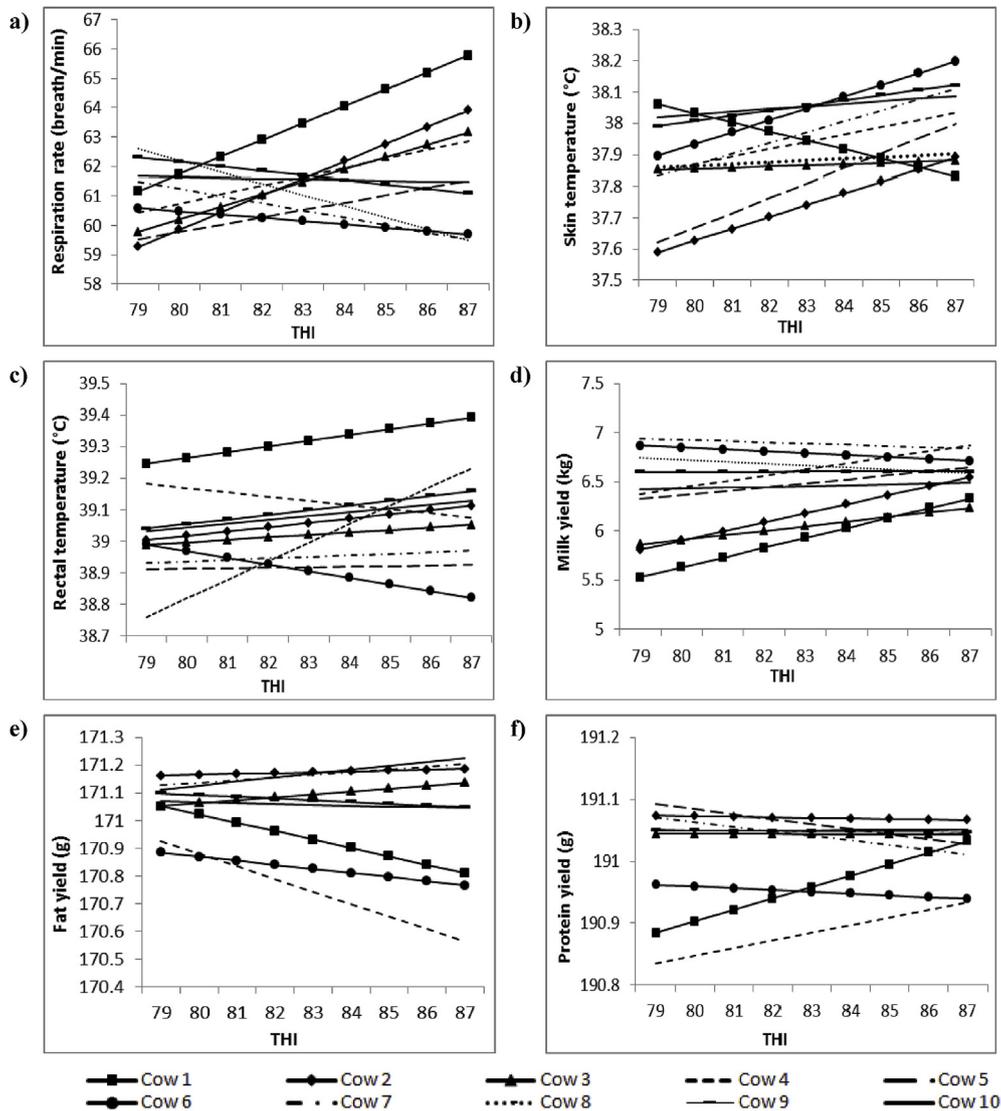


Fig. 5. Pattern of individual responses of the reaction norms of 10 representative sampled cows based on their estimated slope deviations from the overall population response through the temperature–humidity index (THI) scale for (a) respiration rate, (b) skin temperature, (c) rectal temperature, (d) milk yield, (e) fat yield and (f) protein yield.

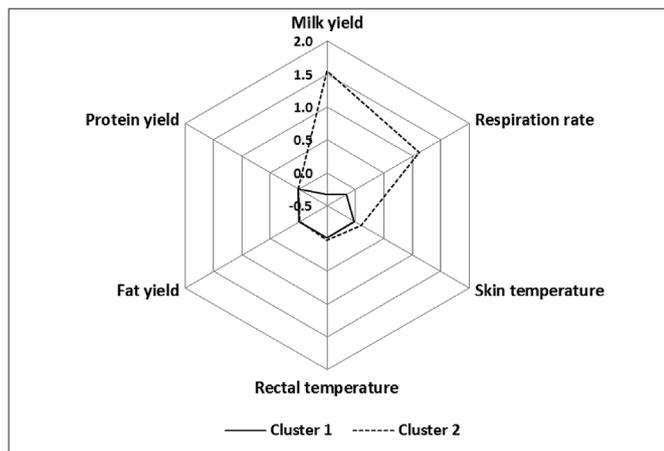


Fig. 6. Individual deviations of slopes from the overall population responses to heat stress between Cluster 1 of heat-sensitive (82%) and Cluster 2 of heat-tolerant (18%) cows.

susceptibility of dairy cows to HS without affecting milk production. In our study, the possibility of using the slopes for individual cow curves responses for HS may provide useful tools for independent selection. Due to large animal-to-animal variation, measurement at the animal level would be useful to manage HS and diseases (Koltés et al., 2018). Based on the cluster analysis using the slopes responses, cows were classified into two clusters (Fig. 6). The largest proportions of cows (82%) belonged to Cluster 1, which showed the type of response with a steeper negative slopes for all studied traits, especially for milk yield, RR, ST and RT by  $-0.33$ ,  $-0.17$ ,  $-0.02$  and  $-0.01$ , respectively. This cluster could be qualified as representing the sensitive cows to HS. In contrast, animals of Cluster 2 tended to show an increase with respect to the mean population response for HS in production and physiological traits (positive slopes) at high THI values, particularly for milk yield, RR, ST and RT (1.55, 1.13, 0.10 and 0.03). Therefore, this cluster could be qualified as grouping the heat tolerant and robust cows. According to Magalhães et al. (2010), every individual exposed to a stressor agent generates adaptive responses to maintain a dynamic balance of thermal homeostasis within its comfort zone. The response of cows of Cluster 2 may be explained by the simultaneous increase in all the physiological traits, especially RR, with increasing THI in order to maintain

homeostasis (Fig. 6). These results still in accordance with those presented in Fig. 5. Responses that allow animals to adapt to high ambient temperatures and dissipate heat are activated when an animal is exposed to high ambient temperatures. According to Blackshaw and Blackshaw (1994), cattle use a variety of strategies such as increases in ST to raise heat loss by convection and radiation and increases in RR to increase respiratory evaporative heat loss to cope with a hot environment. Numerous studies have shown that Jersey cows had a considerably higher RR than Holsteins, which was attributed to the better ability of Jerseys to dissipate heat compared to that of Holsteins (Kadzere et al., 2002; Kibler and Brody, 1954). In conclusion, animals of Cluster 2 are more tolerant to heat stress and therefore are expected to cope better with high heat loads, mainly because they show less effects of high THI values on their production levels by intensifying physiological mechanisms, especially RR, to dissipate heat and to re-establish thermal homeostasis. On the other hand, animals of Cluster 1 would be expected to suffer the consequences of HS more intensely and show larger than average declines in production level.

#### 4. Implications

Improving the ability of animals to cope with environmental stressors by selecting animals adapted to certain environmental conditions and by proposing programs for the herd management is based on heat tolerance indicators. Direct measurements on animal (e.g. body temperature, respiration rate) defined as direct indicators of heat resilience, could improve performance prediction. Despite the large individual variation among animals to heat tolerance, studying production and physiological individual responses of dairy cows to develop thermotolerance indicators confirms that ST and RR are good early indicators of heat stress in dairy cattle. This knowledge could be helpful for farmers to identify the instantaneous thermal status of cows and to apply immediate intervention strategies, adapted to their farm system, to limit the effects of HS on animals. Also, RR may be a good indicator for heat tolerance and the ability of animals to dissipate heat under unfavourable climate conditions, as intensifying the RR of certain cows allows increases in heat-tolerance without a negative effect on production level. Selection for regulation of RR is a potential strategy to mitigate the effects of HS on dairy cows. However, the use of physiological traits was restricted on an experimental level as their use in heat management or genetic selection for heat tolerance at commercial and extended field level was limited due to the difficulty and the cost of their systematic accurate recording. Indicators for heat resilience are of particular interest as long as the associated phenotypes remain easily to collect in a cost-effectiveness way. Ideally, these indicators should be obtained from readily accessible samples, preferably none invasively or minimally invasive. In this perspective, this study may be a first step to continue our researches that aim to identify thermotolerance indicators for adaptation to heat stress.

#### 5. Conclusions

The combination between ambient and animal measurements was necessary to determine which measurements are better in predicting animal performance under heat stress conditions. Generally, there was a decrease in production traits at high THI levels. In counterpart, physiological traits increased with THI. Specifically, with addressing individual animal variability, THI produces an early change in ST and RR, which are related to changes in the thermal status of the animals associated with changes in milk yield. It was interesting to find that among the production and physiological traits, RR was a reliable indicator for thermotolerance to mitigate the impact of heat stress on dairy cow production. These results can be considered as a long-term solution to cope with climate change-related risks and will be an important aspect of heat resilience in the future.

#### Conflicts of interest

No conflicts of interest to declare.

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