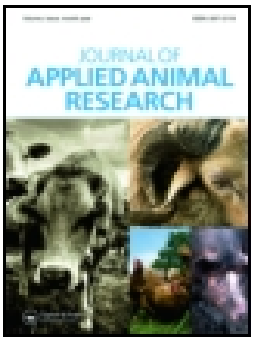

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ABSTRACT
Physiological and productive responses were studied in five Holstein cows in thermal comfort (T1), stress by exogenous adrenocorticotropic hormone (ACTH) administration (T2) and heat stress (T3) to compare acute and punctual stress (ACTH) and prolonged stress (heat stress). During T1 and T2, cows were housed in a climatic-free stall barn. In T3, the animals were kept in a climatic room (air temperature of 37°C from 08:00 to 13:00 h, and of 26°C from 14:00 to 07:00 h) for 7 days. Milk yield, rectal temperature (RT), respiratory rate (RR) and blood samples were obtained before, during and after all treatments. In T1 at 08:00 h, RT and RR were below the upper critical limit. Simultaneously, cortisol and insulin growth-factor I (IGF-I) were within the normal limits. After ACTH administration (T2), cortisol significantly increased, reaching maximum levels at 60 min and returning to basal levels at 300 min. However, IGF-I was not affected. During T3, Holstein cows did not effectively dissipate their body temperature and RT, RR and cortisol significantly increased. There was a 26.6% reduction in milk production after heat stress ($P < .05$). Prolonged heat stress was more stressful and cows had higher levels of CORT in T3 than in T2 even before the increase in body temperature. Although the total amount of cortisol and IGF-I presented a negative and significant Pearson correlation ($r = -0.79$), IGF-I was not significantly influenced by heat stress or ACTH administration, and the relationship between IGF-I and heat stress remains controversial.

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Climatic room; lactation; milk yield

1. Introduction

In tropical regions, solar radiation, air temperature, relative humidity and wind speed can contribute to heat stress in dairy cattle (West et al. 2003; Spiers et al. 2004; Tapki & Sahin 2006). Consequently, high producing cows do not effectively adjust their physiologic and behavioural response to dissipate body heat when exposed to high air temperature (Srikandakumar & Johnson 2004; Shehab-El-Deen et al. 2010). Furthermore, global warming negatively influences the heat exchange between dairy cow and their environment (Baumgard & Rhoads 2012).

In fact, heat could be considered as a chronic stress or prolonged stress (Shehab-El-Deen et al. 2010). During heat stress, dairy cows expend more energy for circulatory adjustments to increase respiratory rate (RR), sweating and panting (Srikandakumar & Johnson 2004). In those animals, heat stress has also been associated with lower food intake, increase in the negative energy balance and loss in milk production (Igono et al. 1992; Chaiyabutr et al. 2008; Shehab-El-Deen et al. 2010). In general, cortisol (CORT) is an appropriate biological endpoint for the investigation of hypothalamic–pituitary–adrenal (HPA) axis, and exogenous administration of adrenocorticotropic hormone (ACTH) can be used to study the relationship between the HPA axis activity, cortisol release and stress in domestic animals (Negrão et al. 2004, 2010). In general, CORT has been associated with physiological adjustments to tolerate stressful conditions (Wise et al. 1988; Ronchi et al. 2001).

Although some studies have demonstrated that CORT levels are not related to heat stress (Ronchi et al. 2001; Zähner et al. 2004; Chaiyabutr et al. 2008), others have associated it with high CORT levels (Satterlee et al. 1977; Du Preez 2000). These results can be explained by the acclimation, which is a process driven by the endocrine system to preserve animal welfare regardless of environmental challenges (Bernabucci et al. 2010). As acclimation involves physiological, behavioural and metabolic mechanisms to maintain productivity and health in ruminants, it is directly related to welfare (Horowitz 2002).

In the same way, while some authors have reported that growth hormone (GH) and insulin-like growth factor I (IGF-I) are negatively influenced by heat stress (Sarko et al. 1994; Rhoads et al. 2010; Wheelock et al. 2010), others mentioned that IGF-I levels during heat stress are similar to those obtained within thermal comfort zone (McGuire et al. 1991; Hirayama et al. 2004; Chaiyabutr et al. 2008; Collier et al. 2008). For these reasons, the interactions between heat stress and CORT and between IGF-I and environmental heat remain controversial.

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In this context, the present study evaluated plasma CORT and IGF-I concentration, rectal temperature (RT), RR and milk yield in lactating Holstein cows submitted to thermal comfort (Treatment 1), stress imposed by ATCH administration (Treatment 2) and heat stress (Treatment 3) in order to compare acute (ACTH) and prolonged stress (heat stress).

2. Materials and methods

The experiment was performed at the Faculty of Animal Sciences and Food Engineering (FZEA) in Pirassununga, Brazil (21°80′00″ S and 47°25′42″ W; 634 m altitude) during the summer. All experimental procedures were approved by the Ethical Committee of Animal Experimentation of the FZEA/USP.

Five non-pregnant Holstein cows in the third lactation and average milk yield of 20 kg/cow/day were used in this study. On average the cows were 63 (±8) days in milk. Feed intake was not measured in this study. All experimental cows were milked twice daily (07:00 and 14:00 h) and the milk production was recorded. The milking routines were constant and the same person performed all the experimental milking. The cows had free access to water and were fed total mixed rations (corn silage, corn, soybeans, mineral and vitamin, according to NRC 2001) after milking at 07:00 and 14:00 h. In the present study, the 25-day experiment was divided into three phases. All animals were subjected to the three treatments in an experimental design of repeated measures.

2.1. *Treatment 1 – thermal comfort (control)*

Experimental cows were kept in free stall under thermal comfort during lactation for 15 days. A cooling system with axial flow fans (125 cm diameter; 300 m^3/min maximum airflow rate) was installed in the east side along the feed passage. The fans were mounted at a height of 2.5 m and angled downward at about 10° from vertical. The system was thermostatically controlled and switched on at 26°C. During this period considered as a control phase, air temperature ranged from 10.4°C to 29.6°C during daylight (06:30–17:30 h) and from 8.9°C to 25.9°C during the night (17:40–06:20 h) with a mean of 19.2 ± 2.8°C, relative humidity with mean of 66.1 ± 1.4% and temperature and humidity index (THI) lower than 72 (Figure 1). THI is a measure of the level of discomfort or stress on living animals calculated as suggested by Thom (1959):

$$THI = (1.8\,x\,T + 32) - (0.55 - 0.0055\,x\,RH\,x\,(1.8\,x\,T - 26)),$$

where T is the air temperature (°C) and RH is the relative humidity (%).

Under thermal comfort, RT and RR were obtained weekly for three consecutive days at 08:00 h and at 13:00 h. Blood samples were collected on the third day at 08:00 and 13:00 h.

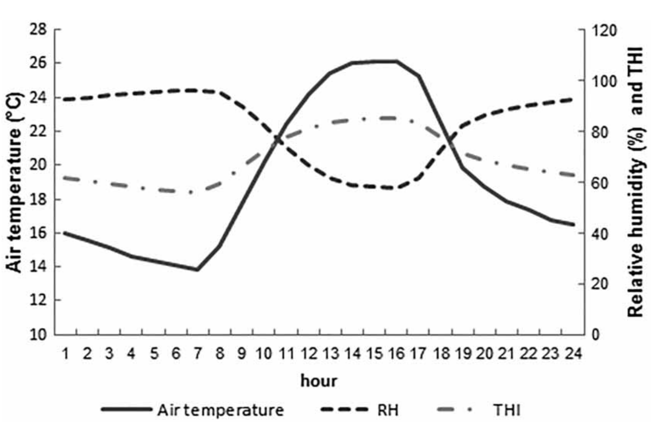

Figure 1. Daily air temperature, relative humidity and THI during thermal comfort (Treatment 1).

2.2. *Treatment 2 – ACTH administration*

On the 15th day, the lactating non-pregnant cows under thermal comfort were injected with 0.6 IU/kg body weight of ACTH (Porcine ACTH 1-24, Sigma®, St. Louis, MO, USA) via mammary vein (time 0) to obtain a controlled response of the adrenal cortex and preserve animal health (Fulkerson & Jamieson 1982; Negrão et al. 2010).

Cows were used for the mammary vein blood sampling. This way the procedure performed by a trained person was fast. If needed, animal restraint was used following the same methods of milking restraint. Blood samples of the same experimental cows were collected before (at −15 and 0 min) and after (at 60, 120 and 300 min) ACTH administration on the 15th day of the experimental period. The 0-min blood sample was taken just before ATCH administration at 08:00 h, and the last blood sample was collected at 13:00 h.

During Treatment 2, the experimental cows were fed at 07:00 h and were kept in free stall barn, restrained by a halter, in thermal comfort for 6 h. During this period, air temperature ranged from 18.4°C to 25.3°C (mean of 23.2 ± 1.1°C), relative humidity variation was from 42% to 62% (with mean of 54.1 ± 1.2%) and THI was lower than 70. Daily meteorological means are presented in Table 1.

2.3. *Treatment 3 – heat stress*

After 15 days in free stall under thermal comfort (T1) and after the ACTH challenge (T2), the same five cows were housed for 7 consecutive days in a climatic room (9 × 10 m × 2.5 m high). Those animals were given a 3-day adaptation period, in thermoneutral conditions, to avoid restraint stress (which was not added to the total experimental period). All the cows were kept unrestrained inside the climatic room, were fed fresh food six times a day and had free access to water. The room was illuminated using artificial light from 06:00 to 18:00 h. Except during machine milking (performed in a milking parlour 50 m away from the laboratory), the cows were

Table 1. Meteorological data monitored 24 h during Treatment 2 and Treatment 3.

Variables	Air temperature (°C)	Relative humidity (%)	THI (index value)
Treatment 2			
Mean	16.5	67.2	63
Min–max	10.2–29.4	45–99	52.2–70.3
Treatment 3			
Mean	29.3	74.1	80
Min–max	26.0–38.0	71–84	75.5–96.6

exposed to high air temperature and humidity in a climatic room for 7 consecutive days (from day 1 to day 22).

The heating system of the climatic room was used to maintain the air temperature at 37 + 1°C from 07:00 to 13:00 h (6 h). After this period, the air temperature and humidity were reduced gradually for 3 h, and subsequently, the air temperature remained at 26 + 1°C for 15 h. Humidity was controlled by ventilation using two axial flow fans (100 cm diameter; 200 m^3/min maximum airflow rate) installed inside the climatic room at a 1.5 m height.

During Treatment 3, blood samples, RT and RR were obtained on 3rd, 5th and 7th day. The blood samples were collected before (at −15 and 0 min) and after (at 60, 120 and 300 min) heat stress. The −15 and 0 min (07:45 h and 08:00 h) blood samples were taken just after the cow entered the climatic room at 08:00 h and the last blood sample was collected at 13:00 h.

The ambient air temperature and relative humidity were recorded with a data logger (LogBox-RHT, Novus, Campinas, SP, Brazil) and the THI was calculated (Table 1).

2.4. *Analytical procedures*

Blood was collected from the mammary vein in heparinized tubes and placed on ice immediately after sampling. Subsequently, the sample was centrifuged at 4°C and 3000*g* for 15 min. The tubes containing plasma were stored at −20°C until CORT and IGF-I were determined using an enzyme immunoassay kit (Diagnostic Systems Laboratory Inc., Webster, TX, USA). All the samples were re-assayed and duplicates were checked for differences higher than 10% between each other. The inter-assay coefficient of variances (CVs) were 9.25% and 11.4% for CORT and IGF-I, respectively, and the intra-assay CVs were 12.1% and 12.9% for CORT and IGF-I, respectively. The kits were validated for cattle by demonstrating parallelism between standard concentrations and serially diluted samples curves (Pauletti et al. 2005; Titto et al. 2013).

Total amounts of CORT and IGF-I measured on experimental period were based on all samples taken during T1, T2 and T3. To measure the area under the curve (AUC) the following formula was applied:

$$\text{AUC} = \frac{1}{2}\sum_{t=1}^{T-1}(t_{t+1} - t_t)(Y_t + Y_{t+1}),$$

where, AUC, is the area under the curve; *T*, the number of measurements; and *Y*, the observation of the outcome variable at time *t*.

RR was measured by visual observation of costal movements for 1 min and is expressed as movements per minute. RT was obtained after RR measurement, using a clinical thermometer (Instrutherm, Sao Paulo, SP, Brazil). This procedure was performed in such a way to avoid compromising animal's welfare.

2.5. *Statistical analysis*

Data were analysed with the mixed procedure of SAS (SAS Institute, Inc. 1998, Cary, NC, USA) using an analysis of variance. The statistical model used the effect of each treatment (thermal comfort, ACTH administration and heat stress), day on milk yield, RT, RR, CORT and IGF-I as main factors; hour and time of sampling was used as repeated measures. All possible interactions among the factors were included. In the case of significant results ($P < .05$) the Student's *t*-test was adopted for multiple comparisons for T1 (RT, RF, CORT, IGF-I); Tukey–Kramer tests were used for T2 and T3 (RT, RF, CORT, IGF-I and milk yield between days) and Newman–Keuls test for T3 (milk yield between morning and afternoon).

All values were presented as the mean (μ) and one standard error of the mean (SEM). The relationships between RT, RR, CORT, IGF-I and milk yield were evaluated by Pearson's correlation coefficients and regression analysis.

3. Results

3.1. *Treatment 1 – thermal comfort (control)*

As presented in Table 2, RT and RR were significantly lower ($P \leq .05$) at 08:00 h than at 13:00 h. However, there were no significant differences for CORT and IGF-I concentrations measured at 08:00 and 13:00 h.

3.2. *Treatment 2 – ACTH administration*

Plasma CORT and IGF-I concentrations measured before and after ACTH administration are shown in Figure 2. When compared with the baseline levels (sampling at −15 and 0 min), CORT increased significantly ($P \leq .05$) after ACTH administration (0-min sampling at 08:00 h), reaching the maximal concentration after 60 min (09:00 h). The CORT concentration decreased at 120 min, and reached basal levels in 300-min sample (at 13:00 h). On the other hand, the IGF-I concentration was not affected by ACTH administration ($P \geq .05$) and there was no significant correlation between the concentrations of CORT and IGF-I ($r = 0.16$, $P = .25$).

3.3. *Treatment 3 – heat stress*

Plasma CORT and IGF-I concentrations in cows submitted to heat stress in a climatic room are shown in Figure 3. When compared wiht the baseline levels (sampling at −15 and 0 min), CORT increased after heat stress (sampling at 60 and 120 min). However, only CORT levels measured at 120 and 300 min on days 18 and 20 were higher ($P \leq .05$) than those measured on day 22. CORT level returned to the baseline levels at 07:45 h in the next day (day 23) after a thermoneutral period. The plasma IGF-I concentration was not affected by heat

Table 2. Least-square means and standard error (SEM) of RT, respiratory frequency (RF), CORT and IGF-I concentration measured in experimental cows under thermal comfort.

Time sampling Data	08:00 h Mean±SEM	13:00 h Mean±SEM
RT (°C)	37.95^a±0.09	38.55^b±0.06
RF (mov/min)	40.42^a±2.28	54.03^b±2.04
Cortisol (ng/ml)	4.97±0.44	4.64±0.39
IGF-I (ng/ml)	64.09±16.12	67.4±11.80

Note: Means within a line labelled with different letters differ ($P \leq .05$).

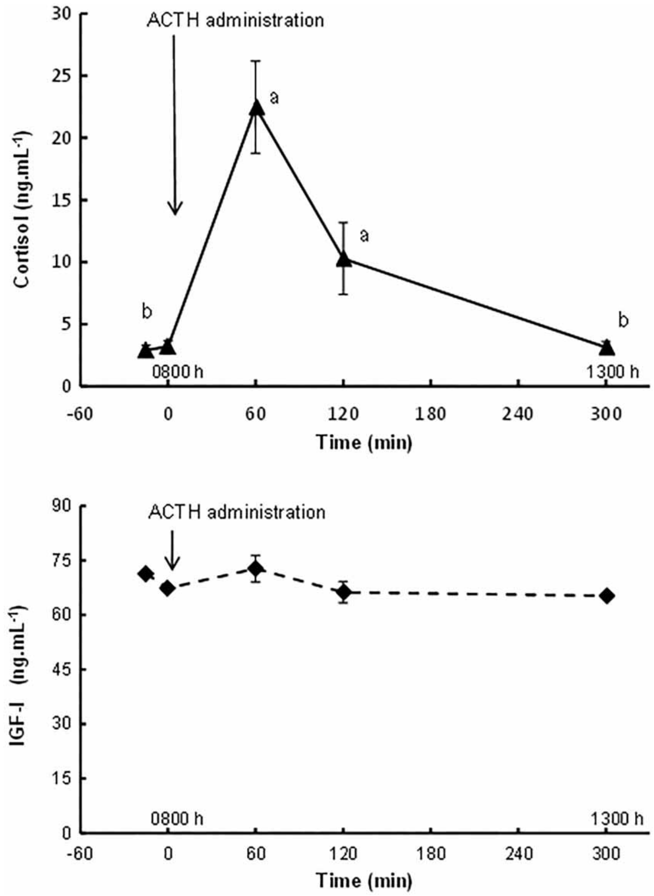

Figure 2. Cortisol and IGF-I levels (ng/ml) before and after ACTH administration in experimental cows. The values are means and standard errors of the mean. Means labelled with different letters differ ($P \leq .05$) within time.

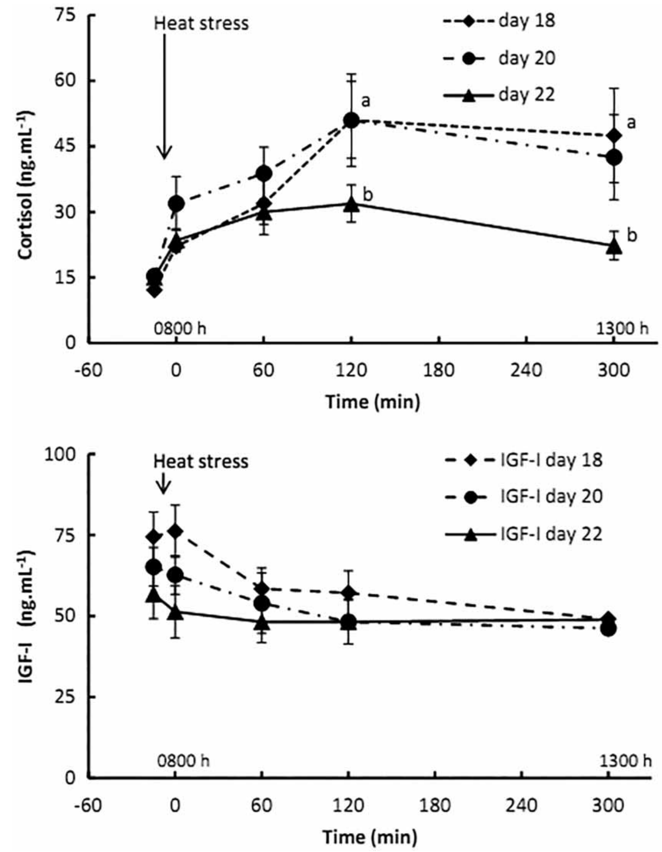

Figure 3. Cortisol and IGF-I levels (ng/ml) measured on 18th, 20th and 22nd day of the experimental period in cows submitted to heat stress during seven consecutive days (from 16th to 22nd). The values are means and standard errors of the mean. Means labelled with different letters differ ($P \leq .05$) on day within sampling time.

stress ($P \geq .05$) and there was no significant correlation between the concentrations of CORT and IGF-I ($r = 0.20$, $P \geq .05$).

The values of RT and RR were not affected by days inside the climatic room, so the mean values at each time point are presented in Table 3. After being exposed to heat stress of 37°C for 60 min (08:00 h), RT and RR increased significantly for all experimental cows. Furthermore, RT and RR remained significantly elevated at 300 min (14:00 h) and presented significant correlations under heat stress ($r = 0.44$, $P \leq .05$).

Milk yields measured before, during and after heat stress in a climatic room are shown in Figure 4. On day 1 after heat stress, the milk yield significantly decreased, when compared with milk production measured before and after heat stress. Milk yield was 26.6% lower on T3. Although all cows were maintained in heat stress for 7 consecutive days, the milk measured during the morning milking did not change significantly during all the periods studied (Figure 4). In contrast, there was a significant reduction in milk production during the afternoon milking from the 17th experimental day on. When the cows had re-established the thermal comfort (free stall), the amount of milk produced in the afternoon increased gradually reaching normal values.

As shown in Figure 5, the total amount of CORT was significantly higher after heat stress (T3) than the amount of CORT after thermal comfort and ACTH test (T1 and T2, respectively). In contrast, there was no significant difference in the total amount of plasma IGF-I concentration during thermal comfort, ATCH administration and heat stress. In fact, CORT and IGF-I changed in opposite ways during heat stress.

Although the total amount of IGF-I concentration during thermal comfort was numerically higher than the total

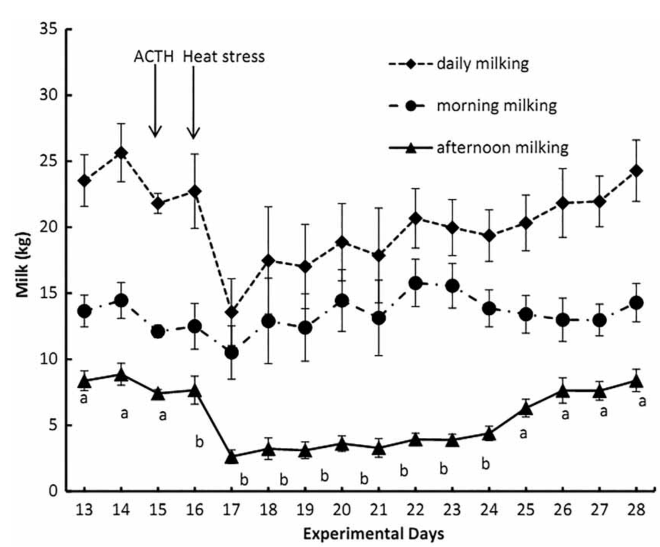

Figure 4. Milk production in five lactating cows before (day 13–15), during (day 16–22) and after (day 23–28) heat stress period. Values are means and standard errors of the mean. Means labelled with different letters differ ($P \leq .05$) within day.

Table 3. Least square means and standard error (SEM) of RT and RF measured in experimental cows under heat stress (T3).

Time sampling	07:45 h	08:00 h	09:00 h	10:00 h	13:00 h
Data	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
RT (°C)	38.02[a]±0.08	38.10[a]±0.14	38.84[b]±0.15	39.81[b]±0.13	39.83[b]±0.12
RF (mov/min)	45.03[a]±2.28	44.42[a]±2.04	65.01[b]±4.22	74.07[b]±4.93	66.08[b]±3.61

Note: Means within a line labelled with different letters differ ($P \leq .05$).

amount concentration during heat stress, the total amount of CORT and IGF-I tended to present a negative and significant Pearson correlation ($r = -0.79$, $P = .12$).

4. Discussion

Different studies have demonstrated that THI is an adequate indicator of climatic conditions, and that a THI higher than 72 is related to heat stress (Igono et al. 1992; Du Preez 2000; West et al. 2003). In fact, during the T1 (thermal comfort/control) and T2 (ACTH administration), the THI values were lower than 72; consequently, the experimental cows were in thermal comfort during these treatments.

However, in indoor climatic room (T3), the THI was higher than 72 from 08:00 at 13:00 h for 7 consecutive days. On the other hand, at the first experimental day, it was necessary to reduce the air temperature and humidity rapidly after 6 h, because RT and RR attained critical values (39°C and 70 mov/min, respectively); as a result, the overnight temperature remained at 26°C. These extreme responses to imposed heat stress demonstrated that high temperature and humidity seriously affected the dairy cows (Du Preez 2000; Shehab-El-Deen et al. 2010).

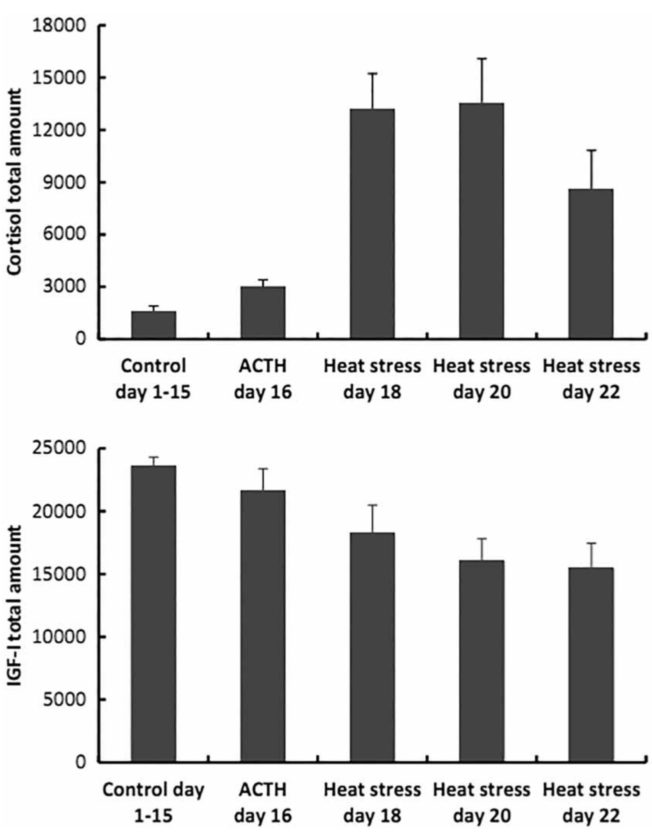

Figure 5. Total amount of CORT and IFG-I measured on experimental tests. Total amount based on all samples taken, the value of the integrated AUC corrected for basal levels. Means labelled with different letters differ ($P \leq .05$).

4.1. *Thermal comfort*

Under thermal comfort (T1), RT and RR were higher in the afternoon (13:00 h) when compared with the morning measurements (08:00 h). However, RT and RR afternoon values were below the upper critical limit (Du Preez 2000), showing that experimental cows were not heat stressed. During thermal comfort, the dairy cows presented effective thermoregulatory characteristics and normal challenges were evaluated when air temperature increased from morning to afternoon (Wise et al. 1988; Ronchi et al. 2001; Kadzere et al. 2002). In this way, CORT and IGF-I levels presented no important variation during the day and hormonal responses were comparable to those reported in studies with dairy cows in thermal comfort.

4.2. *ACTH administration*

On the day of ACTH administration, THI was lower than 72, so the cows were not under heat stress. At the same time, CORT levels measured before, during and after ACTH administration were similar to those observed by other authors (Negrão et al. 2004). In fact, plasma CORT concentration after ACTH administration is an appropriate characteristic to study stress response in dairy animals (Fulkerson & Jamieson 1982; Negrão et al. 2010). Interestingly, many authors have associated a similar increase in CORT with different kinds of stress (Rushen et al. 2001; Negrão & Marnet 2006), including heat stress (Satterlee et al. 1977; Du Preez 2000).

Some authors demonstrated that IGF-I had a slight decrease during heat stress (Sarko et al. 1994; Rhoads et al. 2009; Wheelock et al. 2010). In the present study, however, IGF-I concentrations were not influenced by ACTH administration or increase in plasma CORT concentration. In the same way, other authors also demonstrated that IGF-I levels during heat stress are similar to those obtained within the zone of thermal comfort (McGuire et al. 1991; Hirayama et al. 2004; Collier et al. 2008). In fact, the relation between IGF-I and heat stress remains controversial.

4.3. *Heat stress*

Although RT was higher during heat stress, its values were within the normal range, below 39.5°C for cows (Du Preez 2000; Silanikove 2000; Kadzere et al. 2002). Under heat stress, the experimental cows were able to prevent further increase in their body temperature (RT) by increasing their RR. In this case, RR measured during our experiment was above the comfort limit value of 60 mov/min (Silanikove 2000; Kadzere et al. 2002).

On the other hand, the experimental cows controlled RT and RR overnight (when air temperature was maintained at 26°C for a long period) and those parameters were normal in the next morning (at 07:45 and 08:00 h). These results are in accordance with those reported by other authors, which demonstrated a cyclic effect of heat stress similar to changes observed in air temperature (Silanikove 2000; Srikandakumar & Johnson 2004).

The RT and RR variations measured in the present study were consistent with the previous studies that submitted dairy cows to heat stress (Du Preez 2000; Kadzere et al. 2002; Tapki & Sahin 2006; Shehab-El-Deen et al. 2010). In general, our result suggests that dairy cows adapted to tropical environment did not show effective adjustment in their body temperature when submitted to environmentally induced hyperthermia and relative humidity for a long period (Srikandakumar & Johnson 2004; Shehab-El-Deen et al. 2010).

CORT levels measured in the present study were higher than those observed during different stressful conditions (Srikandakumar & Johnson 2004; Shehab-El-Deen et al. 2010; Comin et al. 2011). The basal CORT concentration in T3 was similar to the peak of CORT in T2. The high cortisol levels observed previously to the heat stress in T3 can be explained by the inability of the animals to maintain thermoneutrality despite the fact of being kept under 26°C. For T3, data were first collected (18 day) on animals submitted to heat stress for three days in a way that it became evident that thermoregulation was clearly compromised by prolonged heat stress. Consequently, heat stress imposed in the present study could be considered an important stressor (Shehab-El-Deen et al. 2010).

In this context, the total amount of CORT measured after heat stress imposed on 3rd and 5th days were higher in our experimental cows, when compared with those observed in T1 and T2 and other studies (Wise et al. 1988; Ronchi et al. 2001). However, on the 7th day, CORT levels were not influenced by heat stress.

These results are in agreement with other studies that have reported CORT decrease some days after heat stress imposition (Collier et al. 2008; Boonkum et al. 2011). The total amount of CORT released during the present study confirmed that CORT was significantly higher during heat stress than comfort and ACTH tests; this indicated that heat stress is a potent stressor.

In contrast, in the present study, the IGF-I concentration was not influenced by heat stress. Similar results were previously described (McGuire et al. 1991; Hirayama et al. 2004; Chaiyabutr et al. 2008). Other authors, in opposition to the aforesaid, reported that bovine somatotropin treatment, under heat stress, increased milk production, IGF-I release and RT in dairy cows (Sarko et al. 1994; Jousan et al. 2007).

Although there was no significant effect of heat stress on IGF-I release, the total amount of IGF-I release during thermal comfort was numerically higher than the total amount released during heat stress. Furthermore, the total amount of CORT and IGF-I tended to present a negative and significant correlation. In the same way, some studies have reported that heat stress caused a numerical decrease in IGF-I release during heat stress (Collier et al. 2008; Rhoads et al. 2010; Wheelock et al. 2010). However, at low air temperatures, CORT are often lower and IGF-I are often higher (Richards et al. 1995; Rensis & Scaramuzzi 2003).

These last results suggest that IGF-I could be involved in milk yield reduction, as hypothesized in earlier studies (Rhoads et al. 2010; Wheelock et al. 2010; Baumgard & Rhoads 2012). Consequently, the effect of heat stress on IGF-I release remains controversial.

It is possible that experimental Holstein cows selected under tropical environment are better adapted and less sensitive to heat stress (Silanikove 2000; Kadzere et al. 2002; Collier et al. 2006). In fact, the decrease in milk yield measured during morning milking was lower than that in afternoon.

The significant reduction in milk yield measured during afternoon milking, when environmental temperature was 37°C is due to the ineffective body temperature dissipation observed in Holstein cows. Lower morning feed intake caused afternoon milking reduction. However, when there was a decrease in air temperature, feed intake was normalized in a way that milking was not affected. On the other hand, during heat stress there is an increase in blood flow to body surfaces to maximize heat loss and ensure thermoregulation. As there is a lower mammary blood flow, the substrate supply for milk synthesis in the mammary gland decreases (Hansen 1994). As the overnight temperature was 26°C, Holstein cows could effectively regulate their body temperature (Wise et al. 1988; Ronchi et al. 2001; Kadzere et al. 2002) and morning milk production was satisfactory.

Comparative analysis of milk production during the 7 days of heat stress revealed the total milk yield was 26.6% lower than milk produced before and after stress imposition. Milk production after imposed stress period was similar to the one observed before heat stress. In this way, it was possible to confirm that milk production was influenced by cyclic challenge of temperature and humidity (Igono et al. 1992), as imposed in this study in a climatic room.

In general, cows are susceptible to heat stress and an 1°C increase is considered sufficient to compromise animal's performance (McDowell et al. 1976), and a THI of 72 is the start point for milk yield decreasing (Zimbelman et al. 2009). Therefore, CORT stimulates gluconeogenesis in the liver, generating new energy from stored reserves (Rhoads et al. 2010), so this hormone could be associated with the extra energy for respiratory adjustments and body temperature regulation (Satterlee et al. 1977; Du Preez 2000; Silanikove 2000; Kadzere et al. 2002).

5. Conclusion

Milk production and CORT release were affected by high temperature and humidity, especially in the beginning of heat stress treatment. However, IGF-I measured under thermal stress did not differ from that measured after ACTH administration or heat stress. Thus, the relationship between IGF-I and heat stress remains controversial.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

Baumgard LH, Rhoads RP. 2012. Ruminant production and metabolic responses to heat stress. J Anim Sci. 90:1855–1865.

Bernabucci U, Lacetera N, Baumgard LH, Rhoads RP, Ronchi B, Nardone A. 2010. Metabolic and hormonal acclimation to heat stress in domesticated ruminants. Animal. 4(7):1167–1183.

Boonkum W, Misztal I, Duangjinda M, Pattarajinda V, Tumwasorn S, Sanpote J. 2011. Genetic effects of heat stress on milk of Thai Holstein crossbreds. J Dairy Sci. 94:487–492.

Chaiyabutr N, Chanpongsang S, Suadsong S. 2008. Effects of evaporative cooling on the regulation of body water and milk production in crossbred Holstein cattle in a tropical environment. Int J Biometeorol. 52:575–585.

Collier RJ, Dahl GE, VanBaale MJ. 2006. Major advances associated with environmental effects on dairy cattle. J Dairy Sci. 89:1244–1253.

Collier RJ, Miller MA, McLaughlin CL, Johnson HD, Baile CA. 2008. Effects of recombinant bovine somatotropin (rbST) and season on plasma and milk insulin-like growth factors I (IGF-I) and II (IGF-II) in lactating dairy cows. Domest Anim Endocrin. 35:16–23.

Comin A, Prandi A, Peric T, Corazzin M, Dovier S, Bovolenta S. 2011. Hair cortisol levels in dairy cows from winter housing to summer highland grazing. Livest Sci. 138:69–73.

Du Preez JH. 2000. Parameters for determination and evaluation of heat stress in dairy cattle in South Africa. Onderstepoort J Vet. 67:263–271.

Fulkerson WJ, Jamieson PA. 1982. Pattern of cortisol release in sheep following administration of synthetic ACTH or imposition of various stressor agents. Aust J Biol Sci. 35:215–222.

Hansen PJ. 1994. Causes and possible solutions to the problem of heat stress in reproductive management of dairy cows. In: Jordan E, editor. Proceedings of the National Reproductive Symposium; 1994 Sep 22–23; Pittsburgh, PA. Dallas, TX: Texas Agricultural Extension Service.

Hirayama T, Katoh K, Obara Y. 2004. Effects of heat exposure on nutrient digestibility, rumen contraction and hormone secretion in goats. Anim Sci J. 75:237–243.

Horowitz M. 2002. From molecular and cellular to integrative heat defence during exposure to chronic heat. Comp Biochem Phys A. 131:475–483.

Igono MO, Bjotvedt G, Sanford-Crane HT. 1992. Environmental profile and critical temperature effects on milk production of Holstein cows in desert climate. Int J Biometeorol. 36:77–87.

Jousan FD, de Castro e Paula LA, Block J, Hansen PJ. 2007. Fertility of lactating dairy cows administered recombinant bovine somatotropin during heat stress. J Dairy Sci. 90:341–351.

Kadzere CT, Murphy MR, Silanikove N, Maltz E. 2002. Heat stress in lactating dairy cows: a review. Livest Sci. 77:59–91.

McDowell RE, Hooven NW, Camoens JK. 1976. Effect of climate or performance of Holstein in first lactation. J Dairy Sci. 59:965–973.

McGuire MA, Beede DK, Collier RJ, Buonomo FC, Delorenzo MA, Wilcox CJ, Huntington GB, Reynolds CK. 1991. Effects of acute thermal stress and amount of feed intake on concentrations of somatotropin, insulin-like growth factor (IGF)-I and IGF-II, and thyroid hormones in plasma of lactating Holstein cows. J Anim Sci. 69:2050–2056.

National Research Council. 2001. Nutrient requirements of dairy cattle. 7th ed. Washington, DC: National Academy Press.

Negrão JA, Marnet PG. 2006. Milk yield, residual milk, oxytocin and cortisol release during machine milking in Gir, Gir x Holstein and Holstein cows. Reprod Nutr Dev. 46:77–85.

Negrão JA, Porcionato MAF, Pasillé AM, Rushen J. 2004. Cortisol in saliva and plasma of cattle after ACTH administration and milking. J Dairy Sci. 87:1713–1718.

Negrão JA, Porcionato MAF, de Passille AM, Rushen J. 2010. Behavioural responses of heifers to ACTH injections. Appl Anim Behav Sci. 128:18–22.

Pauletti P, Bagaldo AR, Kindlein L, Paz CCP, Lanna DPD, Machado Neto R. 2005. IGF-I and IgG serum concentrations and in mammary secretions in cows treated with rbST prepartum. R Bras Zootec. 34 (3):976–986.

Rensis F, Scaramuzzi RJ. 2003. Heat stress and seasonal effects on reproduction in the dairy cow – a review. Theriogenology. 60(6):1139–1151.

Rhoads ML, Kim JW, Collier RJ, Crooker BA, Boisclair YR, Baumgard LH, Rhoads RP. 2010. Effect of heat stress and nutrition on lactating Holstein cows: II. Aspects of hepatic growth hormone responsiveness. J Dairy Sci. 93:170–179.

Rhoads ML, Rhoads RP, VanBaale MJ, Collier RJ, Sanders SR, Weber WJ, Crooker BA, Baumgard LH. 2009. Effects of heat stress and plane of nutrition on lactating Holstein cows: I. Production, metabolism, and aspects of circulating somatotropin. J Dairy Sci. 92(5):1986–1997.

Richards MW, Spicer LJ, Wettemann RP. 1995. Influence of diet and ambient temperature on bovine serum insulin-like growth factor-I and thyroxine: relationships with non-esterified fatty acids, glucose, insulin, luteinizing hormone and progesterone. Anim Reprod Sci. 37:267–279.

Ronchi B, Stradaioli G, Verini Supplizi A, Bernabucci U, Lacetera N, Accorsi PA, Nardone A, Seren E. 2001. Influence of heat stress or feed restriction on plasma progesterone, oestradiol-17, LH, FSH, prolactin and cortisol in Holstein heifers. Livest Pro Sci. 68:231–241.

Rushen J, Munksgaard L, Marnet PG, De Passilé AM. 2001. Human contact and the effects of acute stress on cows at milking. Appl Anim Behav Sci. 73:1–14.

Sarko TA, Bishop MD, Davis ME. 1994. Relationship of air temperature, relative humidity, precipitation, photoperiod, wind speed and solar radiation with serum insulin-like growth factor I (IGF-I) concentration in Angus beef cattle. Domest Anim Endocrin. 11:281–290.

Satterlee D, Roussel JD, Gomila LF, Segura ET. 1977. Effect of exogenous corticotropin and climatic conditions on bovine adrenal cortical function. J Dairy Sci. 60:1612–1616.

Shehab-El-Deen MAMM, Fadel MS, Soom AV, Saleh SY, Maes D, Leroy JLMR. 2010. Circadian rhythm of metabolic changes associated with summer heat stress in high-producing dairy cattle. Trop Anim Health Pro. 42:1119–1125.

Silanikove N. 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. Livest Pro Sci. 67:1–18.

Spiers DE, Vogt DW, Johnson HD, Garner GB, Murphy CN. 2004. Use of physiological parameters to predict milk yield and feed intake in heat-stressed dairy cows. J Therm Biol. 29:759–764.

Srikandakumar A, Johnson EH. 2004. Effect of heat stress on milk production, rectal temperature, respiratory rate and blood chemistry in Holstein, Jersey and Australian Milking Zebu cows. Trop Anim Health Pro. 36:685–692.

Tapki I, Sahin A. 2006. Comparison of the thermoregulatory behaviours of low and high producing dairy cows in a hot environment. Appl Anim Behav Sci. 99:1–11.

Thom EC. 1959. The discomfort index. Weatherwise. 12:57–61.

Titto CG, Negrão JA, Titto EAL, Canaes, TS, Titto RM, Pereira AMF. 2013. Effects of an evaporative cooling system on plasma cortisol, IGF-I, and milk production in dairy cows in a tropical environment. Int J Biometeorol. 57(2):299–306.

West J, Mullinix BG, Bernard JK. 2003. Effects of hot, humid weather on milk temperature, dry matter intake, and milk yield of lactating dairy cows. J Anim Sci. 86:232–242.

Wheelock JB, Rhoads RP, VanBaale MJ, Sanders SR, Baumgard LH. 2010. Effects of heat stress on energetic metabolism in lactating Holstein cows. J Dairy Sci. 93:644–655.

Wise ME, Armstrong DV, Huber JT, Hunter R, Wiersma F. 1988. Hormonal alterations in the lactating dairy cow in response to thermal stress. J Dairy Sci. 71:2480–2485.

Zähner M, Schrader L, Hauser R, Keck M, Langhans W, Wechsler B. 2004. The influence of climatic conditions on physiological and behavioural parameters in dairy cows kept in open stables. Anim Sci. 78:139–147.

Zimbelman RB, Rhoads RP, Rhoads ML, Duff GC, Baumgard LH, Collier RJ. 2009. A re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. Proceedings of the 24th Annual Southwest Nutrition and Management Conference; 26 & 27 February; Tempe. Arizona: University of Arizona; p. 158–168.