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The relationship of temperature-humidity index with milk production of dairy cows in a Mediterranean climate

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Abstract — Two experiments were conducted using lactating Friesian-Holstein cows to measure the effects of heat stress, using temperature-humidity index (THI), on milk production, milk composition and dry matter intake (DMI) under the Mediterranean climate. These trials were carried out in two periods differing in average THI values (68 ± 3.75 vs. 78 ± 3.23 for the spring and summer periods, respectively). Daily THI was negatively correlated to milk yield (r = –0.76) and feed intake (r = –0.24). When the THI value increased from 68 to 78, milk production decreased by 21% and DMI by 9.6%. Milk yield decreased by 0.41 kg per cow per day for each point increase in the THI values above 69. Milk fat (3.24 vs. 3.58%) and milk protein (2.88 vs. 2.96%) were lower for the summer group. THI was positively correlated to respiration rate (RR) (r = 0.89), heart rate (HR) (r = 0.88), rectal temperature (RT) (r = 0.85) and cortisol (0.31), and negatively with free thyroxin (–0.43). As the THI values increased from 68 to 78, RT increased by 0.5 °C, HR by 6 beats, and RR by 5 inspirations per min. The average concentration of cortisol increased from 21.75 to 23.5 nmol·L–1 (P > 0.05), while that of free thyroxin decreased from 15.5 to 14.5 pmol·L–1, (P > 0.05). Summer heat stress reduced milk yield and DMI, altered milk composition and affected the physiological functions of confined lactating Holstein cows managed under Mediterranean climatic conditions.

dairy cow / temperature-humidity index / milk production / intake / physiology


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1. INTRODUCTION

Dairy cattle in many subtropical, tropical and semi-arid regions are subject to high ambient temperatures (Ta), relative humidity (RH) and solar radiation for extended periods. This compromises the ability of the lactating cow to dissipate heat, resulting in heat stress. As a result, the cow develops numerous physiological mechanisms for coping with this stress. Unfortunately, these responses have negative effects on the physiology of the cow and milk yield. The Temperature-Humidity Index (THI) is widely used in hot areas all over the world to assess the impact of heat stress on dairy cows. According to Johnson [12] and Du Preez et al. [6], milk production is not affected by heat stress when mean THI values are between 35 and 72. However, milk production and feed intake begin to decline when THI reaches 72 and continue to decline sharply at a THI value of 76 or greater [11]. Milk yield decreases of 10 to 40% have been reported for Holstein cows during the summer as compared to the winter [7]. Moreover, heat stress is associated with changes in milk composition, milk somatic cell counts (SCC) and mastitis frequencies [7, 25, 26]. Research also indicates that rectal temperature (RT), respiration rate (RR), thyroid hormones and cortisol are affected by heat stress [17, 23, 36].

Research on THI as an indicator of heat stress and its effects on milk production of cows managed under the Mediterranean climatic conditions is limited and the effects of heat stress on milk yield and milk composition are, in most cases, assessed using days with a maximum temperature exceeding 27 °C as the index [22]. However, when high Ta is coupled with a high RH, stress intensity increases. Thus, THI may more precisely describe the effect of the environment on the cow’s ability to dissipate heat [34]. The objective was to measure the effects of heat stress on milk production and composition and to examine the relationship between THI and milk yield in confined lactating cows in a Mediterranean climate in central Tunisia.

2. MATERIALS AND METHODS

2.1. Cows, feeding and management

Two experiments were conducted at the El Alem dairy farm, Kairouan (central Tunisia), which is situated at 35°40' north latitude and 10°6' east longitude. One
experiment was carried out under spring conditions (mean daily THI value 68 ± 3.75, no heat stress), and the second during the summer season (mean daily THI value 78 ± 3.23, stress conditions). Fourteen mid-lactating Friesian–Holstein cows, 144 to 150 d postpartum, were randomly assigned to two equal groups according to milk production, lactation number, days in milk and body weight so that both groups of cows were similar at the beginning of the experiments \((P > 0.05)\). Cows in the spring experiment calved from mid-November to early December and had an average lactation number of 2.29 ± 0.48 and a daily milk yield of 20 ± 3 kg. Those used in the summer calved from late January to late February and had an average lactation number of 2.42 ± 0.53 and a daily milk yield of 19 ± 4 kg. Prior to the experiments, the cows were housed in a covered free stall barn with the remaining herd. They were fed oat and/or corn silages for ad libitum and concentrate according to the production level. Experimental periods were 25 days each, preceded by two weeks of adaptation to the experimental conditions. The cows in both groups were managed similarly, and experiments were conducted with similar protocols.

The cows were housed in a covered tie stall barn with straw bedding (3 m² per cow). Metal roofs covered the stalls. The cows were fed individually in mangers (0.70 m wide and 1.2 m long) in front of the stalls. The diets were typical of those in the region and consisted of about 53% oat silage and 47% concentrate mix on a dry matter (DM) basis. Corn, barley, soybean meal, and a mineral and vitamin supplement were the feed ingredients in the concentrate mixture. The concentrate contained, on average, 89.2% DM, 17.8% crude protein (CP), and 4.2% crude fiber (CF) on a DM basis. The percentages of DM, CP and CF in oat silage were 28.5, 6.7 and 35.1% and 30, 7.11 and 34% for the spring and summer periods, respectively. Oat silage was fed ad libitum. The concentrate (9 kg per cow per day as fed) was fed in three equal meals daily. The amounts were calculated according to the milk production level. Forage and concentrate were separately provided to the cows to allow for the measurement of individual refusals. Drinking water was made available at all times.

### 2.2. Measurement, sampling and laboratory analysis

To determine daily DMI, the amounts of the feed offered and refused were recorded daily throughout the experiments. Refused feed was removed and weighed daily just prior to the morning feeding. All cows consumed all of the concentrate; therefore, weigh-back consisted of only oat silage. The samples of feed and refusal were taken daily and one fraction was used for DM determination by drying at 105 °C in a forced air oven for 24 h. The other fractions of samples were stored and later composited by week within the period. Composites were dried at 50 °C and ground through a 1 mm-screen for subsequent analyses for DM, organic matter, CF and CP according to AOAC [1].

The cows were milked three times a day (7:00, 13:00 and 22:00 h) and milk yield of the individual cows was recorded at each milking on all test days. A weekly composite sample from the three milkings was analyzed for protein, fat and somatic cells at the office of livestock and pasture central milk testing laboratory using a Foss 4000 milko Scan (Foss electronic, France). Milk yield and milk fat percentage was used to calculate 4% fat-corrected milk (FCM). The yields of milk fat and protein were calculated from the contents of milk fat and proteins and milk yield. Food efficiency (yield of kg FCM per kg DMI) was calculated for each period.

Ambient temperature (Ta), RH, RT, heart and respiration rates were recorded daily. Daily maximum and minimum
temperatures were recorded in the feeding area at 16:00 h, using maximum-minimum thermometers. Maximum and minimum RH were recorded each day at a weather station approximately 3 km from the experimental site. RT, HR and RR of cows were measured just prior to each milking. RT was measured by inserting a veterinary digital thermometer (Jorgen Kruuse A/S, China, model: MT 1681) approximately 60 mm into the rectum for 60 s and the temperatures were recorded with one decimal. HR was determined using a stethoscope for one minute. RR was measured by counting the flank movements of the individual cows for a 1-min period of uninterrupted breathing and reported as the number of inspirations per minute.

Monthly THI values for the experimental site were calculated over a 10-year period (1988–1997). Daily THI values were also determined for the experimental period using the equation, \( \text{THI} = 1.8 \times \text{Ta} - (1 - \text{RH}) \times (\text{Ta} - 14.3) + 32 \), as described by Kibler [14] where \( \text{Ta} \) is the average ambient temperature in °C and RH is the average relative humidity as a fraction of the unit. Monthly averages were used for the 10-year period whereas daily averages were used for the trial periods.

Blood samples were taken twice per day for two days from the tail vein using heparinized vacutainer tubes (10 mL). Collections were made at 7:00 and 13:00 h before the cows had been milked and fed. The samples were kept in an ice bath for a few hours until centrifugation at 4 °C to recover plasma. Plasma samples were then shipped the same day in vacutainer systems (Becton-Dickinson) to the veterinary school of Nantes (France) for hormone analysis. Blood plasma was analyzed for free thyroxin (T) using a radioimmunnoassay kit (Immunotech, ref. 1363). The total cortisol concentration was determined using the \(^{125}\text{I} \) RIA kit (DiaSorin, catalog No./REF. CA-1529, CA-1549) suitable for the quantitative determination of cortisol levels in serum, plasma or urine.

### 2.3. Statistical analysis

To determine the effect of period on DMI, roughage intake, milk production, RT, HR and RR, we used a mixed model where repeated measurements per cow were considered random and auto-correlated. The statistical model was:

\[
Y_{ijk} = \mu + P_i + V_j(P_i) + U_k(V_j) + e_{ijk}
\]

where

- \( Y_{ijk} \) = the kth day observation on the jth cow in the ith period;
- \( \mu \) = mean effect;
- \( P_i \) = effect of period i;
- \( V_j(P_i) \) = effect of cow j nested within period i;
- \( U_k(V_j) \) = effect of day k nested within cow j; and
- \( e_{ijk} \) = residual

with

- \( U_k(V_j) = e_k \) for \( k = 1 \);
- or \( U_k(V_j) = r U_{k-1}(V_j) \) for \( k > 1 \);

where

- \( E[U_k(V_j)] = 0 \), \( \text{Var}[U_k(V_j)] = s^2_k \) and \( \text{Cov}[U_k(V_j), U_{k-1}(V_j)] = r s^2_k \);
- \( r \) = correlation coefficient, and \( e_k \) = random error.

Period differences for all measured variables were tested using contrasts. Pearson correlations were determined between the different parameters.

We used the following model to determine the effect of period on milk fat and protein contents, 4% FCM, yields of milk fat and protein, food efficiency, SCC, cortisol and T\(_4\) concentrations:

\[
Y_{ijk} = \mu + P_i + V_j(P_i) + e_{ijk}
\]
where

\[ Y_{ijk} = \text{the kth observation on the jth cow in the ith period}; \]
\[ \mu = \text{mean effect}; \]
\[ P_i = \text{effect of period } i; \]
\[ V_j(P_i) = \text{effect of cow } j \text{ nested within period } i; \]
\[ e_{ijk} = \text{residual} \]

where \( E[e_{ijk}] = 0 \) and \( V[e_{ijk}] = s_e^2 \).

Period differences for these parameters were tested using the student test. Finally, a regression equation was developed between milk production and THI. All analyses were conducted using SAS [30]. The differences were considered to be significant at \( P < 0.05 \), unless otherwise indicated.

### 3. RESULTS AND DISCUSSION

#### 3.1. Environmental conditions during the experimental periods

Mean maximum and minimum Ta, RH and calculated THI by the experimental period are shown in Table I. The upper critical temperature for Holsteins is 25 to 26 °C [3]; cows decrease milk production when THI exceeds the critical comfort level of 72 [11]. The spring period was characterized by a lack of heat stress conditions; mean maximum and minimum Ta and RH were 29.1 and 14.7 °C and 28.1 and 55.7%, respectively. Average THI was 68 ± 3.75. Average Ta and average THI were respectively higher than the critical values of 25 °C and 72 on only 4 and 8% of all test days for this period. In contrast to the spring period, the summer period was characterized by heat stress conditions; mean maximum Ta and maximum RH were 38.9 °C and 73%, respectively. Average Ta and THI exceeded the 25 °C and 72 critical points, respectively, on all and 96% of test days for this period indicating that cows were exposed to heat stress during the summer trial. This was consistent with long-term data (1988-1997) for the region which indicates the presence of summer heat stress as depicted by the THI values varying between 71 and 76 from June to September [4]. Average daily Ta, RH and THI were 21.6 and 29.8 °C, 55.7 and 45.9% and 68 and 78 for the spring and summer periods, respectively. Day to day average temperature,

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Spring</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature, minimum (°C)</td>
<td>14.7 (2.55)</td>
<td>21.9 (1.87)</td>
</tr>
<tr>
<td>Temperature, maximum (°C)</td>
<td>29.1 (3.56)</td>
<td>38.9 (3.48)</td>
</tr>
<tr>
<td>Average temperature (°C)</td>
<td>21.6 (2.69)</td>
<td>29.8 (2.5 )</td>
</tr>
<tr>
<td>Days average temperature &gt; 25 °C</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>RH, minimum (%)</td>
<td>28.1 (6.87)</td>
<td>18.5 (5.36)</td>
</tr>
<tr>
<td>RH, maximum (%)</td>
<td>82.4 (11.3)</td>
<td>73.0 (11.71)</td>
</tr>
<tr>
<td>RH, average (%)</td>
<td>55.7 (0.07)</td>
<td>45.9 (0.06)</td>
</tr>
<tr>
<td>Average daily THI</td>
<td>68 (3.75)</td>
<td>78 (3.23)</td>
</tr>
<tr>
<td>Number days daily THI &gt; 72</td>
<td>2</td>
<td>24</td>
</tr>
</tbody>
</table>

( ): standard deviation; RH: relative humidity; THI: temperature-humidity index.
humidity and THI variations across each period are given in Figures 1 and 2. Variation between days, within a period, was low as indicated by the standard deviation for each period, particularly for Ta (Tab. I).

3.2. The effects of heat stress on dry matter intake, milk production and milk composition

The results in Table II showed a significant ($P < 0.05$) heat stress effect on DMI,
milk production, and milk composition. The reductions in voluntary intake and the subsequent declines in milk production are consistent responses to heat stress in lactating dairy cows [2, 21]. In the present study, heat stress reduced daily milk yield by 21% as the THI values went from 68 in the spring period to 78 in the summer period. Lower milk yields were recorded for confined Holstein cows in a Mediterranean climate during the spring as compared to summer [22]. The adverse effect on milk yield was most likely mediated through a reduction in DMI, which decreased by 1.73 kg or 9.6%, and changes that occurred in body temperature and plasma hormone concentrations. RT is a sensitive indicator of thermal balance and may be used to assess the negative effects of hot environments on growth, lactation and reproduction of dairy cows [11, 34]. It has been shown that a rise of 1 °C or less in rectal temperature is enough to reduce intake and production in dairy cows [10]. Johnson et al. [10] reported that milk yield declines when body temperature exceeds 38.9 °C, and, for each 0.55 °C increase in RT, milk yield and intake of total digestible nutrient decline by 1.8 and 1.4 kg, respectively. However, THI may describe more precisely the effect of the environment on the cow’s ability to dissipate heat [34]. In this study, RT increased by 0.5 °C when the THI value increased from 68 to 78. Meanwhile, milk yield decreased from 18.73 to 14.75 kg, a 21% loss. This confirms the reported findings by Johnson et al. [11], which indicate that milk yield declines when THI exceeds 72. The 21% drop in milk production was similar to declines noted by Mallonee et al. [18] and Du Preez [7].

Feed intake was negatively correlated to daily minimum, mean and maximum THI values and to mean Ta for the same day of measurement, with the highest correlation being with the mean Ta. The respective correlations were –0.16; –0.23; –0.15 and –0.26. Other authors have reported similar results [13, 18]. Higher correlations were, however, observed with temperatures

**Table II. Heat stress (THI) effect on feed intake, milk yield, milk composition, food efficiency.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Period</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring (THI 68)</td>
<td>Summer (THI 78)</td>
<td></td>
</tr>
<tr>
<td>Dry matter intake (kg·d⁻¹)</td>
<td>18.00 (0.24)</td>
<td>16.27 (0.16)</td>
<td></td>
</tr>
<tr>
<td>Forage intake (kg·d⁻¹)</td>
<td>9.98 (0.24)</td>
<td>8.25 (0.16)</td>
<td></td>
</tr>
<tr>
<td>Milk (kg·d⁻¹)</td>
<td>18.73 (0.18)</td>
<td>14.75 (0.18)</td>
<td></td>
</tr>
<tr>
<td>Milk fat (%)</td>
<td>3.58 (0.06)</td>
<td>3.24 (0.06)</td>
<td></td>
</tr>
<tr>
<td>4% FCM (kg·d⁻¹)</td>
<td>17.83 (0.36)</td>
<td>13.25 (0.36)</td>
<td></td>
</tr>
<tr>
<td>Food efficiency (kg FCM per kg DMI)</td>
<td>0.99 (0.02)</td>
<td>0.82 (0.02)</td>
<td></td>
</tr>
<tr>
<td>Milk protein (%)</td>
<td>2.96 (0.03)</td>
<td>2.88 (0.03)</td>
<td></td>
</tr>
<tr>
<td>Fat yield (g·d⁻¹)</td>
<td>681 (15)</td>
<td>480 (15)</td>
<td></td>
</tr>
<tr>
<td>Protein yield (g·d⁻¹)</td>
<td>562 (11)</td>
<td>433 (11)</td>
<td></td>
</tr>
<tr>
<td>Somatic cell counts × 10⁵</td>
<td>4.1 (0.9)</td>
<td>8.6 (0.8)</td>
<td></td>
</tr>
</tbody>
</table>

Least squares means on the same row with the same letter are not significantly different (P > 0.05); ( ) : standard error; THI: temperature-humidity index; FCM: fat-corrected milk; DMI: dry matter intake.
and THI \((-0.24; \ -0.25; \ -0.32)\) respectively for days 1, 2 and 3 prior to the measurement. This agreed with results reported by West [34], which indicate that weather conditions during the 2 to 3 days preceding the day of temperature measurement are most closely associated with milk yield and composition.

Forage accounted for the decrease in DMI, since all cows consumed all of the allocated concentrate in both experiments (Tab. II). Beede and Collier [2] reported that, if cows are fed diets that allow sorting during high environmental temperatures, they selectively will decrease forage intake relative to concentrates in an attempt to reduce body core temperature through reduced heat production from fermentation, digestion and other metabolic processes. The gross efficiency of conversion of feed to milk (kg FCM per kg DMI) was lower \((P < 0.05)\) for heat-stressed cows \((0.82 \text{ vs. } 0.99)\). This suggested that an adaptive mechanism must have occurred in the heat stressed cows, resulting in higher maintenance requirements [24] and lower efficiency of energy use for milk production. This, combined with the decrease in DMI, would explain the decreased milk yield for these cows [31]. Reduced efficiency of energy utilization for milk production by 30 to 50% have been reported for dairy cows in hotter environments [20].

There was a significant effect of heat stress on milk composition and SCC. Heat stress significantly reduced milk fat content from 3.58% during the spring to 3.24% during the summer. Heat stress environments have been associated with depressions in milk fat percentages [26]. However, other authors [15, 27] found no significant decrease in fat percentage for cows under heat stress. The depressed fat percentage observed in the present study could be attributed to the decrease in forage intake (17%) which may have resulted in an inadequate fiber level in the diet to maintain normal rumen function. The reason for the discrepancy between our results and those reported by Knapp and Grummer [15] may have resulted from the differences in experimental conditions. These latter authors fed total mixed rations (TMR), and cows were unable to sort the TMR. This appeared to alleviate milk fat depression commonly associated with heat stress by maintaining the intended forage to concentrate intake and, ensuring adequate fiber for proper rumen fermentation.

Milk protein percentage significantly decreased as a result of summer heat stress \((2.96 \text{ vs. } 2.88\%, \text{ respectively for the spring and summer})\). The data in the literature are conflicting. Our results were in agreement with those reported by Rodriguez et al. [26] and Knapp and Grummer [15] which indicate a decreased milk protein with increased maximum daily temperature. The reduction in milk protein is probably caused by a decreased DMI and energy intake. Decreased levels of food intake during lactation are usually associated with a decreased protein content [8].

The yields of milk fat and protein were lower \((P < 0.05)\) for the summer than for the spring group because both milk yield and milk fat and protein percentages decreased as a result of heat stress. SCC significantly \((P < 0.05)\) increased from \(4.1 \times 10^5\) in the spring to \(8.6 \times 10^5\) in the summer confirming the tendencies already observed by others which indicate negative effects of heat stress on SCC through impaired mammary defense mechanisms [5, 7, 23].

3.3. The effects of heat stress on the physiological responses and some plasma hormones of cows

Research has indicated that RT, RR and changes in some hormonal concentrations may be used as indicators of climatic stress. In the present study, heat stress, as indicated by THI, altered \((P < 0.05)\) all the measured physiological parameters. A daily increase of 0.5 °C was observed for RT when the
THI value increased from 68 to 78. Similarly, heart and respiration rates increased by 6 beats and 5 inspirations per min, respectively. Such response changes are adaptive mechanisms initiated by the cow in an attempt to restore its thermal balance.

Various studies under field conditions have demonstrated that body temperature increases with increasing environmental temperature above 21 °C in European breeds [19]. Little effect on RT and RR was observed at temperatures below 25 °C (spring group). However, a higher rise in RT was evident at a Ta greater than 25 °C. This temperature was suggested to be the upper limit of temperature at which Holstein cows may maintain their thermal balance [3]. Our data indicated that RT increased significantly \((P < 0.05)\) from the spring \((38.36 \, ^oC)\) to the summer \((38.86 \, ^oC)\). Others have reported increases in RT when lactating cows are subject to temperatures above their thermoneutral zone \([21, 27, 36]\).

The respiration and heart rates of cows during the spring and the summer experimental periods were significantly different \((P < 0.05)\). The overall averages for both rates were significantly higher for the summer cows (Tab. III). These cows exhibited increased RT, respiration and heart rates from 07:00 to 22:00 h with peaks at mid-day (13:00 h). This suggests that the cows were mainly subject to heat stress during the day-light hours. Similar results were observed by Muller et al. [23] for Friesian cows in South Africa. However, higher RR

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Period</th>
<th>Spring (THI 68)</th>
<th>Summer (THI 78)</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT per day ( (^oC) )</td>
<td>38.36 (0.03)(^a)</td>
<td>38.86 (0.03)(^b)</td>
<td>+1.2</td>
<td></td>
</tr>
<tr>
<td>RT at 7:00 h ( (^oC) )</td>
<td>38.24 (0.03)(^a)</td>
<td>38.31 (0.03)(^a)</td>
<td>+0.2</td>
<td></td>
</tr>
<tr>
<td>RT at 13:00 h ( (^oC) )</td>
<td>38.51 (0.04)(^a)</td>
<td>39.18 (0.04)(^b)</td>
<td>+1.7</td>
<td></td>
</tr>
<tr>
<td>RT at 22:00 h ( (^oC) )</td>
<td>38.32 (0.04)(^a)</td>
<td>39.10 (0.04)(^b)</td>
<td>+2.0</td>
<td></td>
</tr>
<tr>
<td>HR per day ( (Beat./min) )</td>
<td>64 (0.21)(^a)</td>
<td>70 (0.21)(^b)</td>
<td>+9.3</td>
<td></td>
</tr>
<tr>
<td>HR at 7:00 h ( (Beat./min) )</td>
<td>63 (0.24)(^a)</td>
<td>66 (0.24)(^b)</td>
<td>+4.8</td>
<td></td>
</tr>
<tr>
<td>HR at 13:00 h ( (Beat./min) )</td>
<td>65 (0.26)(^a)</td>
<td>74 (0.27)(^b)</td>
<td>+13.8</td>
<td></td>
</tr>
<tr>
<td>HR at 22:00 h ( (Beat./min) )</td>
<td>64 (0.27)(^a)</td>
<td>69 (0.28)(^b)</td>
<td>+7.8</td>
<td></td>
</tr>
<tr>
<td>RR per day ( (Insp./min) )</td>
<td>31 (0.29)(^a)</td>
<td>36 (0.29)(^b)</td>
<td>+16.1</td>
<td></td>
</tr>
<tr>
<td>RR at 7:00 h ( (Insp./min) )</td>
<td>28 (0.38)(^a)</td>
<td>28 (0.38)(^a)</td>
<td>+0.0</td>
<td></td>
</tr>
<tr>
<td>RR at 13:00 h ( (Insp./min) )</td>
<td>31 (0.37)(^a)</td>
<td>41 (0.38)(^b)</td>
<td>+32.3</td>
<td></td>
</tr>
<tr>
<td>RR at 22:00 h ( (Insp./min) )</td>
<td>32 (0.37)(^a)</td>
<td>37 (0.38)(^b)</td>
<td>+15.6</td>
<td></td>
</tr>
<tr>
<td>Cortisol ( (nmol·L^{-1}) )</td>
<td>21.75 (8.30)(^a)</td>
<td>23.5 (4.60)(^a)</td>
<td>+8.0</td>
<td></td>
</tr>
<tr>
<td>Thyroxin ( (pmol·L^{-1}) )</td>
<td>15.5 (0.69)(^a)</td>
<td>14.5 (0.43)(^a)</td>
<td>−6.4</td>
<td></td>
</tr>
</tbody>
</table>

Least squares means on the same row with the same letter are not significantly different \((P > 0.05); \) ( ): standard error; THI: temperature-humidity index; Beat./min: beats per minute; Insp./min: inspirations per minute.
were reported by Knapp and Grummer [15] for cows housed in environmentally controlled chambers. Stress conditions in the latter study were severe and differed from those in our study as a result of higher Ta and RH.

Hormonal changes that occurred in response to heat stress may have played a role in the decline of milk yield as THI increased. Plasma cortisol and thyroxin concentrations of cows were different in both periods but the differences were not significant ($P > 0.05$). THI was negatively correlated with $T_4$ ($r = -0.43$) but positively with cortisol concentration ($r = 0.31$). As THI increased from 68 to 78, the free thyroxin concentration decreased from 15.5 to 14.5 pmol·L$^{-1}$, while the average cortisol concentration went from 21.75 to 23.5 nmol·L$^{-1}$. Generally, cows that are heat stressed have decreased concentrations of thyroid hormones [17], but increased plasma concentrations of corticoids [28, 29]. The lack of significance in hormone concentrations between the two periods could be attributed to the previous exposure of summer cows to high temperatures prior to the trial. Thompson [33] concluded that adaptation to high temperatures results in an increased body temperature and a decreased thyroid activity.

Heat stress at the trial site starts in June and goes through September [4]. Our summer trial was conducted in July, and cows may have already been exposed to one month of an increasing heat stress. They may have been adapted by the time of assignment to the experiment and thus responded differently than cows subjected to an abruptly imposed heat stress [17].

3.4. THI-milk production relationship

Figure 3 shows that milk production is a function of THI. The negative slope of the regression line indicates that milk production decreases as THI increases. This is best expressed by the following equation:

$$\text{milk production (kg per cow per day)} = \frac{47.722 - 0.4129 \times \text{THI}}{R^2 = 0.76}.$$  

This regression indicates that, in general, for each point increase in the THI value above 69, there was a decrease in milk yield of 0.41 kg per cow per day. The value of this relationship for predictive purposes is relatively high, as depicted by an $R^2$ value of 0.76. A large part of the variation in daily milk yield could therefore be attributed to heat stress. The drop in daily milk production in our study was higher than the 0.32

![Figure 3. Relation of milk yield to average THI.](image-url)
and 0.26 kg per cow reported by Ingraham et al. [9] and Johnson [11] for each point increase of the THI values beyond 70.

The present results (Tab. IV) also indicated that the decrease in milk yield started at a THI value of 69, which is similar to the upper point favorable for milk production reported by Silanikove [32], but lower than the critical index of 72 suggested to be the upper limit of the comfort zone for milk production [6, 12]. For THI values between 70 and 73, milk yield dropped by 9% while it dropped by almost 20% when the THI values reached 74. Losses were 23 to 28% when THI were between 76 and 79. The maximum decrease in milk yield occurred when THI reached values of 80 or above. This agreed with the previous results, which indicate that milk yield declines slightly when THI exceeds 72 and declines sharply when 76 is exceeded [11, 16, 35].

The calculated correlations indicated that milk yield was negatively correlated to THI ($r = -0.75, P < 0.01$) and to measured physiological parameters ($r = -0.56; -0.84$ and $-0.72$ for RT, HR and RR, respectively). Moreover, the THI values one, two and three days prior had a greater effect on milk yield than the same day measure. The respective correlation values between milk yield and THI were $-0.83, -0.87, -0.89$.

4. CONCLUSION

Summer heat stress significantly decreased milk yield and milk fat and protein contents in lactating dairy cows managed under Mediterranean climatic conditions. As the THI values increased from 68 to 78, DMI decreased by 1.73 kg and milk production by 4 kg. The regression equation obtained under the conditions of the present work indicates that milk yield drops by 0.41 kg per cow per day for each point increase in the value of THI above 69. Management strategies are needed to minimize heat stress and attain optimal animal productivity.

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