2013

Evaluation of the endocrine response of cattle during the relocation process

S.M. Falkenberg
USDA, Agricultural Research Service

J.A. Carroll
USDA, Agricultural Research Service, jeff.carroll@ars.usda.gov

D.H. Keisler
University of Missouri, KeislerD@missouri.edu

J.L. Sartin
College of Veterinary Medicine, Auburn

T.H. Elsasser
USDA-ARS, THEODORE.ELSASSER@ARS.USDA.GOV

See next page for additional authors

Livestock Science 151 (2013) 203–212

Contents lists available at SciVerse ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

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S.M. Falkenberg [a], J.A. Carroll [b], D.H. Keisler [c], J.L. Sartin [d], T.H. Elsasser [e], J.O. Buntyn [f], P.R. Broadway [g], T.B. Schmidt [h,*]

[a] USDA, Agricultural Research Service, National Animal Disease Center, Ames, IA 50010, USA
[b] USDA, Agricultural Research Service, Livestock Issues Research Unit, Lubbock, TX 79403, USA
[c] Division of Animal Science, University of Missouri, MO 65211, USA
[d] College of Veterinary Medicine, Auburn, AL 36849, USA
[e] Bovine Functional Genomics, USDA-ARS, Beltsville, MD 20705, USA
[f] Department of Animal and Dairy Sciences, Mississippi State University, MS 39762, USA
[g] Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79403, USA
[h] Animal Science Department, A213, University of Nebraska, P.O. Box 830908, Lincoln, NE 68583, USA

ARTICLE INFO

Article history:
Received 5 March 2012
Received in revised form
7 September 2012
Accepted 8 September 2012

Keywords:
Transportation stress
Cortisol
Rectal temperature
Cattle
Growth hormone

ABSTRACT

To evaluate the endocrine responses associated with the relocation process, 22 Holstein heifers (326.4 ± 46.8 kg BW) were randomly assigned to control (CON) or relocation (RELOC) treatment groups. On d 0, heifers were weighed and fitted with indwelling rectal temperature (RT) monitoring devices and jugular catheters. On d 1, baseline blood samples were collected from all heifers for 2 h prior to the transportation event, then weighed. Controls were returned to tie stalls and RELOC were loaded into a modified stock trailer (12 individual stanchions) for a 4 h transportation event. Simultaneous blood samples were obtained at 30-min intervals from both groups throughout the 4 h transport event (TE-I). After transport, RELOC were unloaded at an unfamiliar location, weighed, and placed in tie stalls for a 2 h post-transportation period. All heifers were then placed into two separate holding paddocks with access to water and hay for 4 h. After 4 h, hay and water was withdrawn for 20 h. On d 2 RELOC heifers were exposed to a second transport event (TE-II); the timeline and procedures of TE-II were identical to those of TE-I (except for the starting point for RELOC heifers). All serum samples were analyzed for concentrations of cortisol, growth hormone (GH), and insulin-like growth factor-I (IGF-I). A 6% reduction in BW for the RELOC as compared to 2.5% reduction in BW for CON ($P < 0.001$) was observed during TE-I. Overall BW loss was 2% greater ($P < 0.02$) for RELOC heifers compared to CON heifers. During TE-I, RELOC heifers had greater RT ($P < 0.05$) compared to CON heifers. There was treatment × time interaction observed for cortisol ($P < 0.003$); RELOC had greater cortisol concentrations at multiple time points throughout TE-I and -II. No differences

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* Corresponding author. Tel.: +1 402 472 6772; fax: +1 402 472 6362.
E-mail address: tschmidt4@unl.edu (T.B. Schmidt).

1871-1413/$ - see front matter
http://dx.doi.org/10.1016/j.livsci.2012.09.009

($P > 0.05$) in area under the curve (AUC) for cortisol were observed during TE-I. However, AUC for total cortisol during TE-II was greater ($P < 0.01$) in the RELOC group compared to CON. There were no differences in AUC for GH between treatment groups for TE-I or -II, but a transient decline ($P < 0.05$) within each group was observed from d 1 to d 2. There were no differences ($P > 0.05$) in IGF-I concentrations or in AUC between the treatment groups during TE-I and -II or from d 1 to d 2. Results provide evidence that the actual processes surrounding the transportation of cattle, can elicit a stress response, as defined by increased concentrations of cortisol, RT, and BW losses.

1. Introduction

One stressor that most, if not all, cattle encounter in a typical management system is stress associated with the relocation process (handling, loading, transporting, and unloading of cattle). The relocation process has been implicated as one of the major stresses to recently received cattle (Grandin, 1997). The biological impact of the animal's response to stress is also one of the factors associated with the multi-factorial etiology of the bovine respiratory disease complex (Loerch and Fluharty, 1999) as it most often is observed in recently transported cattle upon arrival at a new facility. The stress associated with transportation and arrival at a new facility can contribute to physiological changes including elevated rectal temperature (RT), transient changes in endocrine hormones, altered metabolic enzymes and metabolites associated with energy and protein metabolism (NEFA, glucose, blood urea nitrogen, creatine phosphokinase, and lactate dehydrogenase), and changes in growth characteristics of newly received cattle (Loerch and Fluharty, 1999).

Burdick et al. (2010) reported an increase in RT of bulls within 30 min of the onset of a transport event, which then declined during transport to a nadir approximately 400 min after initiation of transportation. Of particular interest in that previous study was a lack of change in serum concentrations of cortisol between pre- vs. 9 h post-transport. The results of the Burdick et al. (2010) study were similar to those reported by Blecha et al. (1984) who utilized an 8–9 h transport event and reported no change in cortisol concentrations pre- vs. post-transport. However, in a more recent transportation trial, Burdick et al. (2011) collected blood samples during the actual transportation event and again reported an increase in RT, but they also reported that bulls classified as temperamentally calm had elevated concentrations of cortisol as a result of transportation. Other researchers have reported an increase in circulating glucocorticoids with transport (Crookshank et al., 1979; Locatelli et al., 1987). The ambiguity in endocrine responses which exists across these studies highlights the importance of timing associated with sample collection in defining the actual changes in physiological and endocrine variables during the relocation process.

Although changes in physiological function have been reported pre- vs. post-transport, there are limited reports detailing the physiological and endocrine response during the actual transportation process. Therefore, given that the relocation process remains a concern within the cattle industry, and since it is generally recognized as a stressful event, the objective of this study was to more accurately characterize several physiological and endocrine parameters in cattle throughout the relocation process.

2. Material and methods

2.1. Experimental design

The use of animals and all animal procedures conducted during this study were reviewed and approved by the IACUC committee at Mississippi State University (IACUC #08-041). This project was conducted in 2008 on 21 October (d 0), 22 October (d 1) and 23 October (d 2) at the Bearden Dairy facilities at Mississippi State University (33.46N, 88.82W, 119.79 m altitude) with the average temperature being 16.11 °C for all three days of the study with lows reaching 8 °C and highs 23.3 °C.

Twenty-two Holstein heifers (326.4 ± 46.8 kg BW) were maintained on pasture at the Bearden Heifer Development Unit for a period of 10 week prior to the start of the study. During this 10-week period, heifers were acclimated to human contact and halter restraint in tie stalls. Seven days prior to initiation of the relocation process, heifers were weighed and randomly assigned to one of two treatment groups: (1) control heifers (CON) that were not relocated, and (2) relocation heifers (RELOC), which experienced two transport events within a period of 23.5 h (Table 1). Control heifers remained at the Heifer Development unit location for the duration of the study. A modified tie stall area was constructed in the same area where the heifers were fed through the headstalls each day prior to the start of the study. This area was in a covered barn open on all sides to allow natural airflow without the use of any fans. The tie stall space allocated for each heifer was similar space to that allowed for the heifers in the RELOC stanchions on the trailer. Heifers in the RELOC group were transported in modified open-air stock trailer with 12 individual stanchions (1.5 m × 0.7 m). The trailer was constructed so that 6 individual stanchions were along each side wall of the trailer with a middle isle between the 2 row of 6 stanchions to allow individuals the ability to traverse the length of the trailer and access the animals for sample collection. The RELOC heifers were transported approximately 402.34 km totaling 4 h (240 min) on a 4-lane highway reaching driving speeds of 112.65 kph and only slowing down to drop off samples or turn.

The first transportation event occurred from 0330 to 0745 h on d 1, followed by a second transport event from 0300 to 0730 h on d 2. During the interval between the two transport events, CON and RELOC were allowed access to

Table 1
Overall timeline for the 2-d study to evaluate the potential endocrine consequences among heifers that experienced two transportation events (RELOC) vs. heifers that were not relocated (CON).

Time (h)	Day 0	Day 1	Day 2
		15 30 45 60	
2400		2 h pre transport blood collection	2 h pre transport blood collection
0100			
0200		Heifers loaded onto trailer	Loaded onto trailer
0300			
0400		Transport Event I	Transport Event II
0500			
0600			
0700			Unloaded
0800	Heifers were weighed and catheters and rectal probes were inserted	Unloaded heifers[a]	2 h post transport blood collection
0900		2 h post transport blood collection	
1000			Heifers were weighed and trial terminated
1100		Heifers were placed into paddocks with access to hay and water (4hr)[b]	
1200			
1300			
1400	Heifers were returned to paddocks and had no contact with humans	Water and hay was removed and heifers were maintained in paddocks for 8 h	9 h without food or water[c]
1500			
1600			
1700			
1800			
1900			
2000			
2100			
2200			
2300	Placed into tie stalls	Placed into tie stalls	

3 h[c]

[a] At the conclusion of the first transportation event, RELOC heifers were unloaded at the Bearden Dairy, a location that was not familiar to the heifers.

[b] Control (CON) heifers were returned to the original paddock and heifers that experienced transportation (RELOC) were placed into an unfamiliar paddock at the Bearden Dairy.

[c] Heifers (CON and RELOC) experienced 12 h depravation from food and water, 9 h on d 1 and an additional 3 h on day 2 (12 consecutive hours).

food and water for a period of 4 h (from 1100 to 1500 h on d 1) after which food and water was removed for 20 h (1500 h on d 1 to 1100 h on d 2).

On d 0, heifers in both treatment groups were weighed and fitted with indwelling rectal temperature monitoring devices (Reuter et al., 2010) and indwelling jugular catheters for serial blood collection. Rectal temperature was recorded at 1-min intervals for each heifer for the duration of the study. Jugular catheters consisted of 15 cm of polytetrafluoroethylene tubing (6417-41 18TW; Cole-Palmer; o.d.= 1.66 mm) that was inserted into the jugular vein using a 14-gauge × 5 cm thin-walled stainless steel needle. The catheter was maintained in place using tag cement and strips of 5 cm wide porous surgical tape. The catheters were fitted with extensions made of sterile plastic tubing (Tygon S-50 HL; VWR Scientific; i.d.=1.59 mm; o.d.=3.18 mm) to enable the collection of blood samples without disturbing the heifers. Prior to collection of each blood sample, approximately 5 ml of fluid was removed from each catheter and discarded to remove any heparinized saline in the tubing. Thereafter, a 14 ml vacutainer sample tube was immediately connected to the catheter and a sample was obtained from each heifer. After each sample was obtained, catheters were flushed with 5 mL of saline followed by 3 mL of heparinized saline (10 IU heparin/ mL saline) to ensure each catheter remained functional for the remaining duration of the trial (2 d).

2.2. Transport event (TE-1)

On d 0 (2300 h), all heifers were weighed, haltered, and placed into individual tie stalls. Once all 22 heifers were in the tie stalls (at 2400 h on d 1), collection of blood samples commenced at 30-min intervals for 2 h (2400–0200 h; baseline samples). At the conclusion of the 2 h pre-transport sampling period, RELOC were loaded onto a modified stock trailer with 12 individual stanchions (1.5 m × 0.7 m). Once heifers were secured in the stanchions and catheter lines positioned, a blood sample was obtained simultaneously from CON in their barn tie stalls and RELOC in their trailer stanchions, at 0330 on d 1 (0 h). The transportation event I (TE-I) for the RELOC heifers

then commenced. For 4 h, RELOC were transported on a four lane highway traveling at highway speeds (from 88.5 to 112 km/h) with simultaneous collection of blood samples at 15-min intervals for the first 60 min, followed by 30-min intervals for the remaining 180 min (3 h) for the duration of the 4 h TE-I. Collection of blood samples was achieved by positioning three trained individuals within the trailer with the heifers; each person had ready access to each jugular catheter extension during transit. Upon completion of TE-I, RELOC were unloaded at Milking Barn at the Bearden Dairy Unit and placed in individual barn tie stalls; the Milking Barn was a location unfamiliar to the heifers. After all heifers were secured in the barn tie stalls, blood samples were again collected for 2 h post-transport at 30-min intervals simultaneously from RELOC at the Bearden Dairy Unit and from the CON at the Bearden Heifer Development Unit which is ~1.6 km from the Bearden Dairy Unit. None of the heifers utilized in this trial had any prior experience at the Bearden Dairy Unit. After the 2 h post-transport blood collection, CON and RELOC heifers were untied, weighed, and placed in dry lot for 12 h. For the first 4 h of the dry lot interval, heifers had ad libitum access to water and Bermudagrass hay (*Cynodon dactylon*; 1100–1500 h). At 1500 h, access to hay and water was removed and CON and RELOC heifers experienced deprivation of food and water for 12 h prior to TE-II (beginning from 1500 on d 1 to 0300 h on d 2).

2.3. Transport event-II (TE-II)

Starting at 2300 h on d 1, CON and RELOC heifers were again weighed, haltered, and moved to tie stalls (RELOC heifers were still located at the Bearden Dairy). Timeline and procedures of TE-II were identical to the timeline and sample collection reported for TE-I. The first blood sample for TE-II (after heifers were loaded onto the trailer) was collected approximately 23.5 h after the initiation of TE-I. Upon conclusion of TE-II, RELOC heifers were returned to the Bearden Dairy Unit, and again the procedures described in TE-I were repeated. At the conclusion of the 2 h post-transport blood collection, all heifers were untied, weighed, catheters and RT monitoring devices removed, and returned to paddocks with access to hay and water. In total both CON and RELOC groups experienced food and water deprivation for a period of 20 h (from 1500 on d 1 to 1100 h on d 2).

2.4. Cortisol, growth hormone and insulin-like growth factor-I

Serum concentrations of cortisol were determined by radioimmunoassay (Coat-A-Count; DPC, Los Angeles, CA) as per the manufacturer's protocol within a single assay. This assay was specific for bovine and previously validated. The detection limit of the assay was 2 ng/mL, and the intra-assay coefficient of variation was less than 5%.

Serum samples were analyzed for GH and IGF-I as previously described (Elsasser et al., 1989). Briefly, for the GH assay, rabbit-anti bGH (R1-1-4) was used at a final dilution of 1:60,000. At this dilution the antibody bound 23% of the tracer counts. Minimal sensitivity of the assay was determined to be 150 pg bGH/assay tube with 50% binding of tracer achieved at 1800 pg/tube. Increasing volumes of plasma displaced tracer counts in a fashion parallel to that of the standard curve. Recovery of non-labeled bGH averaged 97% for 300, 600, and 1200 pg added to 200 ml serum. Intra- and inter-assay coefficients of variation were $\leq 10\%$.

Concentrations of IGF-I were determined following acidification of the samples for 36 h with glycyl-glycine buffer to achieve a final pH of 3.6. Following 36 h in acid, each individual sample was diluted and neutralized with a 1:80 dilution of assay buffer. Anti-human/bovine IGF-I primary serum was purchased from GroPep (Adalaide, Australia) and used at a final dilution of 1:10,000. Dilutions of plasma displaced the radioactive tracer (^{125}I-recombinant human/bovine IGF-1, GE Healthcare Life Sciences, Piscataway, NJ) in a manner parallel with the displacement generated in the standard curve. The minimal detectable mass of IGF-1 was 32 pg/tube; recovery of non-labeled IGF-1 added to plasma before acidification averaged 95% with intra- and inter-assay coefficients of variation $\leq 10\%$.

2.5. Statistical analysis

The data consisted of repeated measurements from heifers over time to evaluate changes in cortisol, GH, IGF-I, as well as RT. The response to the transportation event over time was analyzed by repeated measures with ANOVA in the MIXED procedure of SAS (Version 9.1, SAS Inst. Inc., Cary, NC). The model included sampling time, treatment, and treatment × sample time as a fixed effect, and a BY statement was used to partition each day. In a separate analysis, day was added to the model to compare variables of interest between TE-I and TE-II. Therefore, the response to the transportation event over time was analyzed by repeated measures with ANOVA in the MIXED procedure of SAS and the model included sampling time, treatment, day, and treatment × sample time × day as a fixed effect. Rectal temperature was recorded at 1-min intervals, but subsequently averaged over 30-min intervals to facilitate comparisons to other physiological measures. When *F*-test statistics were significant ($P < 0.05$) for treatment × sample time on each day, means were separated using LSD.

Absolute area under the curve (AAUC) for cortisol or GH was calculated by trapezoidal summation of the individual segments bounded by time and concentration [[(Time 1 + Time 2) × (time between Time 1 and Time 2)]/2] to determine the ng h/mL concentration of each hormone over a given time period. Response area under the curve (RAUC) was calculated in the same method, but baseline concentrations (2 h prior to transport) were subtracted from the absolute area under the curve to reflect the response above baseline. Results from these calculations were analyzed by ANOVA with the MIXED procedure of SAS. The model included treatment as a fixed effect for each variable of interest.

3. Results

3.1. Body weight comparisons

During TE-I, RELOC heifers had a 6% loss in BW as compared to the 2.5% loss for CON heifers ($P \leq 0.001$;

Table 2). There was no difference in percent of BW loss between RELOC and CON heifers during TE-II ($P > 0.05$). When total weight loss associated with both TE-I and TE-II was compared, BW loss was 2% greater ($P = 0.02$) for RELOC heifers as compared to CON heifers.

3.2. Rectal temperature

Differences in RT were observed at specific time points during TE-I between RELOC and CON heifers. Prior to start of TE-I, there were no differences ($P > 0.05$) in RT between RELOC and CON heifers (Fig. 1). During the first 60 min of TE-I, RT for RELOC heifers began to increase and peaked at 39 °C approximately 90 min into the event. Initial time points during TE-I (< 1 h) RT measures were greater ($P < 0.05$) than CON heifers. From a peak temperature of 39 °C, RT of RELOC heifers began to gradually decline approximately 90 min into TE-I. While RELOC heifers had a peak in RT early in the transport period, the difference in RT ($P < 0.05$) remained between the RELOC and CON heifers from 30 min after TE-I began until 10 min after the 240-min TE-I period ended. The treatment × time interaction observed for RT was significant ($P < 0.001$).

During TE-II, there were no differences ($P > 0.05$) in RT prior to transport, during transport, and post-transport (Fig. 2) for CON and RELOC heifers. Rectal temperature gradually declined for both CON and RELOC heifers from initial RT observed at −120 min pre-transport (39.05 °C and 38.9 °C, respectively) to 390 min post-transport (38.7 °C and 38.65 °C, respectively).

Table 2
Least squares means for weight loss (kg) for control (CON) heifers and heifers experiencing two transport events (RELOC) over a 23.5 h period.

Variable of interest	Treatment groups		SEM	*P*-value[a]
	CON	RELOC		
Transport event-I[b]	8.20	18.83	5.27	0.001
Transport event-II[c]	3.30	4.25	3.79	0.45
Total loss for transport event-I plus -II	26.70	33.00	7.31	0.02

[a] Means within a row differed between treatment groups if $P \leq 0.05$.
[b] Transport period from 0 to 4.25 h on d 1.
[c] Transport period from 23.5 to 27.75 h on d 2.

3.3. Cortisol

A significant treatment × time interaction ($P = 0.003$) was observed for cortisol during TE-I (Fig. 3), with RELOC heifers having greater circulating concentrations of cortisol. During TE-I samples taken 3 h into the transport period were not utilized for CON or RELOC heifers. A feed truck pulled up to the barn where the CON heifers were located and since this event was not duplicated with the RELOC heifers, samples were not reported. During the collection of baseline samples and up to approximately 90 min into TE-I, increases in serum cortisol observed in each group and there was no difference ($P > 0.05$) in cortisol concentrations between the two treatment groups. Cortisol concentrations for RELOC heifers were greater ~120 min after initiation of transport, and remained greater ($P < 0.05$) until ~240 min of transportation. While heifers in the RELOC treatment group had greater cortisol concentrations at the conclusion of the 240 min TE-I ($P = 0.02$), no statistical differences ($P > 0.05$) were observed at any other time points for post-transport cortisol concentrations between RELOC and CON heifers.

A treatment × time interaction was observed for cortisol during TE-II. There were no differences ($P > 0.05$) in pre-transport cortisol concentrations between RELOC and CON heifers (Fig. 4). At the onset of TE-II (25 h after

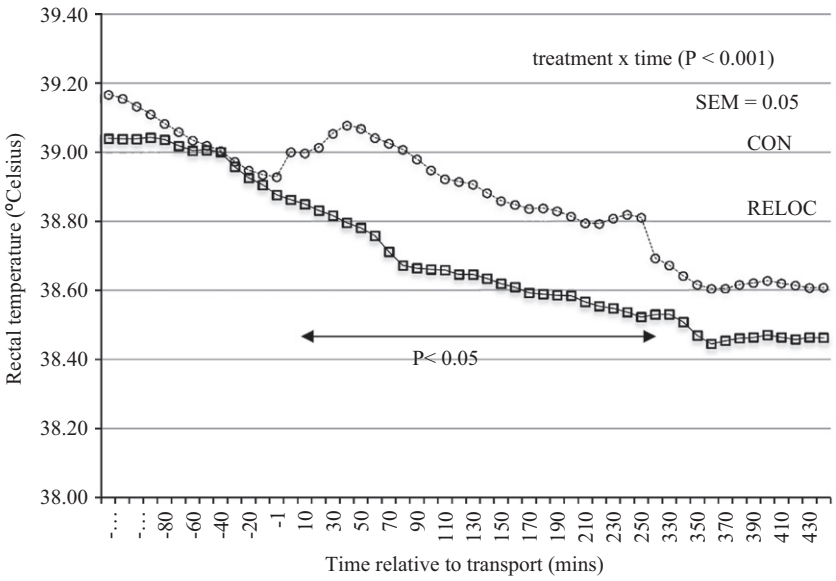

Fig. 1. Mean rectal temperature (RT) for control and RELOC heifers on d 1. The transportation event elicited a change in RT ($P < 0.001$) over time. Transport event-I began at minute 0 and ended at minute 240 on d 1.

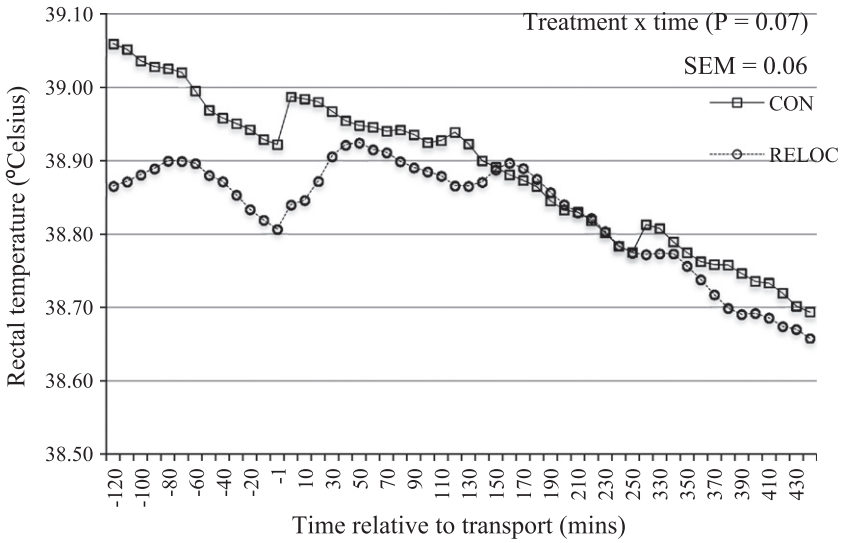

Fig. 2. Mean rectal temperature (RT) for control and RELOC heifers on d 2. The transportation event did not elicit a change in RT ($P < 0.07$) over time. Transport event-I began at minute 0 and ended at minute 240 on d 2.

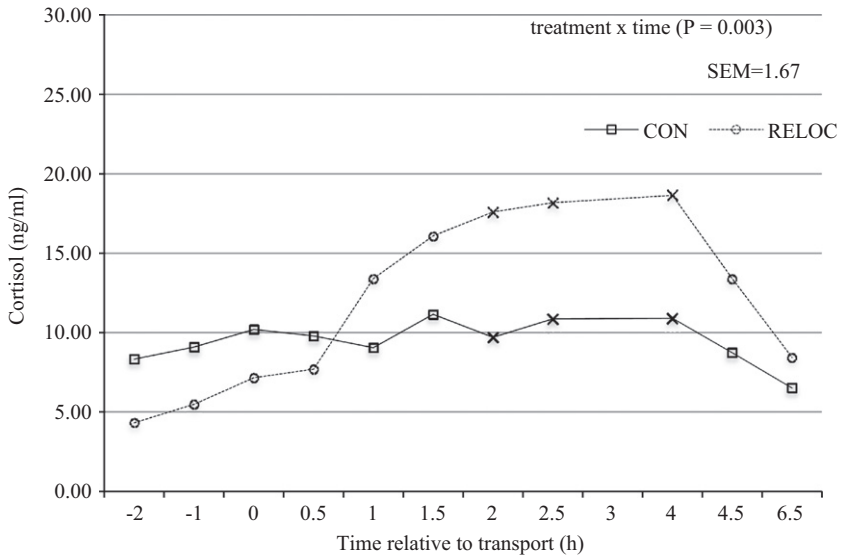

Fig. 3. Mean serum concentrations of cortisol for control and RELOC heifers experiencing the initial transport event (TE-I). Transport event-I elicited a change in cortisol ($P = 0.003$) over time and began at hour 0 and ended at hour 4 on d 1. x denotes differences ($P \leq 0.05$) between treatment groups at the specific time points.

initiation of TE-I), there were no differences in cortisol concentrations ($P > 0.05$) between treatment groups; however, 60 min into TE-II (26 h), RELOC heifers had greater ($P < 0.05$) cortisol concentrations when compared to CON heifers. This increase in cortisol continued until ~120 min into TE-II, with cortisol concentrations in the RELOC heifers remaining greater ($P < 0.05$) than the CON heifers for 120 min (2 h) during TE-II (26–28 h). Thirty min after the conclusion of TE-II (h 29.5) RELOC heifers had greater cortisol concentrations ($P = 0.03$), but no differences ($P > 0.05$) were observed at any other time points for post-transport cortisol concentrations between RELOC and CON heifers.

3.4. Cortisol—area under the curve (AUC)

While differences were observed at specific time points during TE-I when the total concentrations of cortisol produced prior to at −2 to 0 h, during at 0–2 and 2–4 h of transport were calculated (area under the curve; AUC), there was no overall difference in AUC between the treatment groups prior to TE-I ($P > 0.05$; Table 3). Cortisol concentration as a function of ng* h/mL was different at time interval 2–4 h as well as total cortisol produced during TE-I. While there was no difference between the treatment groups prior to the onset of transportation, when the response AUC (RAUC) was

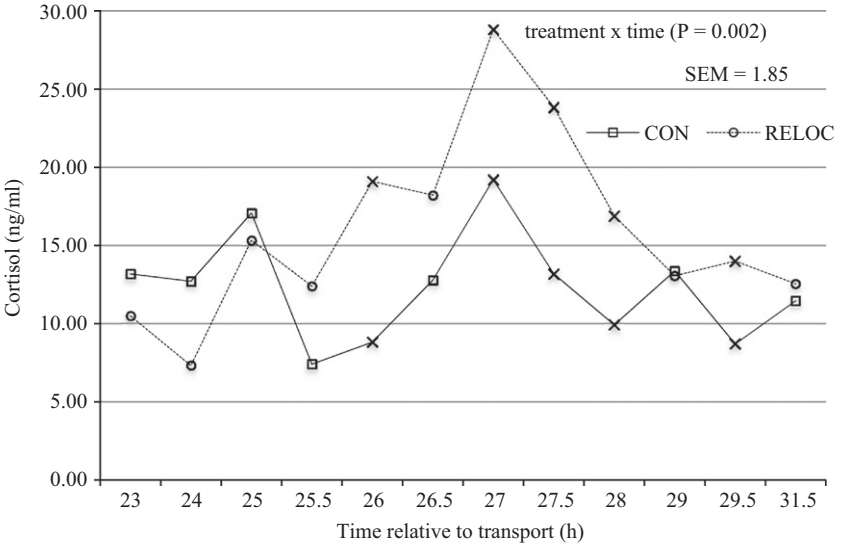

Fig. 4. Mean serum concentrations of cortisol for control and RELOC heifers experiencing the second transport event (TE-II). Transport event-II elicited a change in cortisol ($P=0.002$) over time and began at hour 25 and ended at hour 29 on d 2. x denotes differences ($P \leq 0.05$) between treatment groups at the specific time points.

Table 3
Least squares means for serum concentrations of cortisol (ng* h/mL) at hour intervals for control (CON) heifers and heifers experiencing two transport events (RELOC) over a 23.5 h period.

Hour intervals	Treatment groups[a]		SEM	*P*-value
	CON	RELOC		
Cortisol AUC (ng* h/mL) transport event-I[b]				
−2–0	18.36	11.22	4.27	0.11
0–2	19.90	24.75	5.48	0.39
2–4	20.60	36.23	5.77	0.01
Total (0–4)	40.50	60.98	10.00	0.05
Cortisol AUC (ng* h/mL) transport event-II[c]				
−23–25	27.84	20.23	3.91	0.07
25–27	36.28	44.14	8.35	0.36
27–29	32.58	41.87	7.66	0.24
Total (25–29)	68.86	86.01	15.41	0.28

[a] Means within a row differed between treatment groups if $P \leq 0.05$.
[b] TE-I = transport period from 0 to 4.25 h on d 1.
[c] TE-II = transport period from 23.5 to 27.75 h on d 2.

Table 4
Least squares means for serum concentrations of cortisol (ng* h/mL) at hour intervals for control (CON) heifers and heifers experiencing two transport events (RELOC) over a 23.5 h period.

Hour intervals	Treatment groups[a]		SEM	P-value
	CON	RELOC		
Cortisol RAUC (ng* h/mL) transport event-I[b]				
0–2	1.55	13.53	3.27	0.002
2–4	2.25	25.01	6.00	0.001
Total (0–4)	3.79	38.54	8.16	0.004
Cortisol RAUC (ng* h/mL) transport event-II[c]				
25–27	8.45	23.91	6.58	0.03
27–29	4.75	21.65	5.38	0.005
Total (25–29)	13.19	45.56	11.19	0.009

[a] Means within a row differed between treatment groups if $P \leq 0.05$.
[b] TE-I = transport period from 0 to 4.25 h on d 1.
[c] TE-II = transport period from 23.5 to 27.75 h on d 2.

calculated by subtracting the baseline circulating concentration prior to TE-I, all RAUC for time intervals 0–2, 2–4, and total (0–4 h) were different between the treatment groups ($P < 0.05$; Table 4). The RELOC heifers had a greater cortisol response as compared to the CON counterparts.

The differences that were observed at specific time points during TE-II did not translate into differences ($P > 0.05$) between the RELOC and CON heifers for total circulating cortisol AUC during the calculated time intervals at −23 to 25, 25–27, 27–29 and total AUC (25–29 h; Table 4) as a function of ng* h/mL. Although there were no differences between the treatment groups prior to the onset of TE-II when baseline cortisol AUC was subtracted and RAUC was calculated, there was a difference in cortisol response between the RELOC and CON heifers (Table 4). The differences between the CON and RELOC heifers were observed at time intervals 25–27, 27–29 h and total (25–29 h).

When the total cortisol response was compared between TE-I and TE-II, there was no difference in RAUC ($P > 0.05$; Table 4) between the two days for both the CON and RELOC heifers. Conversely, there was a difference ($P < 0.05$; Table 3) between TE-I and TE-II for total circulating cortisol AUC for both the CON and RELOC heifers.

3.5. Growth hormone comparisons

There was treatment × time interaction for GH between the RELOC and CON heifers; however there was no clear

pattern to the changes in GH. Evaluation of AUC for total concentration of GH at time intervals of 0–60, 60–120, 120–180, and 180–240 min during TE-I also indicated that there were no differences between CON and RELOC ($P > 0.05$; Table 5).

There was no main effect interaction of treatment × time for GH during TE-II and both the RELOC and CON heifers had very similar patterns of circulating GH concentrations. Therefore, there were no differences ($P > 0.05$) in GH concentrations as a function of ng* h/mL (AUC) at any of the one-h intervals (0–60, 60–120, 120–180, and 180–240 min), nor was there any difference in total GH during TE-II for RELOC and CON heifers.

The total concentration of GH AUC during TE-I was less ($P < 0.05$) when compared to the total GH AUC during TE-II within each treatment group (Table 5). It would appear that withdrawing feed and water for 12 h prior to TE-II impacted GH AUC in both the CON and RELOC heifers, regardless of being exposed to a transportation event. Growth hormone AUC was 66% for the CON and 35% greater for the RELOC heifers on d 2 as compared to values on d 1.

Table 5
Least squares means for serum concentrations of growth hormone (ng h/mL) at hour intervals for control (CON) heifers and heifers experiencing two transport events (RELOC) over a 23.5 h period.

Hour intervals	Treatment groups		SEM	*P*-value
	CON	RELOC		
Growth hormone AUC (ng h/mL) transport event-I[c]				
0–1	5.33	6.75	0.95	0.28
1–2	4.46	5.63	0.82	0.29
2–3	3.85	4.80	0.80	0.40
3–4	6.23	5.32	0.96	0.48
Total (0–4)	19.87[a]	23.71[a]	2.71	0.33
Growth hormone AUC (ng h/mL) transport event-II[d]				
25–26	9.94	8.72	1.28	0.50
26–27	9.29	7.26	1.11	0.22
27–28	7.46	7.34	0.92	0.92
28–29	6.43	6.99	0.87	0.65
Total (25–29)	33.13[b]	32.07[b]	2.92	0.78

[a] Means within a column are differed within treatment groups if $P \leq 0.05$.
[b] Means within a column are differed within treatment groups if $P \leq 0.05$.
[c] TE-I = transport period from 0 to 4.25 h on d 1.
[d] TE-II = transport period from 23.5 to 27.75 h on d 2.

3.6. Insulin-like growth factor-I comparisons

There was no main plot effect of treatment × time interaction observed for IGF-I on d 1 or 2 ($P > 0.05$). There were no differences in initial IGF-I concentrations between the treatment groups, as well as no difference for change in pre- and post-transport concentrations between the groups (Table 6). Neither transportation on d 1 nor transportation on d 2 following the 20 h food and water deprivation altered IGF-I concentrations.

4. Discussion

In the present study heifers were subjected to a relocation event that included two 4-h transport events (TEs) 23.5 h apart to examine the impact the relocation process on physiological and endocrine parameters. The two TEs were used to simulate what cattle would experience if transported to the sale barn and then held for a period of time at the sale barn prior to being transported to another location (e.g., background or feedlot facility). The relocation process is generally regarded as stressful to cattle and includes both physical and psychological stimuli that can cause detrimental physiological and endocrine changes. These physiological and endocrine changes can often potentiate or alter other physiological, immunological, or endocrine responses. Consequently, the release of cortisol associated with the relocation process can cause cattle to be more susceptible to disease through immunosuppression (Blecha et al., 1984), thus resulting in cattle prone to an increased risk of morbidity or mortality. Preventive measures have been studied to help mitigate the detrimental effects associated with transportation stress. While research has focused on pre- and post-transport management strategies for newly received incoming cattle that have been transported to the feedlot

Table 6
Least squares means for serum concentrations of insulin-like growth factor-I at specific time points and time intervals for control (CON) heifers and heifers experiencing two transport events (RELOC) over a 23.5 h period.

	Treatment groups[a]		SEM	*P*-value
	CON	RELOC		
Insulin-like growth factor-I AUC (ng* h/mL)				
Transport event-I[b]	1229.59	1285.05	104.23	0.68
Transport event-II[c]	883.95	804.75	74.30	0.16
Serum Insulin-like growth factor-I (ng/ml)				
−120 min	159.23	139.91	12.55	0.26
390 min	140.61	162.46	11.16	0.18
1440 min	145.96	145.85	14.17	0.99
1770 min	173.87	140.54	13.57	0.10

[a] Means within a row differed between treatment groups if $P \leq 0.05$.
[b] TE-I = transport period from 0 to 4.25 h on d 1.
[c] TE-II = transport period from 23.5 to 27.75 h on d 2.

or stocker facility, limited data is available regarding the changes that occur during the actual transportation process. Therefore, while the relocation process can cause increased susceptibility the actual process of transportation may only provide a brief increase in cortisol. This brief increase in cortisol for a limited time could reflect the intact homeostatic compensation of a healthy animal with the brief increase serving as a needed stimulus for the animal to be ready to mobilize resources that might be needed if the stress continued. Data from this study helps elucidate the hormonal changes associated with transportation and the potential contribution transportation has on cattle that go through the relocation process.

Rectal temperature observed during TE-I was similar to data reported by Burdick et al. (2010) in which Brahman bulls transported for a period of 8 h displayed a peak in RT 30 min after the onset of transportation. Similarly, the decrease in RT observed over time by Burdick et al. (2010) is consistent with data in the current study for both TE-I and -II. While there was not an initial increase in RT during TE-II, this could be associated with acclimation to the handling procedures the cattle experienced on d 1. Mean values for RT during TE-I and -II during the current study declined for both CON and RELOC heifers over the course of the transport period. This decline in RT could be attributed to the drop in ambient temperatures during the transport period, which was conducted early in the morning. The early morning temperature could have had a cooling effect on the heifers and attributed to the decline in RT. Conversely, Burdick et al. (2011) observed an increase in RT overtime beginning prior to the transport event and continuing through the post-transport event but some of the increase in RT during the trial could be attributed to the correlation with the increase in ambient temperature and/or the handling of cattle prior to transport. Other research using a digital thermometer did not detect differences before or after a 9 h transport of bulls (Buckham Sporer et al., 2008). However, RT was not measured during transportation, therefore, it is unknown if any changes during transport actually occurred in the study by Buckham Sporer et al. (2008).

A limited number of reports are available in which the hormone response associated with transportation during the actual transportation process was measured. Burdick et al. (2011) evaluated calm and temperamental bulls during a transportation event and only reported a difference in cortisol for the calm bulls. Other studies have evaluated pre- and post-transportation cortisol concentrations in cattle and have determined that there were no differences in cortisol concentrations due to transportation (Blecha et al., 1984; Burdick et al., 2010). Conversely, other research has suggested that pre-transport samples differ from samples taken at different time points during transportation (Buckham Sporer et al., 2008; Crookshank et al., 1979; Odore et al., 2004), but a complete profile of the physiological and endocrine response during the entire transportation process was not determined. The conflicting results observed between these studies suggest that transportation time or length as well as sampling time and intervals can confound the overall conclusions. As reported by Burdick et al. (2010), temperament can also impact cortisol concentrations; therefore docile Holstein cattle that were acclimated to being handled were used in the current study to help minimize the amount of variability in the stress response. While it is important to understand the fluctuations in the cortisol during the sample period, the total amount of circulating cortisol at different time intervals during the transportation process should be considered. There were differences in cortisol concentrations at specific time points during TE-I, but these spikes in cortisol differed between the RELOC and CON heifers and did not translate into differences in the total amount of cortisol (i.e., AUC) during specific time intervals during the transportation process. Whereas during TE-II cortisol concentrations were greater than concentrations observed during TE-I and the fluctuations or degree of change from initial concentrations at the different time points during TE-II were also greater. The differences in fluctuations of cortisol coincided with differences in cortisol AUC during specific time intervals during the transportation process. The greater cortisol AUC over a period of time for the RELOC heifers during the transportation process could be more concerning, rather than small fluctuations in cortisol that are not sustained. The sustained response of cortisol over a period of time needs to be evaluated in comparison to short lasting perturbation and fluctuations to determine when greater cortisol responses over time impact performance of cattle. Data from the current study would suggest that transportation does cause activation of the HPA axis and transported animals have greater concentrations of cortisol during the transportation process. However, cortisol did return to baseline concentrations by the end of a 4 h transport. Therefore if samples were only obtained pre- and post-transport, an erroneous conclusion would have been made that there was no change in cortisol concentrations due to being transported. Furthermore, the initial response during the onset of transportation could be coupled with the handling and loading of the cattle onto the trailer, which is an unfamiliar space. The CON heifers were handled to obtain a weight at the same time the RELOC heifers were weighed and loaded on the trailer, but handling the CON heifers did not appear to impact cortisol concentrations, possibly due to returning the heifers to their original tie stalls. Whereas, the transported heifers exhibited greater RT and cortisol concentrations early in the transportation process it would appear the handling at loading could have impacted the response to transportation since they were put in an unfamiliar stall.

Transportation stress has been implicated to be one of the major stressors associated with newly received cattle (Grandin, 1997). While transportation is part of the relocation process, there are multiple factors such as pathogen exposure, commingling, diet changes, handling, processing, and acclimation to a novel environment that contribute to the shipping stress complex generally associated with newly received cattle. Therefore, after evaluating the initial data it would appear that the term "transportation or shipping stress" should be regarded as relocation stress to encompass the whole process associated with arrival and acclimation to a new environment. 6518 gateway ave

The increase in cortisol due to transportation has been implicated in alteration of metabolic functions in beef cattle. Buckham Sporer et al. (2008) reported metabolic

alterations in beef calves demonstrated by the differences observed in plasma concentrations of albumin, globulin, urea, total protein, and creatine kinase due to the effects of transportation. Immobilization stress in rats has been reported to cause suppression of GH; but increased concentrations of corticosterone were observed for prolonged periods (Kant et al., 1983). Similarly, GH suppression is observed in humans that received prolonged exposure to glucocorticoid treatment which lead to IGF-I insensitivity (Allen et al., 1998; Jux et al., 1998). While results from these prior studies indicate that transportation stress can alter protein metabolism, our data from TE-I and -II suggests that transportation did not elicit differences in GH or IGF-I concentrations. Given that cortisol concentrations did not remain increased for prolonged periods of time during the transport events, it is not surprising that no changes in GH or IGF were observed for TE-I or -II. Collectively, our data indicates that intermittent stimulation of cortisol release during transportation in either fed or fasted cattle does not impact GH and IGF-1 concentrations in cattle. However, withdrawal of feed and water for a 12-h period does alter circulating concentrations of GH. Therefore, we cannot dismiss the possibility that the changes in circulating concentrations of GH associated with feed and water withdrawal may have masked any potential changes that could have been induced by the actual transportation process.

5. Conclusion

In conclusion, RT and cortisol do increase during the initial relocation process. While there were differences observed during the 4-h transportation event, there were no differences in samples taken pre- and post-transport. Therefore, while our current data does not completely elucidate the stress response associated with transportation, it does highlight the need for a more robust protocol for sampling to detect subtle changes in the hormone milieu that may have significant implications towards a better understanding of how animal stress could be assessed, managed and minimized toward the well-being of animals. It would also appear that the short time the heifers were subjected to greater cortisol concentrations did not impact endocrine factors associated with the overt stability of the somatotrophic axis. While transportation is a component of the relocation process, the multiple stressors associated with the overall relocation process that result in transient changes in endocrine hormones and changes in growth rate do not appear to be directly related to the transport event itself. A better understanding of the components associated with the relocation process is needed to identify the major stressors and at what point multiple stressors begin to impact endocrine, immunological and metabolic functions in a manner that jeopardizes the health and well-being of cattle.

Conflict of interest statement

The authors of this manuscript have no financial or other relationship with other people or organizations that may inappropriately influence the work that has been reported in this manuscript

Acknowledgment

The authors acknowledge the technical support of D. Sykes, K. Cooley, K. Graves, D.T. Dawson, J.W. Dailey, L.E. Hulbert, E. Keiss, and S. Kahl.

References

Allen, D.B., Julius, J.R., Breen, T.J., Attie, K.M., 1998. Treatment of glucocorticoids-induced growth suppression with growth hormone. J. Clin. Endocrinol. Metab. 83, 2824–2829.

Blecha, F., Boyles, S.L., Riley, J.G., 1984. Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman × Angus feeder calves. J. Anim. Sci. 59, 576–583.

Buckham Sporer, K.R., Weber, P.S., Burton, J.L., Earley, B., Crowe, M.A., 2008. Transportation of young beef bulls alters circulating physiological parameters that may be effective biomarkers of stress. J. Anim. Sci. 86, 1325–1334.

Burdick, N.C., Carroll, J.A., Randel, R.D., Willard, S.T., Vann, R.C., Chase Jr., C.C., Lawhon, S.D., Hulbert, L.E., Welsh Jr., T.H., 2011. Influence of temperament and transportation on physiological and endocrinological parameters in bulls. Livest. Sci. 139, 213–221.

Burdick, N.C., Carroll, J.A., Hulbert, L.E., Dailey, J.W., Willard, S.T., Vann, R.C., Welsh Jr., T.H., Randel, R.D., 2010. Relationships between temperament and transportation with rectal temperature and serum concentrations of cortisol and epinephrine in bulls. Livest. Sci. 129, 166–172.

Crookshank, H.R., Elissalde, M.H., White, R.G., Clanton, D.C., Smalley, H.E., 1979. Effect of transportation and handling of calves upon blood serum composition. J. Anim. Sci. 48, 430.

Elsasser, T.H., Rumsey, T.S., Hammond, A.C., 1989. Influence of diet on basal and growth hormone-stimulated plasma concentrations of IGF-I in beef cattle. J. Anim. Sci. 67, 128–141.

Grandin, T., 1997. Assessment of stress during handling and transport. J. Anim. Sci. 75, 249–257.

Jux, C., Leiber, K., Hugel, U., Blum, W., Ohlsson, C., Klaus, G., Mehls, O., 1998. Dexamethasone impairs growth hormone (GH)-stimulated growth by suppression of local insulin-like growth factor (IGF)-I production and expression of GH- and IGF-I-receptor in cultured rat chondrocytes. Endocrinology 139, 3296–3305.

Kant, G.J., Lenox, R.H., Bunnell, B.N., Mougey, E.H., Pennington, L.L., Meyerhoff, J.L., 1983. Comparison of stress response in male and female rats: pituitary cyclic AMP and plasma prolactin, growth hormone and corticosterone. Psychoneurondocrinology 4, 421–428.

Locatelli, A., Agnes, F., Sartorelli, P., 1987. Effects of road and simulated transportation for 30 min. on blood values in calves. Arch. Vet. Ital. 38, 81.

Loerch, S.C., Fluharty, F.L., 1999. Physiological changes and digestive capabilities of newly received feedlot cattle. J. Anim. Sci. 77, 1113–1119.

Odore, R., D'Angelo, A.D., Badino, P., Bellino, C., Pagliasso, S., Re, G., 2004. Road transportation affects blood hormone levels and lymphocyte glucocorticoid and β-adrenergic receptor concentrations in calves. Vet. J. 168, 297–303.

Reuter, R.R., Carroll, J.A., Hulbert, L.E., Dailey, J.W., Galyean, M.L., 2010. Technical note: development of a self-contained, indwelling rectal temperature probe for cattle research. J. Anim. Sci. 88, 3291–3295.