Effects of chronic heat stress on plasma concentration of secreted heat shock protein 70 in growing feedlot cattle

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ABSTRACT: Sixty Angus steers (449.2 ± 11.0 kg) with implanted body temperature (BT) transmitters were used in a 110-d study to determine the effect of chronic stress (housing, diet, and climate) on extracellular heat shock protein 70 (eHsp70) concentration in plasma. The steers were a subset of a larger study involving 164 steers. Before the start of the study (d –31), 63 steers were implanted with a BT transmitter between the internal abdominal muscle and the peritoneum at the right side flank. Steers were housed in 20 pens (10 with shade and 10 without). Within each pen, 3 steers had a transmitter, and BT was recorded at 30-min intervals throughout the study. On d 0, 30, 60, 90, and 110, steers were weighed, BCS assessed (1 to 9 scale in which 1 = emaciated and 9 = obese), and 10 mL of blood from the coccygeal vein was collected for determination of inducible heat shock protein 70 (Hsp70) concentration by ELISA. Climatic variables (ambient temperature, relative humidity, solar radiation, black globe temperature, and wind speed) were obtained every 30 min from an on-site weather station. The relationship between the climatic variables and Hsp70 concentration were examined. As we failed to detect an effect of shade, all data were pooled. Mean BT over the duration of the study was 39.6 ± 0.10°C. Mean BT was lowest (38.7 ± 0.10°C) on d 0 and highest on d 110 (40.2°C ± 0.10). The Hsp70 concentration was least on d 0 (2.33 ± 0.47 ng/mL) and greatest on d 30 (8.08 ± 0.78 ng/mL). The Hsp70 concentration decreased from d 30 but remained above the d-0 concentrations on d 60, 90, and 110. There was a strong relationship between Hsp70 concentration and ambient temperature ($r^2 = 0.86$; $P < 0.0001$) and Hsp70 concentration and photoperiod ($r^2 = 0.94$; $P < 0.0001$) and no relationship with BT ($r^2 = 0.06$; $P < 0.0001$). When assessed with both BCS and BT, the relationship was moderate ($r^2 = 0.48$; $P < 0.001$). The relationship between Hsp70 and change in BT (BTΔ) above 38.6°C was also moderate ($r^2 = 0.54$; $P < 0.0001$). The BT at a given time does not appear to be related to Hsp70 concentration. However, Hsp70 expression may be a useful indicator for BTΔ when BT > 38.6°C. The Hsp70 concentration is a reliable indicator of chronic stress but is not a reliable indicator of a single stressor when animals are exposed to multiple chronic stressors.

Key words: chronic stress, feedlot cattle, heat shock protein 70

INTRODUCTION

Heat shock proteins (HSP) are a highly conserved family of molecular chaperons (Hecker and McGarvey, 2011) and are named according to their molecular weight expressed in kilodaltons [e.g., heat shock protein 70 (Hsp70), heat shock protein 90; Marruchella et al., 2004]. The HSP are activated in response to stressors such as heat, ischemia, physical strain, and oxidative stress (Iwaki et al., 1993). The HSP act cognitively in cellular and tissue homeostasis (Thompson et al., 2002; de Jong et al., 2009) and are released intracellularly and extracellularly in an inducible form in response to stress (Hightower and Guidon, 1989; Hecker and McGarvey, 2011). Among the HSP, Hsp70 has a significant role in cell thermotolerance (Beckham et al., 2004) and animal survival (King et al., 2002). The source of extracellular Hsp70 (eHsp70) during heat stress has not been fully elucidated (Hom et al., 2012). However, studies suggest that the source may be from damaged intestinal cells.
Table 1. Composition of diets fed to Angus steers

<table>
<thead>
<tr>
<th>Item</th>
<th>Starter</th>
<th>Intermediate 1</th>
<th>Intermediate 2</th>
<th>Finisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ingredients, kg/t (as fed)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheat, dry rolled</td>
<td>450</td>
<td>540</td>
<td>625</td>
<td>700</td>
</tr>
<tr>
<td>Molasses, cane</td>
<td>125</td>
<td>100</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Cottonseed meal, solvent</td>
<td>55</td>
<td>55</td>
<td>25</td>
<td>–</td>
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<tr>
<td>Cottonseed, high lint</td>
<td>70</td>
<td>80</td>
<td>80</td>
<td>90</td>
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<tr>
<td>Wheat straw</td>
<td>85</td>
<td>85</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Sorghum silage</td>
<td>70</td>
<td>110</td>
<td>110</td>
<td>90</td>
</tr>
<tr>
<td>Alfalfa hay</td>
<td>120</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vegetable oil</td>
<td>–</td>
<td>–</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Protein–mineral supplement</td>
<td>25.3</td>
<td>30</td>
<td>40</td>
<td>45</td>
</tr>
</tbody>
</table>

Nutrient content, on a DM basis

<table>
<thead>
<tr>
<th>Item</th>
<th>NEg, Mcal/kg</th>
<th>CP, %</th>
<th>Fat, %</th>
<th>Ca, %</th>
<th>P, %</th>
<th>NaCl, %</th>
<th>S, %</th>
<th>K, %</th>
<th>Monensin, g/t</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starter</td>
<td>1.18</td>
<td>14.3</td>
<td>3.42</td>
<td>0.78</td>
<td>0.40</td>
<td>0.11</td>
<td>0.28</td>
<td>1.38</td>
<td>12.4</td>
</tr>
<tr>
<td>Intermediate 1</td>
<td>1.26</td>
<td>13.9</td>
<td>3.67</td>
<td>0.69</td>
<td>0.42</td>
<td>0.14</td>
<td>0.26</td>
<td>1.10</td>
<td>15.2</td>
</tr>
<tr>
<td>Intermediate 2</td>
<td>1.35</td>
<td>13.5</td>
<td>4.85</td>
<td>0.80</td>
<td>0.42</td>
<td>0.18</td>
<td>0.26</td>
<td>0.88</td>
<td>20.1</td>
</tr>
<tr>
<td>Finisher</td>
<td>1.44</td>
<td>13.2</td>
<td>6.18</td>
<td>0.83</td>
<td>0.42</td>
<td>0.20</td>
<td>0.25</td>
<td>0.71</td>
<td>22.2</td>
</tr>
</tbody>
</table>

1Contained on a DM basis: 42.6% CP, 7742.3 IU/kg vitamin A, 193.6 IU/kg vitamin E, 14.4% Ca, 0.40% P, 3.91% salt, 0.72% K, 1.85% S, 0.81% Mg, 618.67 mg/kg Zn, 1361.91 mg/kg Fe, and 139.92 mg/kg Cu.
2Elanco Animal Health, West Ryde, Australia.

The study was undertaken in Central Queensland, Australia, at the Department of Employment Economic Development and Innovations (DEEDI) Brigalow Research Station feedlot (24°84′ S, 149°78′ E, and 168 m above mean sea level) during the Southern Hemisphere summer (November to March) with the approval of the DEEDI Animal Ethics Committee.

The study described herein was part of a larger study of 164 Black Angus steers. A full description of the feedlot, animals, and nutritional management was presented previously (Gaughan et al., 2010). In the study described in this paper, 60 steers out of the 164 steers of the larger study, ranging in chronological age from 390 to 480 d at the commencement of the study, were used. The 60 steers were implanted with a body temperature (BT) transmitter (see below) and of these, 3 were allocated to each pen. Twenty outdoor pens with an earthen floor were used, 16 of which had an area of 144 m2 (8 steers/pen; 18 m2/steer) and 4 had an area of 168 m2 (9 steers/pen; 18.7 m2/steer). Eight of the 144-m2 pens and 2 of 168-m2 pens had shade (3.3 m2/steer). The remaining 10 pens were unshaded. The shade was provided by 80% solar block shade cloth (Darling Downs Tarpaulins, Toowoomba, Australia). The structure was 4 m high and had a north–south orientation. The earthen floor had a 2% slope from the feed bunk (eastern end) to the rear of the pen (western end). Concrete feed bunks with a 3-m concrete apron were located at the front of each pen. Linear feed bunk and water trough space per steer were 583 and 279 mm/steer, respectively. The water trough was located at the rear of each pen.

Diets and Feeding

The feed intake of the steers was managed using a modified “clean bunk at midday” program (Lawrence, 1998). A starter diet was fed on d 0 to 3 followed by 2 intermediate diets (intermediate 1: d 4 to 10; intermediate 2: d 11 to 16). From d 17 a finisher diet was fed (Table 1).

Body Temperature

Thirty-three days before the commencement of the feedlot study, 63 steers were surgically implanted [between the internal abdominal (abdominal oblique) muscle layer and the peritoneum at the right side flank] with a digital BT transmitter (Sirtrack Ltd, Havelock North, New Zealand) as described by Gaughan et al. (2010). Data from 60 of the implanted steers were used in the study described herein. The remaining 3 were alternate animals and were not used in the study.

Each transmitter was calibrated at 40°C before the commencement of the study and reassessed at the end of the study. Each transmitter (30 mm in diam. by 95 mm long) operated on a different radio frequency (150.10 to 151.36 MHz), which were detected and logged onto a radio receiver (TR-5 Receiver; Telonics, Mesa, AZ) that was programmed to acquire BT data from each transmitter at 30-min intervals on a 24-h cycle. At the end of each 24-h cycle, temperature acquisitions from each transmitter were

(Shapiro et al., 1986).

Redirection of blood to the periphery for enhanced heat dissipation is a primary response to a heat challenge (Cronje, 2007). Concurrently, blood flow to the intestines is reduced. If exposure to a thermal challenge persists, the reduced supply of O2 and nutrients results in cell damage (Hall et al., 2001; Cronje, 2007), leading to a loss of intestinal barrier integrity (Doklandy et al., 2006; Lambert, 2009) and increased intestinal permeability, which facilitates the penetration of endotoxins, thereby causing an inflammatory response (Shapiro et al., 1986; Lambert, 2009). Extracellular Hsp70 has important functions in pro-inflammatory immune response (Pockley, 2003); therefore, changes in eHsp70 may be an indication of cellular damage within the intestines (Doklandy et al., 2006). The aim of the current study was to investigate changes in inducible Hsp70 concentration in the plasma of feedlot cattle exposed to chronic heat stress.

MATERIALS AND METHODS

The study was undertaken in Central Queensland, Australia, at the Department of Employment Economic Development and Innovations (DEEDI) Brigalow Research Station feedlot (24°84′ S, 149°78′ E, and 168 m above mean sea level) during the Southern Hemisphere summer (November to March) with the approval of the
downloaded. Each transmitter was switched off for approximately 10 min as data were downloaded to a computer (TR-5 interface software; Telonics). After data downloads, BT acquisition recommenced. This cycle continued for the duration of the study. On blood collection days (see below), the data downloads corresponded with the time cattle were being weighed (approximately 5 min before blood collection). This allowed for individual BT to be obtained as close as possible to the time of blood collection.

**Weighing and Blood Collection**

Before the commencement of the study, the cattle were managed in a 40-ha grazing paddock. On d –1 the steers were moved from the paddock to the feedlot. The steers were weighed and individual BT obtained from the transmitters. This procedure was repeated on d 0, and the steers were then randomly allocated to a feedlot pen based on stratification of BW and BT. The steers had access to feed upon arrival in each pen.

Body condition score was assessed using a 1 to 9 scale in which 1 = emaciated and 9 = obese (Herd and Sprott, 1996), and cattle were weighed immediately before blood collection on d 0, 30, 60, 90, and 110, which corresponded to these dates: November 11, December 12, January 11, February 10, and March 1 (the study was undertaken in a leap year so there were 29 d in February). On each of these days, the procedures commenced at approximately 0600 h and were completed by approximately 0900 h. The same pen sequence was used for each blood collection (always started with pen furthest from the weighing area).

The steers were removed from their respective pens and walked as a pen group 10 to 30 m (distance determined by pen location within the feedlot) to an open-sided barn with a weighing crate and squeeze chute. Within the barn, the steers were weighed and blood was collected. After the steers were weighed, they were restrained in a head bail and squeeze chute for blood collection (approximately 5 min per steer). After blood collection, the steers were returned to their respective pens.

Blood for Hsp70 determination was collected from the coccygeal vein using 10-mL lithium heparin-coated vacutainers (BD Vacutainer, Franklin Lakes, NJ). Immediately after collection, the samples were chilled (approximately 2 to 4°C) for 30 min and then underwent centrifugation at 1,328 × g for 10 min at 2°C. After centrifugation, the plasma was separated into 2 equal aliquots into 2- by 5-mL specimen containers and placed on ice for 30 min. The samples were then frozen (–20°C for 24 h) and stored at –80°C until assayed.

**Heat Shock Protein 70 Analysis**

The Hsp70 concentration was determined using ELISA. All diluents and buffers were brought to room temperature before use (approximately 24°C). All incubation steps were carried out in a humid container. Samples were diluted 1:40 in carbonate/bicarbonate buffer (pH 9.2; TropBio, Townsville, Queensland, Australia) and thoroughly mixed. After dilution, 100 μL of diluted sample were added to each well of a 96-well, flat bottom microtitre plate, which was incubated overnight at room temperature. After incubation, excess reagent was decanted; the plate was tapped dry with no wash step, and 120 μL of postcoating buffer (TropBio) were added to each well and incubated at room temperature for 2 h. Excess blocking buffer was shaken out and the plate was then dried for 2 h at 37°C. Mouse anti-human Hsp70 monoclonal antibodies (mAb; BioScientific, Gymea, NSW, Australia) were diluted in TEN-TC buffer (TropBio) to a dilution of 1 μg/mL. Then 100 μL were added for each well of diluted mAb and incubated for 1 h at room temperature. Excess mAb was shaken out and wells were rinsed 3 times with 10 M CaCl₂ (TC) buffer (TropBio). Goat anti-mouse horseradish peroxidase conjugated antibody (TropBio) was diluted in TC buffer to 1:1,000. After this process, 100 μL of diluted mAb conjugate were added to each well and incubated for 1 h at room temperature. Excess mAb solution was shaken out and afterwards the wells were rinsed 3 times with TEN-TW buffer (50 mM Tris, 1 mM EDTA, 0.15 M NaCl, 0.2% casein, 0.05% Tween 20, pH 8.0; TropBio) and 100 μL of 2,2'-azino-bis [3-ethylbenzthiazoline-6-sulphonic acid] were added to each well, which was then incubated in the dark at room temperature for 1 h. [Note: blank control = carbonate/bicarbonate buffer; negative control = fetal bovine serum at 1:40 dilution; positive control protein standard at 0.04 μg/mL = human Hsp70 protein (BioScientific)].

The assay was validated using the method of Cimino et al. (2002). The optical density (OD) of each plasma sample was measured in duplicate at a dual absorbance of 414 and 492 nm. The average sample OD was then corrected to incorporate blank control values and colorimetric substrate (dual-wavelength readings). The OD concentrations, determined by ELISA, were logit transformed and a standard curve of logit values vs. human Hsp70 concentration was developed. Parallelism between the human Hsp70 standard and the bovine plasma samples was obtained at OD measurements between 1.815 and 0.004, with the slope of both plots being 0.979. Regression analysis (PROC REG; SAS Inst. Inc., Cary, NC) was used to determine the concentration of human Hsp70 equivalents (y = 97.97x – 0.053; r² = 0.97). When OD measurements from the bovine samples were in the range showing parallelism, the OD values were divided by the dilution and specific concentrations of human Hsp70 equivalents were determined. The plasma Hsp70 detection range was 0.031...
Heat shock protein expression in cattle

The plasma concentration of Hsp70 and the BT on the day of sampling were analyzed using repeated measures in PROC MIXED of SAS. Data were analyzed for blood collection day (DAY: d 0, 30, 60, 90, and 110) × animal effects (BCS, Hsp70 expression, change in BT (BTΔ) relative to d 0, and actual BT) and included fixed effects for DAY, animal, BWG, BWG × DAY, and animal × DAY. Animal was considered the experimental unit. Where effects were significant (P < 0.05), pairwise comparisons of the least squares means were carried out within each DAY.

Regression analysis was undertaken using PROC REG of SAS to determine the relationship between Hsp70 concentration, BT, BW, and BTΔ. Regression analysis was also used to determine the relationship between Hsp70 expression and 1) 7-d (168 h before blood sampling) means, 2) 7-d mean maximums, and 3) 24-h means (24 h before sampling) for PP, TA, BG, HLI, and THI. The model was also run to determine the relationship between BT and the aforementioned climatic variables and also to determine to what extent the animal and climatic measures could predict Hsp70 concentration and vice versa. Average daily gain and the change in BCS from d 0 were analyzed using PROC GLM of SAS. Least squares means were estimated for the various treatment effects. Statistical significance was declared at P < 0.05.

RESULTS

Animal Health

The animals had fully recovered from surgery before the commencement of the study. On d 0, 18 steers, and on d 4, 2 steers were treated for eye infection using cloxacillin benzathine (Orbenin Eye Ointment; Pfizer Australia P/L., West Ryde, NSW, Australia). On d 14 and 59, 1 steer each day was treated with oxytetracycline hydrochloride (Engemycin 100; Intervet Australia P/L., Bendigo, VIC, Australia). The BT and Hsp70 data from the steer that was treated on d 59 was excluded from the d-60 dataset. Although there were some occasional incidences of nasal discharge and sore feet, no other veterinary treatment was administered throughout the study.

Weather Data

The weather conditions were sufficient to induce a heat stress response on most days of the study. On average, the weather conditions were suitable for 76.0% of the study period (36.7 h). The averages for 4 periods representing d 0 to 30 (period 1), d 30 to 60 (period 2), d 60 to 90 (period 3), and d 90 to 110 (period 4) are presented in Table 2. A 21-d heat wave ensued from d 71 to 91, which resulted in significant heat stress. Steers were exposed to TΔ > 23°C for 10.9 h/d (3.6 h was >30°C) and 8.3 h/d when TΔ < 23°C, which included 2.6 h/d when TΔ < 20°C. The averages for 4 periods representing d 0 to 30 (period 1), d 30 to 60 (period 2), d 60 to 90 (period 3), and d 90 to 110 (period 4) are presented in Table 2. A 21-d heat wave ensued from d 71 to 91, which resulted in significant heat stress. Steers were exposed to TΔ > 30°C for 8 to 10 h each day during the heat wave with only minimal nighttime relief (Gaughan et al., 2010).

The weather data (TA, BG, and RH), for each 24 h before weighing and blood collection and HLI and THI for each 7-d period before weighing and blood collection are presented in Table 3. The weather conditions in the 24 h before each blood collection were also suf-
efficient to induce a heat-stress response in the steers. During the 7 d before blood collection on d 30, 60, 90, and 110, the cattle were exposed to extreme heat loads (HLI > 96). The mean HLI values suggest that the cattle were exposed to moderate heat load for much of the time. As previously mentioned, d 91 was the end of an intense 21-d period of high heat load. During this 21-d period, the cattle were exposed to daily maximum HLI in excess of 96. The mean monthly PP were 785.8 min in November, 802.9 min in December, 801.9 min in January, 783.9 min in February, and 767.2 min in March (11 d).

Growth Performance

The ADG, BCS, and BW at d 0, 30, 60, 90, and 110 are presented in Table 4. Average daily gain from induction (1.53 ± 0.17 kg/d), BCS at d 110 (7.16 ± 0.09), and BW at d 110 (569.8 ± 11.0 kg) were within the expected range for the type of cattle used in the study when fed a grain-based diet for 110 d.

Body Temperature

The diurnal BT (all steers) for the duration of the study is presented in Fig. 1. Body temperature on sampling days was a function of the mean $T_A$ over the previous 7 d ($P = 0.02$). There were no relationships between BT and mean $T_A$ over the previous 24 h ($P = 0.51$), the 7-d mean maximum $T_A$ ($P = 0.31$), BW ($P = 0.35$) on sampling day, or BCS ($P = 0.18$) on sampling day; however, there was a strong linear relationship ($r^2 = 0.95$) between BT and BGW. The mathematical relationship is shown below as Eq. 1:

$$y = (0.27 \times a) + 38.71,$$

in which $y$ = BT (°C) and $a$ = BW group (1 to 6).

The mean BT over the duration of the study (pooled values for each collection day) was 39.6 ± 0.10°C (range 38.2 to 41.6°C). The mean BT on d 0, 30, 60, 90, and 110 were 38.8 ± 0.06, 39.9 ± 0.08, 39.4 ± 0.08, 39.7 ± 0.10, and 39.8 ± 0.10°C, respectively.

Table 2. Hours per day that dry bulb temperature was >25°C, <25°C, <20°C, and >30°C across the 4 periods of the study

<table>
<thead>
<tr>
<th>Period1</th>
<th>&gt;25°C, h/d</th>
<th>&gt;30°C, h/d</th>
<th>&lt;20°C, h/d</th>
<th>&lt;23°C, h/d</th>
<th>&lt;20°C, h/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (30 d)</td>
<td>10.4</td>
<td>3.3</td>
<td>9.8</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>2 (30 d)</td>
<td>12.5</td>
<td>4.6</td>
<td>6.8</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td>3 (30 d)</td>
<td>11.3</td>
<td>4.0</td>
<td>6.9</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>4 (20 d)</td>
<td>8.7</td>
<td>1.8</td>
<td>10.2</td>
<td>3.6</td>
<td></td>
</tr>
</tbody>
</table>

1Period 1 = d 0 to 30; period 2 = d 30 to 60; period 3 = d 60 to 90; period 4 = d 90 to 110. Day commenced at 0901 h.

Table 3. Mean, maximum, and minimum values for ambient temperature ($T_A$; °C), black-globe temperature (BG; °C), relative humidity (RH; %), for the 24 h before weighing and blood collection and the heat load index (HLI) and temperature humidity index (THI) for the 7 d before weighing and blood collection on d 0, 30, 60, 90, and 110

<table>
<thead>
<tr>
<th>Period</th>
<th>$T_A$, °C</th>
<th>BG, °C</th>
<th>RH, %</th>
<th>HLI2</th>
<th>THI3</th>
</tr>
</thead>
<tbody>
<tr>
<td>d 0</td>
<td>19.9 ± 3.4</td>
<td>21.9 ± 6.74</td>
<td>75.7 ± 11.74</td>
<td>69.9 ± 3.4</td>
<td>68.3 ± 4.4</td>
</tr>
<tr>
<td>Maximum</td>
<td>28.5</td>
<td>36.9</td>
<td>91.0</td>
<td>93.9</td>
<td>77.3</td>
</tr>
<tr>
<td>Minimum</td>
<td>14.2</td>
<td>12.4</td>
<td>46.0</td>
<td>51.3</td>
<td>56.2</td>
</tr>
<tr>
<td>d 30</td>
<td>27.2 ± 4.1</td>
<td>30.4 ± 7.64</td>
<td>62.1 ± 15.6</td>
<td>76.0 ± 12.5</td>
<td>75.5 ± 3.8</td>
</tr>
<tr>
<td>Maximum</td>
<td>35.1</td>
<td>47.4</td>
<td>93.0</td>
<td>100.4</td>
<td>81.9</td>
</tr>
<tr>
<td>Minimum</td>
<td>19.9</td>
<td>19.2</td>
<td>33.0</td>
<td>57.9</td>
<td>67.1</td>
</tr>
<tr>
<td>d 60</td>
<td>26.2 ± 4.7</td>
<td>29.2 ± 8.3</td>
<td>66.9 ± 18.7</td>
<td>75.4 ± 13.3</td>
<td>74.5 ± 4.7</td>
</tr>
<tr>
<td>Maximum</td>
<td>36.7</td>
<td>48.5</td>
<td>96.0</td>
<td>101.6</td>
<td>83.2</td>
</tr>
<tr>
<td>Minimum</td>
<td>18.3</td>
<td>17.6</td>
<td>30.3</td>
<td>56.2</td>
<td>64.5</td>
</tr>
<tr>
<td>d 90</td>
<td>25.6 ± 4.3</td>
<td>28.5 ± 8.1</td>
<td>68.1 ± 20.8</td>
<td>74.2 ± 14.4</td>
<td>73.9 ± 4.6</td>
</tr>
<tr>
<td>Maximum</td>
<td>34.4</td>
<td>47.5</td>
<td>97.0</td>
<td>105.7</td>
<td>82.1</td>
</tr>
<tr>
<td>Minimum</td>
<td>17.1</td>
<td>16.1</td>
<td>29.0</td>
<td>48.9</td>
<td>61.9</td>
</tr>
</tbody>
</table>

1The HLI and THI data were calculated using 10-min weather data from 0900 h (d –7) to 0900 h on the day of weighing and bleeding.

Table 4. Growth performance of grain-fed Angus steers (n = 60) over 110 d

<table>
<thead>
<tr>
<th>Variable</th>
<th>d 0</th>
<th>d 301</th>
<th>d 602</th>
<th>d 903</th>
<th>d 1104</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temp, °C</td>
<td>38.7a</td>
<td>39.9b</td>
<td>39.5b</td>
<td>39.8b</td>
<td>40.2c</td>
<td>0.10</td>
</tr>
<tr>
<td>BW, kg</td>
<td>401.4</td>
<td>449.2</td>
<td>504.3</td>
<td>544.5</td>
<td>569.8</td>
<td>11.00</td>
</tr>
<tr>
<td>BCS5</td>
<td>5.22</td>
<td>5.59</td>
<td>6.15</td>
<td>6.97</td>
<td>7.16</td>
<td>0.09</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>–</td>
<td>1.60</td>
<td>1.72</td>
<td>1.59</td>
<td>1.53</td>
<td>0.17</td>
</tr>
</tbody>
</table>

1ADG from d 0 to 30.

2ADG from d 0 to 60.

3ADG from d 0 to 90.

4ADG from d 0 to 110.

5On each day, body temperature was measured immediately before weighing.

6BCS was on a 0 to 9 scale in which 1 = emaciated and 9 = obese (Herd and Sprott, 1996).
Heat shock protein expression in cattle

± 0.08, and 40.2 ± 0.06°C, respectively. The overall changes in BT (pooled values for d 30, 60, 90, and 110 relative to d 0) ranged from –0.34 to 2.76°C, which was a mean increase of 1.12°C. There was considerable BT variation among animals. On d 0, the range was 38.2 to 40.2°C (2.0°C variation). On d 110, which corresponded with the highest mean BT, the range was 39.5 to 41.1°C (a 1.6°C variation).

Heat Shock Protein 70 Concentration

Two hundred thirteen plasma samples were analyzed. Measurable concentrations of Hsp70 were detected in 209 of these samples. The concentration of Hsp70 in 10 of the 209 samples was outside the validated detection range (0.33 to 20.00 ng/mL); these 10 samples were excluded from the data set, leaving a total of 199 valid samples.

The least mean (± SE) Hsp70 concentration was on d 0 (2.33 ± 0.47 ng/mL), and the greatest concentration was on d 30 (8.08 ± 0.78 ng/mL), which was a 3.47-fold increase relative to d 0 (Fig. 2). The Hsp70 concentration then decreased on each test day relative to d 30. From d 0 to 60 there was a 2.92-fold increase in Hsp70 concentration (6.80 ± 0.78 ng/mL). By d 90 and 110, Hsp70 concentration, although elevated relative to d 0 (4.69 ± 0.76 and 4.04 ± 0.49 ng/mL, respectively), had decreased relative to d 30 (1.72- and 2.00-fold reductions, respectively).

Over the 5 blood collection d, the Hsp70 concentration for the population ranged from 0.54 to 19.75 ng/mL (mean 5.22 ng/mL), with 10 animals exceeding 10 ng/mL on at least 1 occasion.

Relationship between Heat Shock Protein 70 Concentration and Other Variables

There were no effects (P > 0.05) of HLI or THI on Hsp70 concentration. The regression equations showing the relationships between Hsp70 concentration, T_A, BT, BT_Δ, BW, and BCS are presented in Table 5.

There was no relationship (P = 0.42) between 7-d mean maximum T_A and Hsp70 concentration. There was, however, a strong relationship (r^2 = 0.86; P = 0.0001) between Hsp70 concentration and mean daily T_A of the 7 d before sampling (T_A7; Table 5). There was a positive linear relationship between Hsp70 concentration and BT (BT; Table 5); however, the coefficient of determination was small (r^2 = 0.06; P < 0.001). When BCS was considered in the regression analysis with BT (BCSBT; Table 5), the coefficient of multiple determination was moderate (r^2 = 0.48; P < 0.001). The relationship between BT and Hsp70 concentration on d 30, 60, 90, and 110 relative to d 0 was moderate (r^2 = 0.54; P < 0.0001). This relationship is presented in Table 5 as BSBT. The inclusion of BW with BSBT resulted in a moderate coefficient of multiple determination (r^2 = 0.63; P < 0.0001; BWBT; Table 5). Lastly, there was a strong PP effect (r^2 = 0.94; P < 0.0001; PP; Table 5) on Hsp70 concentration.

DISCUSSION

Animals are often exposed to a multitude of stressors in both natural and built environments. The stressors may be chronic, lasting from a few weeks to months (e.g., summer heat load), or acute (e.g., heat waves), lasting from a few minutes to a few days. Two adaptation responses that are evoked to reduce the effects of high heat load have been proposed: the heat shock response and heat acclimation (Maloyan et al., 1999). Heat acclimation was defined by McClung et al. (2008) as a biological adaptation that reduces physiological strain. The heat shock response leads to the development of rapid but transient thermotolerance after acute heat stress (Maloyan et al., 1999). This response commences within minutes of exposure to high heat load; however, it takes approximately 24 h after a stressful heat event for thermotolerance to be observed in animals (Maloyan et al., 1999). The heat shock response may also improve the ability of an animal to withstand subsequent heat stress events. McClung et al. (2008) used the term “acquired thermal tolerance” to explain this phenomenon. Acquired heat tolerance refers to the increased...
Table 5. Relationship between heat shock protein 70 (Hsp70) concentration of Angus steers on blood collection d 0, 30, 60, 90, and 110 and mean daily ambient temperature over the 7 d before sampling (T_A), body temperature (BT; °C) at time of sampling, BCS on day of sampling, and BT interaction (BCS_BT), the change in BT (BT_A) at time of sampling relative to BT on d 0, and BW, BT_A interaction (BW_BT_A) on day of sampling, and photoperiod (PP; min).

<table>
<thead>
<tr>
<th>Item</th>
<th>Equation</th>
<th>r^2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TA</td>
<td>y = (0.68 × a) – 11.79 + 1</td>
<td>0.86</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BT</td>
<td>y = 39.42 + (0.034 × a)^2</td>
<td>0.06</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BCS_BT</td>
<td>y = 35.93 + (0.03 × a) + (0.57 × a)^3</td>
<td>0.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BT_A</td>
<td>y = 0.99 + (6.38 × a)^4</td>
<td>0.54</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BW_BT_A</td>
<td>y = (7.20 × a) + (0.019 × a) – 9.04 + 2168</td>
<td>0.63</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PP</td>
<td>y = (0.0036 × a^2) – (5.62 × a) + 2168</td>
<td>0.94</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Thermotolerance of cells as a result of repeated exposure to heat by which the cells are able to withstand a subsequent potentially lethal heat challenge. For example, rats with acquired thermal tolerance can survive a 60% greater heat load compared with rats that have not been previously exposed to thermal stress (Maloyan et al., 1999). In comparison with acquired thermal tolerance, heat acclimation is a slower process and is evoked by chronic exposure to moderate or gradual temperature changes (Maloyan et al., 1999; Angilletta, 2009).

It is common for feedlot cattle to be exposed to a number of low-level stressors over a period of time, potentially leading to chronic stress. In feedlot production, chronic heat stress is common in many regions over summer and is often the only major stressor for healthy cattle. The weather conditions experienced during the study presented here were sufficient to elicit a heat-stress response (e.g., increased respiration rate, elevated BT) on most days. When Bos taurus cattle are exposed to a T_A > 24°C, they are generally considered to be outside of their thermoneutral zone (Hahn et al., 1992). When cattle are exposed to temperatures above the thermoneutral zone, there may be a heat-stress response; however, T_A on its own is somewhat arbitrary, as other factors may also influence the response of an animal. These include but are not limited to environmental factors such as air movement and RH, animal factors such as prior exposure to higher temperatures, level of performance, and health status, and management factors such as access to shade and nutritional strategies (Gaughan et al., 2008). In the current study, cattle were exposed to a temperature >25°C on average for 10.9 h/d and an average of 8.3 h/d when T_A was <23°C. It has been suggested by Hahn and Mader (1997) that for effective BT regulation, cattle need to be exposed to nighttime temperatures below 23°C for adequate relief from high daytime temperatures. The actual number of hours required has not been adequately determined, largely because of the relationship between intensity of the heat load, the duration of the heat event, and various animal factors that are difficult to quantify. However, based on Hahn and Mader (1997) and Mader et al. (2010), it would appear that an exposure time below 23°C of between 6 and 8 h is required for cattle to recover from daytime heat load. It is probable that there was adequate nighttime relief for much of the current study, apart from the 21-d heat wave.

In the current study, mean BT (38.8 ± 0.06°C) on d 0 was within the normal range for healthy grazing cattle (Robertshaw, 2004) and grain-fed cattle in a nonstressful environment (Gaughan and Mader, 2009). Body temperatures on d 30, 60, 90, and 110 and the BT_A relative to d 0 were indicative of a heat-stress response in grain-fed cattle (Gaughan and Mader, 2009). There was a strong relationship between T_A and Hsp70 concentration but no relationship between BT and Hsp70 concentration. These findings are in agreement with Horowitz (2001) who suggested that changes in HSP transcription are due to intermediate messengers responding to changes in ambient heat and not BT. It would appear that once animals receive a significant stress challenge the HSP response is elicited; however, that response may not be apparent with further challenges. This may be due to HSP only being associated with an initial stress challenge or the process of acclimation is taking place whereby subsequent stress events do not invoke a HSP response to the same extend as the initial stressor. Studies with rats (Sareh et al., 2011) and humans (Thompson et al., 2002; Ogura et al., 2008; Hom et al., 2012; Périard et al., 2012) showed a reduction in Hsp70 expression when subjects were exposed to repeated bouts of exercise-induced heat stress. It has been postulated that the reduction in Hsp70 expression may be due to acclimation to the imposed stressor. This may explain the reduction in eHsp70 over time that was seen in the current study.

To our knowledge, the Hsp70 response to BT_A has not been reported previously. The relationship between BT_A and Hsp70 expression may be a reflection of the BT response to increasing T_A and not a direct response of BT_A on Hsp70 concentration. Further studies are required to elucidate this relationship.

Although individual stressors may be mild, a HSP response could be induced when multiple stressors act in combination. Exposure to stressors (including nonheat stressors) will induce a HSP response resulting in intracellular concentrations of Hsp70 (as well as other HSP; Feder and Hofmann, 1999; Johnson et al., 2005; Collier et al., 2006). More recently, eHsp70 has been found
They are a response to a nonspecific stressor or if the increase induces a specific biological function (Anje et al., 2006), such as activation of the innate immune system (Asea et al., 2000; Tsan and Gao, 2004; Anje et al., 2006). In addition, it is possible that damaged intestinal cells, due to the animal being exposed to prolonged heat stress, may be the causal factor leading to eHsp70 expression (Dokland et al., 2006; Lambert, 2009). Extracellular concentrations of HSP tend to be less than the concentration of HSP found in specific organs (e.g., liver and muscle tissue; King et al., 2002; Kristensen and Løvendahl, 2006; Sørensen, 2010). Moreover, there may be little correlation between HSP concentration in muscle tissue and plasma for cattle exposed to heat stress (Kristensen and Løvendahl, 2006).

The mean (5.22 ± 0.62 ng/mL) and range (0.54 to 19.75 ng/mL) in Hsp70 concentration in the current study was similar to the mean (4.46 ± 0.17 ng/mL) and range (0.24 to 26.47 ng/mL) of heat shock protein 72 (Hsp72) reported by Kristensen et al. (2004) for Holstein-Friesian cows. However, considerably less concentrations of plasma Hsp72 (0 to 1.3 ng/mL) were reported by Kristensen and Løvendahl (2006) for heat-stressed Jersey calves (7.6 ± 4.5 d of age).

An age-related decrease in HSP expression was reported for rats and Rhesus monkeys (Pahlavani et al., 1995) and humans (Rea et al., 2001). In contrast, Kristensen et al. (2004) reported that younger cows (<305 d of age) had reduced Hsp72 concentration compared with older cows (305 to 560 d of age). At the end of the current study, the age of the cattle ranged from 560 to 650 d. Although the precise age of individuals could not be determined, it is possible that the reduction in Hsp70 concentration was related to increasing age of the animal; however, adaptation to a periodic stressor would also be expected over time. Therefore, it is difficult to determine if the increase in the concentration in Hsp70 over time for animals in the current study is related to age, due to adaptation to the multiple stressors, or due to other factors.

The positive correlation with Hsp70 concentration with increasing BCS seen in the current study suggests a response to changing body mass or due to increasing body fat rather than BW. An evaluation of cattle deaths during a heat-wave event in Australia in 2000 during which over 1,200 feedlot cattle died found that there was a strong correlation between days on feed, body size and body condition, and mortality (Entwistle et al., 2000). Similarly, an increase in mortality of heavier-BW cattle was reported by Busby and Loy (1996). In their report of 3,750 feedlot cattle deaths in the United States in 1995, they reported mortality rates across 46 feedlots of 3.4 and 5.9%, respectively, for cattle within a BW range of 362 to 476 kg and 487 to 535 kg.

The PP effect on HSP concentration was not expected. Photoperiod effects on HSP expression have been suggested for fish (Fader et al., 1994); however, most studies investigating HSP responses have been done under controlled PP (e.g., 12 h on 12 h off). Clearly, there is a need for further studies in this area.

It has been postulated that differences in HSP concentrations between species may be due to differences in thermotolerance (Agnew and Colditz, 2008). This may also apply within a species. The differences in HSP concentration among individuals in the current study may reflect within-breed variations with respect to thermotolerance or stress in general. Further studies are required to explore this hypothesis.

Interpreting results from biological data collected in the field can be difficult. This is due, in part, to the dynamics of the natural and the built environment, which contain a mixture of acute (heat waves) and chronic (housing system, nutrition, hot ambient conditions, commingling cattle) stressors. In addition, interactions between the acute and chronic stressors affect expression of the stress response (Sørensen, 2010). Therefore, it is important that future studies are undertaken in an attempt to determine whether the environmental factors assumed to contribute to the stress induction are actually responsible for the measured levels of HSP concentration (Sørensen, 2010) and whether HSP concentration is a useful biomarker for heat tolerance in cattle.

**LITERATURE CITED**


Cimino, E. J., L. Owens, E. Bromage, and T. A. Anderson. 2002. A newly developed ELISA showing the effect of environmental levels of stress on hsp86 in Cherax quadricarinatus and Pan-


Livestock Conservation Incorporated (LCI). 1970. Patterns of transient losses. LCI, Omaha, NE.


