

Cattle handling technique can induce fatigued cattle syndrome in cattle not fed a beta adrenergic agonist¹

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ABSTRACT: Angus crossbred steers ($n = 40$; 563 ± 44 kg) were used to examine the effects of handling method and fat thickness on the blood chemistry and physiology of market steers. Steers were blocked by backfat (BF) thickness and were randomly assigned to treatment groups: low-stress handling (LSH) and aggressive handling (AH). Cattle were then randomly assigned to one of 5 blocks containing 4 steers from the LSH and AH treatments. Steers in the LSH treatment were walked and AH cattle were run through a course of 1,540 m. Blood samples were obtained via jugular venipuncture before handling (BASE), at 770 m (LAP1), at 1,540 m (LAP2), and at 1 h (1H) and 2 h (2H) after finishing the course. Blood samples were analyzed for plasma lactate (LAC), creatinine kinase (CK), base excess (BE), blood pH (pH), serum cortisol (CORT) concentrations, and venous carbon dioxide (PvCO₂) and oxygen (PvO₂) pressures. Heart rate (HR), respiratory rate (RR), and rectal temperature (TEMP) were measured at the same intervals. Cattle in the AH treatment had greater ($P < 0.05$) LAC than those in LSH at BASE (4.1 vs. 3.0 mmol/L), LAP1 (16.5 vs. 2.3 mmol/L), LAP2 (22.3 vs. 2.4 mmol/L), 1H (7.2 vs. 2.7 mmol/L), and 2H (4.0 vs. 2.5 mmol/L), respectively. Creatinine kinase and RR

were not different ($P > 0.14$). Blood pH in AH cattle was decreased compared with that in LSH cattle ($P < 0.05$) at LAP1 (7.25 vs. 7.45) and LAP2 (7.19 vs. 7.48) but was not different ($P > 0.13$) at BASE, 1H, or 2H. Heart rate and TEMP were increased in AH cattle compared to LSH ($P > 0.01$). Serum cortisol was increased ($P < 0.05$) in AH compared to that in LSH cattle at LAP1 (87.5 vs. 58.9 nmol/L), LAP2 (144.4 vs. 93.1 nmol/L), and 1H (113.5 vs. 53.1 nmol/L). Although RR was not different between LSH and AH, PvCO₂ was decreased in AH compared to that in LSH ($P < 0.05$) at LAP2 (30.6 vs. 39.3 mmHg) and PvO₂ was increased at LAP1 (42.7 vs. 33.5 mmHg) and at LAP2 (51.5 vs. 36.6 mmHg). Lactate was increased in AH cattle in the thicker BF group at 1H ($P < 0.05$), and blood pH was decreased at LAP1, LAP2, and 1H ($P < 0.05$) compared to the thinner BF cohorts. Four AH steers became exhausted (EXH) and did not complete the course. Increased CK, decreased PvCO₂, and muscle tremors occurred in EXH steers compared to non-exhausted AH cohorts. Results of this study show that AH causes physiologic and blood chemistry changes in steers, which can be potentially detrimental to cattle, emphasizing the need for low-stress handling practices.

Key words: cattle, exercise, fatigue, lactate, locomotion

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doi:10.2527/jas2015-9824

INTRODUCTION

Cattle welfare is a high priority for the beef industry (Verbeke and Viaene, 2000). Recently, ab-

normalities in cattle mobility at abattoirs has gained considerable attention, with the greatest focus occurring in the fall of 2013 when cattle fed β -adrenergic agonists (β AA) were the focus of an adverse welfare event at an abattoir (Vance, 2013). The clinical signs and serum biochemistry of the cattle involved with this event have been termed fatigued cattle syndrome (FCS; Thomson et al., 2015). Clinical signs and the biochemistry of FCS include tachypnea with abdom-

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Received September 7, 2015.

Accepted November 25, 2015.

inal breathing, muscle tremors, stiff gait, reluctance to move, and increased serum lactate (**LAC**) and creatinine kinase (**CK**; Thomson et al., 2015). The clinical signs and serum biochemical abnormalities observed in FCS are similar to those observed in pigs with fatigued pig syndrome (**FPS**; Ritter et al., 2005).

Fatigued pig syndrome is caused by multiple additive stressors, including aggressive animal handling, and is characterized clinically by vocalization, blotchy skin, reluctance or inability to move, and muscle tremors (Ritter et al., 2005; Fitzgerald et al., 2009; Ritter et al., 2009a). Greater serum LAC concentration has been identified as a consistent characteristic of FPS pigs that become reluctant to move or nonambulatory (Anderson et al., 2002; Ritter et al., 2005). Research into FPS has led to mitigation strategies that include management changes, such as improvements in animal handling and transportation methods (Benjamin, 2005; Ritter et al., 2005). The similarities between swine diagnosed with FPS and cattle diagnosed with FCS have led to the hypothesis that high-stress, or aggressive, handling may contribute to FCS, particularly in fatter cattle. This study was designed similarly to the FPS model with the objective to determine if this model could induce the clinical signs and biochemistry abnormalities of FCS in finished feedlot cattle by aggressive animal handling.

MATERIALS AND METHODS

The protocol and procedures for this study were reviewed and approved by the Institutional Animal Care and Use Committee of Kansas State University (number 3465).

Angus-crossbred steers ($n = 40$; BW = 563 ± 44 kg) were selected from a single cohort of cattle with 127 d on feed and were fed at a commercial feeding facility in central Kansas. These selected steers were then used to evaluate the effects of 2 handling treatments: 1) low-stress handling (**LSH**) with cattle walked approximately 1,540 m, and 2) aggressive handling (**AH**) with cattle run the same distance.

The day before the study, the cattle were weighed and ultrasounds were performed to determine backfat (**BF**) thickness (10.2 ± 2.7 mm) and estimated LM area. Ultrasound measurements were made with an Aloka SSD-500 ultrasound with a 3.5-MHz 10-cm linear probe (Hitachi-Aloka Medical USA, Ltd., Wallingford, CT) to capture an image of the sagittal section of the LM between the 10th and 13th ribs, approximately 1/2 the distance laterally over the muscle from midline. This image was subsequently analyzed using Image Capture 3.1.0 (Cattle Performance Enhancement Company, Oakley, KS) to estimate LM area, BF, and marbling score. Cattle were then placed into 1 of 2 groups based

on BF thickness. The first group contained the 20 steers with the least BF, and the second group contained the 20 steers with the greatest BF. Cattle within the same BF thickness group were stratified by BW within BF thickness group. Cattle were paired by stratification order, 1 from the greatest BF group (**FATBF**; 13.5 ± 0.5 mm) and 1 from the least BF group (**THINBF**; 9.1 ± 2.8 mm). Pairs of cattle were then randomly assigned to treatment. After assignment to treatment, each BF pair of steers was randomly assigned to 1 of 5 blocks. Each block contained 2 pairs of steers from each treatment so that 2 steers from each BF group in each treatment were in each block ($n = 8$). Exercise order of the 5 blocks was determined by random number generator. Treatment exercise order within a block was determined by coin flip. Both treatment groups within a block were exercised consecutively. The study was conducted over 2 consecutive days with the processing order of blocks of cattle randomly assigned with the first 3 blocks exercised on d 1 and the final 2 blocks on d 2.

Cattle Handling

On each study day, cattle were individually restrained in a hydraulic chute, and rectal temperature (**TEMP**), respiration rate (**RR**), and heart rate (**HR**) were recorded; blood was sampled via jugular venipuncture, and cattle were sorted into their respective treatment cohorts. Each treatment cohort of 4 steers was moved along a course of approximately 770 m including length of cattle chute and alley (**LAPI**). The course was shaped generally in a square with the handling facility in the middle of the square, allowing cattle to be moved continuously throughout the course. After a treatment cohort of cattle completed the 770-m course, they were individually restrained in a hydraulic chute, and HR, RR, and TEMP were measured, a blood sample was collected, and the animal was released. Following sampling of all cattle in a treatment cohort, the cattle were moved through the course a second time (**LAP2**), followed by blood sampling and recording of vital measures. Cattle were then placed in a nearby pen and resampled in an identical manner following each of two 1-h rest periods (**1H** and **2H**). Cattle had ad libitum access to water during the rest period.

Cattle in the LSH treatment were walked the entire distance with a lead and trail rider each using an all-terrain vehicle (**ATV**). Cattle in the AH treatment were forced to run the entire course distance by 2 people riding ATVs behind the cattle. The amount of time running (excluding sampling time) the entire 1,540-m course was between 7 and 8 min for AH cattle. Exercise was stopped on individual cattle meeting exercise stop criteria as described below. These cattle were allowed to

recover with their treatment cohort and were sampled at 1H and 2H rest periods with the treatment cohort.

Exercise-Stop Criteria

To ensure the welfare of the animals involved with the study, exercise-stop criteria were established. Exercise of a steer was to be discontinued if an animal was deemed exhausted (**EXH**) by the assigned supervising veterinarian based on the existence of any of the following conditions: 1) The steer becomes extremely reluctant to move with a marked decrease in flight zone or becomes recumbent; 2) The steer exhibits open-mouth breathing with excessive salivation; 3) An audible inspiratory or expiratory stridor is present; 4) The steer displays agitation and agonistic behavior toward handlers; 5) The steer becomes lame and presents a lameness score of >1 during the exercise procedure as previously described (Terrell et al., 2014); 6) The steer has a HR greater than 170 beats/min; 7) The steer has a RR greater than 120 breaths/min; or 8) The steer has a TEMP greater than 42.2°C.

Blood Sampling and Processing

Blood samples were obtained via jugular venipuncture using a 60-mL syringe fitted with a 16-gauge 3.8-cm hypodermic needle. Blood was immediately transferred to three 10-mL pre-labeled blood tubes containing 1) coated potassium EDTA with 100 μ L of 100 mM benzamidine solution, 2) coated lithium heparin, or 3) no anticoagulant. Blood tubes were stored in an ice bath and transported to an onsite laboratory within 10 min of sample collection. After laboratory processing, blood was stored on dry ice until transportation to permanent storage facilities at the end of the second study day.

Substance P

Blood samples in a tube coated with potassium EDTA with 100 μ L of 100 mM benzamidine solution were designated for testing for substance p (**SUBP**). Following receipt at the onsite laboratory, samples were placed on ice until centrifugation at 3,000 \times g for 15 min at 4°C. Following centrifugation, plasma was separated into 2 aliquots and was stored in cryovials on dry ice until transfer to permanent storage at -80°C. Samples were processed as expeditiously as possible with a mean time from sample collection to freezing of 45 min (\pm 15 min). Following transfer to permanent storage, 1 sample aliquot was placed on dry ice and shipped via overnight to Iowa State University College of Veterinary Medicine, Department of Biomedical Sciences. Assays were subsequently performed as previously described (Van Engen et al., 2014).

On-Site Blood Analysis

Blood samples collected in a 10-mL tube containing coated lithium heparin were analyzed for blood pH, LAC, base excess (**BE**), partial venous pressure of O₂ (**P_vO₂**), partial venous pressure of CO₂ (**P_vCO₂**), and bicarbonate (**HCO₃⁻**) using an i-Stat clinical analyzer with the CG4⁺ cartridge (Abaxis North America, Union City, CA). Cartridges were loaded and analyses performed using procedures described by the manufacturer. Following confirmation of analysis, the tube was centrifuged at 3,000 \times g for 15 min at 4°C. Following centrifugation, plasma was separated, placed in a 5-mL cryovial, and stored on dry ice until transfer to permanent storage at -80°C.

Serum Cortisol

Blood collected in a 10-mL coagulation tube was allowed to clot in the tube and was then placed in a centrifuge at 3,000 \times g for 15 min at 4°C. Serum was separated, placed in a cryovial, and stored on dry ice until transfer to permanent storage at -80°C. Samples were analyzed using Immulite 1000 Cortisol chemiluminescent enzyme immunoassay per manufacturer instructions (Siemens, Malvern, PA).

Plasma Lactate and Creatinine Kinase

Cryovials containing lithium heparinized plasma stored as previously described were submitted to the Kansas State University Veterinary Diagnostic Laboratory and were analyzed for LAC using a Nova CCX analyzer (Nova Biomedical, Waltham, MA) and CK using a Cobas c501 analyzer (Roche Diagnostics, Indianapolis, IN).

Pedometer Application

An IceTag pedometer (IceRobotics Ltd., Edinburgh, UK) was secured to the right rear leg just proximal to the metatarsophalangeal joint of the steer using the applicator band with the device attached while the steer was restrained in the hydraulic chute at the time of randomization. Data were recorded in 15-min intervals. Individual files were combined, and data were truncated to include only the 48 h immediately following the conclusion of exercise and summarized by hour. Seven pedometers were excluded from the study because they either failed to stay on the animal or failed to record data. Steps taken and number of lying bouts were recorded as count data, and time spent standing was recorded as the percentage of the hour spent standing. Lying bouts are defined as the number of times an animal changes standing or lying positions.

Statistical Analysis

All data were analyzed using the GLIMMIX procedure of SAS 9.3 (SAS Inst. Inc., Cary, NC) accounting for repeated measures by steer when appropriate; the model included the fixed effects of treatment, BF group, sampling time, and their interactions. All interactions found to be nonsignificant ($\alpha = 0.05$) were dropped from the final model. The random effects included in the model were block and day. Degrees of freedom were calculated using the Kenward-Rogers method. Transformation of LAC and CK data were performed by analyzing the natural logarithm of the original data. The model was selected using the maximum-likelihood estimation and the Akaike information criterion for best fit. Body weight and LM area were tested as potential covariates but were dropped from the final models because they were not significant ($\alpha = 0.05$).

One steer from the AH cattle was removed from the analysis as an outlier using Cook's distance test. Data from LAP2 samples on steers that met exercise-stop criteria before sample collection were treated as missing data. Serum SUBP concentrations below the assay detection threshold of 5 pg/mL were assigned a value of 2.5 pg/mL (Helsel, 2005).

Within the AH treatment, 4 steers did not finish the course or displayed clinical symptoms of muscle fatigue and exhaustion as per exercise stop criteria. These EXH steers were compared to the 15 remaining non-exhausted (NEXH) steers using the same procedures described above for each measured variable. Values of $P \leq 0.05$ were considered significant. Values of $P \leq 0.10$ were considered a trend. The interaction of the BF group and handling method was analyzed for LAC, CK, and blood pH. Values of $P \leq 0.05$ were considered significant. Values of $P \leq 0.10$ were considered a trend.

Analysis of pedometer steps taken was performed using a Poisson distribution. The full model included the fixed effects of handling, the number of hours post-handling (TREATHOUR), the BF group, and all of their interactions were considered. Time of day was used as a covariate to correct for the effect of time of day on the outcome variables as the handling time was different between blocks. All interactions found to be nonsignificant ($\alpha = 0.05$) were dropped from the final model. Seven steers were removed from the analysis, 3 from the AH treatment and 4 from the LSH treatment, due to faulty pedometers or because the pedometer failed to remain on the leg. One EXH and 2 NEXH steers were removed due to failed pedometers. Values of $P \leq 0.05$ were considered significant. Data for steps taken were analyzed by adding 1 to the total number of steps taken using a Poisson distribution. Analysis of time standing (STAND) was performed using a binomial probability distribution. Analysis of standing and

lying position changes (POS) was performed using a Poisson distribution and the Bonferroni method of adjustment for multiple comparisons.

RESULTS

Plasma LAC concentrations were greater ($P < 0.05$) in AH cattle than in LSH cattle at baseline (BASE). Plasma LAC concentration at the end of LAP1, at the end of LAP2, following 1H, and following 2H were greater ($P < 0.01$) in AH cattle than in LSH cattle (Table 1). In LSH cattle, a trend was found for greater LAC at LAP1 ($P = 0.06$) compared to BASE, but no effect of sample time on LAC was detected at other sample times ($P > 0.16$). Base excess was decreased ($P < 0.05$) in AH cattle compared to that in LSH cattle at LAP1, LAP2, and 1H but not at BASE or 2H. Serum cortisol (CORT) concentrations were greater ($P < 0.05$) in AH cattle compared to those of LSH cattle at LAP1, LAP2, 1H, and 2H.

Compared to BASE, CK concentrations were greater ($P < 0.05$) for LSH cattle at LAP2, 1H, and 2H, and at LAP1, LAP2, 1H, and 2H for AH cattle (Table 1). However, no differences in CK ($P > 0.42$) concentrations were detected between LSH and AH for any sample time.

Heart rate and TEMP were greater ($P < 0.05$) in AH cattle compared to those in LSH cattle at LAP1, LAP2, and 1H but not at BASE and 2H. Respiratory rate was not different between LSH and AH ($P > 0.25$) at BASE, LAP2, and the 1H and 2H rest periods. However, AH cattle displayed a trend for greater RR ($P = 0.06$) at LAP1 compared to that of LSH cattle. Compared to BASE, TEMP was greater in LSH cattle at 1H and 2H ($P < 0.05$) but not at LAP1 and LAP2. Temperature in LSH cattle was decreased at 2H compared to AH cattle. Peak TEMP in both AH and LSH cattle occurred at the final 2H reading (Table 1).

Blood bicarbonate concentration and blood pH were decreased in AH cattle compared to those in LSH cattle ($P < 0.01$) at LAP1, LAP2, and 1H but were not different between treatments by 2H. Blood gas values for P_{vO_2} were greater at LAP1 and LAP2 in AH cattle compared to those in LSH cattle, while P_{vCO_2} for AH was decreased at LAP1 and LAP2 compared to that for LSH.

Blood CORT concentration was greater ($P < 0.05$) in AH cattle compared to that in LSH cattle at LAP1, LAP2, and 1H. Cortisol was greater ($P < 0.05$) at LAP2 in AH cattle compared to that at all other sample times (Table 1). No differences in SUBP were found ($P > 0.14$) between LSH and AH cattle within any sample time.

Table 1. Blood chemistry and vital signs of Aggressive (AH) vs. low-stress handling (LSH) of finishing steers during exercise of 1540 m¹

Item	BASE ²		LAP1 ³		LAP2 ⁴		1 h rest ⁵		2 h rest ⁶		SEM
	LSH ⁷	AH ⁸	LSH	AH	LSH	AH	LSH	AH	LSH	AH	
Heart rate, bpm	76.6 ^a	75.0 ^a	80.8 ^{a,b}	124.1 ^c	90.1 ^b	140.9 ^c	81.9 ^{a,b}	105.8 ^d	89.9 ^b	100.6 ^d	3.7
Respiration rate, bpm	48.0 ^a	46.3 ^a	57.7 ^b	67.8 ^b	72.1 ^c	74.3 ^c	59.1 ^b	65.6 ^b	65.3 ^b	65.4 ^b	3.7
Temperature, C	39.4 ^a	39.6 ^a	39.7 ^b	39.9 ^b	40.0 ^b	40.6 ^c	39.6 ^a	39.9 ^b	39.6 ^a	39.8 ^a	0.7
Plasma lactate, mmol/L	3.0 ^a	4.1 ^b	2.3 ^a	16.5 ^c	2.4 ^a	22.3 ^c	2.7 ^a	7.2 ^c	2.5 ^a	4.0 ^b	0.7
Creatinine kinase, U/L	424 ^{a,b}	346 ^a	588 ^{b,c}	544 ^b	681 ^c	648 ^c	829 ^c	1,006 ^c	1,034 ^c	1,430 ^c	1,160
pH	7.42 ^a	7.43 ^{a,e}	7.45 ^{a,c}	7.25 ^c	7.48 ^{b,c}	7.19 ^d	7.47 ^a	7.43 ^a	7.46 ^a	7.46 ^a	0.02
P _v CO ₂ ⁹ , mmHg	46.1 ^a	44.3 ^a	43.1 ^{a,b}	41.1 ^b	39.3 ^c	30.6 ^d	40.8 ^{b,c,f}	38.8 ^f	42.3 ^{b,c}	43.7 ^{a,b}	1.4
P _v O ₂ ¹⁰ , mmHg	30.6 ^a	35.5 ^a	33.5 ^{a,b}	42.7 ^c	36.6 ^b	51.5 ^d	33.3 ^a	33.8 ^a	33.8 ^a	32.8 ^a	3.7
HCO ₃ ⁻¹¹	30.2 ^a	29.2 ^a	30.2 ^a	17.7 ^b	29.4 ^a	12.3 ^c	29.7 ^a	25.9 ^d	30.3 ^a	30.4 ^a	1.6
Base excess	5.8 ^a	4.9 ^a	6.2 ^a	-9.6 ^b	5.9 ^a	-16.0 ^c	5.9 ^a	1.5 ^d	6.6 ^a	6.7 ^a	0.7
Cortisol, nmol/L	69.6 ^{a,b}	80.6 ^{a,c}	58.9 ^b	87.5 ^a	93.1 ^c	144.4 ^d	53.1 ^b	113.5 ^d	72.5 ^a	97.0 ^{c,d}	15.3
Substance P, pg/mL	26.4	32.8	22.9	26.1	24.4	24.5	24.6	28.7	24.2	29.9	4.9

^{a-c}Values within rows without a common superscript differ ($P \leq 0.05$).

¹Angus crossbred steers ($n = 40$; 563 ± 44 kg).

²BASE = baseline measurements taken before application of treatment.

³LAP1 = measurements following a distance of 770 m.

⁴LAP2 = measurements following a distance of 1,540 m.

⁵Measurements following 1 h of rest following the completion of animal handling.

⁶Measurements following 2 h of rest following the completion of animal handling.

⁷Low-stress animal handling methods. Cattle were walked with a lead rider.

⁸Aggressive animal handling methods. Cattle were run without a lead rider up to the course distance of 1,540 m in 7 to 8 min.

⁹P_vCO₂ = partial pressure venous carbon dioxide.

¹⁰P_vO₂ = partial pressure venous oxygen.

¹¹HCO₃⁻ = bicarbonate.

Exhausted Steers

Two steers in the AH treatment, with 1 from each BF group, did not finish the course to the end of LAP2 because they met the exercise-stop criteria. One steer collapsed and then recovered after a brief rest period, and the second steer became extremely reluctant to proceed. Both steers completed LAP1. A third steer from the AH treatment, from the THINBF group, was observed with epaxial muscle fasciculations at LAP2 sampling, and a fourth from the AH treatment FATBF strata developed clinical signs consistent with rhabdomyolysis in the alleyway leading to the chute for LAP2 sampling. There was a main effect of EXH cattle for a decreased ($P < 0.01$) P_vCO₂, and there were trends for greater ($P < 0.10$) P_vO₂ and CORT and decreased TEMP and SUBP ($P < 0.10$) than those in NEXH cattle. However, there were no main effects detected in LAC, CK, pH, BE, HCO₃⁻, RR, and HR for EXH cattle compared to NEXH cattle. All variables displayed similar changes with sample time as described for AH cattle. The EXH cattle had greater CK ($P < 0.05$) at 2H compared to NEXH but not at other sample times (Table 2). Exhausted cattle had greater ($P < 0.05$) LM area than that of NEXH cattle. However, no effect of LM area was detected between NEXH and EXH on the variables measured in this study.

Backfat Group

A main effect of BF group ($P < 0.05$) increased LAC concentration by 1.1 mmol/L. Backfat group interacted with sample time for pH and treatment and sample time for HCO₃⁻. Additionally, an interaction between handling treatment and BF group was detected ($P < 0.05$) for LAC, pH, HCO₃⁻, and BE.

Backfat strata within LSH cattle showed no differences in LAC, blood pH, HCO₃⁻, or BE. AH cattle in the FATBF group had a greater LAC ($P < 0.05$) at LAP2 and 1H than cattle in the THINBF group (Table 3). Additionally pH was decreased at LAP2 and at 1H in FATBF AH cattle compared to THINBF AH ($P < 0.01$) cattle. It should be noted that BW is also different ($P \leq 0.05$) between THINBF (528 ± 14.7 kg) and FATBF (581 ± 14.7 kg) AH cattle. Backfat grouping tended to place lighter cattle in the THINBF group although BW between treatments and blocks were not different.

Pedometers

An interaction of cattle handling and TREATHOUR ($P < 0.05$) was observed. There was a trend for cattle in the FATBF group to take more steps than those in the THINBF group ($P < 0.07$). There was no interaction of

Table 2. Blood chemistry and vital signs of non-exhausted (NEXH) vs. exhausted (EXH) aggressively handled finishing steers during exercise up to 1,540 m¹

Item	BASE ²		LAP1 ³		LAP2 ⁴		1 h rest ⁵		2 h rest ⁶		SEM
	NEXH ⁷	EXH ⁸	NEXH	EXH	NEXH	EXH	NEXH	EXH	NEXH	EXH	
Heart rate, bpm	74.5 ^a	70.3 ^a	124.4 ^b	112.1 ^{a,b}	134.3 ^b	167.3 ^c	114.5 ^{b,d}	81.9 ^{a,d}	105.3 ^{b,d}	87.3 ^{a,d}	5.3
Respiration rate, bpm	47.3 ^a	46.4 ^a	68.7 ^b	65.7 ^b	76.0 ^b	70.0 ^b	66.9 ^{b,d}	58.9 ^{a,c,d}	67.7 ^{b,d}	56.3 ^{a,c,d}	3.8
Temperature, C	39.6 ^a	39.4 ^a	40.0 ^b	39.7 ^{a,b}	40.8 ^c	40.2 ^b	40.0 ^b	39.7 ^{a,b}	39.8 ^{a,b}	40.0 ^{a,b}	0.5
Plasma lactate, mmol/L	4.1 ^a	4.1 ^a	16.3 ^b	18.5 ^b	21.9 ^b	24.6 ^b	7.2 ^c	7.6 ^{a,c}	3.9 ^a	4.3 ^a	1.5
Creatinine kinase, U/L	294 ^a	272 ^a	443 ^{a,b}	541 ^{a,c}	522 ^{b,c}	507 ^{a,c}	777 ^{c,d}	1,145 ^d	1,049 ^d	1,980 ^d	3,414
pH	7.41 ^a	7.48 ^a	7.25 ^b	7.24 ^b	7.18 ^c	7.19 ^{b,c}	7.41 ^a	7.47 ^a	7.43 ^a	7.50 ^a	0.05
P _v CO ₂ ⁹ , mmHg	45.2 ^a	40.2 ^a	42.3 ^a	35.3 ^b	31.4 ^c	27.1 ^c	40.1 ^{a,b}	34.2 ^b	45.0 ^a	39.3 ^a	2.2
P _v O ₂ ¹⁰ , mmHg	35.3 ^{a,b}	36.0 ^a	40.4 ^b	51.0 ^b	51.05 ^c	53.2 ^c	32.5 ^a	38.5 ^a	31.3 ^a	38.5 ^a	3.3
HCO ₃ ⁻¹¹	29.3 ^a	29.5 ^a	18.9 ^b	15.3 ^{b,c}	12.7 ^c	10.8 ^c	26.0 ^d	24.9 ^{a,d}	30.3 ^a	29.9 ^{a,d}	1.1
Base excess	4.8 ^a	6.0 ^a	-8.3 ^b	-12.0 ^{b,c}	-15.6 ^c	-17.3 ^c	1.3 ^d	1.2 ^d	6.1 ^a	7.0 ^b	1.5
Cortisol, nmol/L	75.6 ^a	90.5 ^{a,c}	78.4 ^{a,b}	117.2 ^{a,c}	140.3 ^d	160.9 ^{c,d}	110.4 ^c	125.0 ^c	88.6 ^a	124.8 ^c	19.0
Substance P, pg/mL	36.5	21.6	28.3	21.3	24.7	19.4	30.1	22.1	31	26.2	4.9

^{a-c}Values within rows without a common superscript differ ($P \leq 0.05$).

¹Angus crossbred steers ($n = 40$; 563 ± 44 kg).

²BASE = baseline measurements taken before application of treatment.

³LAP1 = measurements following a distance of 770 m.

⁴LAP2 = measurements following a distance of 1,540 m.

⁵Measurements following a 1 h of rest following the completion of animal handling.

⁶Measurements following a 2 h of rest following the completion of animal handling.

⁷Non-exhausted aggressively handled steers ($n = 15$). Cattle were walked with a lead rider.

⁸Exhausted aggressively handled steers ($n = 4$). Cattle were run without a lead rider up to the course distance of 1,540 m in 7 to 8 min.

⁹P_vCO₂ = partial pressure venous carbon dioxide.

¹⁰P_vO₂ = partial pressure venous oxygen.

¹¹HCO₃⁻ = bicarbonate.

BF strata ($P > 0.32$) with any other factor. Cattle in the AH group walked less than LSH cattle (49.8 vs. 68.9 steps/h; $P < 0.01$). Cattle in the AH group were not decreased in steps taken until 20 h post-handling when compared to the LSH group ($P < 0.05$; Fig. 1 and 2). Briefly, LSH cattle took fewer steps at TREATHOUR 2, 4, 16, 22, 39, 40, and 45 to 48 after handling ($P < 0.05$). Cattle in the AH group took fewer steps in 9 of the first 24 h and at all time points except h 36 and 40 after 24 h ($P < 0.05$). There was a main effect of TREATHOUR ($P < 0.05$) on EXH or NEXH cattle, but no interactions of TREATHOUR with handling or with BF strata were detected ($P > 0.36$).

Briefly, STAND was decreased for AH cattle compared to LSH cattle (31.7 vs. 44.3 ± 1.1 min/h; $P < 0.01$; Table 4) and a main effect of TREATHOUR was observed ($P < 0.01$), but there were no handling by TREATHOUR interactions. There was no effect of BF strata or its interactions with TREATHOUR or handling on STAND ($P > 0.26$).

There was no difference in POS (0.63 vs. 0.61 changes/h; Table 4) due to handling, BF strata, or their interaction ($P > 0.22$). The range of POS was 0 to 4 and 0 to 3 changes/h, with a median of 1, for LSH and AH, respectively.

DISCUSSION

Animal welfare is a major concern for all who are involved in the cattle industry (Verbeke and Viaene, 2000). During the summer of 2013, various cattle abattoirs throughout the United States reported concerns with cattle that were slow or difficult to move (Thomson et al., 2015). These cattle exhibited reluctance to move, stiff and shortened gait, and lagging behind cohorts. These animals required greater human-animal interaction to initiate movement. Periods of greater environmental temperature, or heat stress, appeared to increase the incidence rates of these slow-moving cattle at the abattoir (Thomson et al., 2015). Upon further investigation, it was noted that these cattle had greater serum lactate, CK concentrations, and muscle tremors, in addition to the above-mentioned clinical symptoms.

The clinical presentation and blood chemistry results reported in this study for the AH cattle are similar to findings in swine suffering from FPS (Ritter et al., 2005). Heart rates in cattle increased from BASE in both the LSH and AH groups and were greater in the AH cattle compared to the LSH cattle. Heart rate peaked in both groups at LAP2 and then decreased but remained increased compared to BASE for the entire rest period

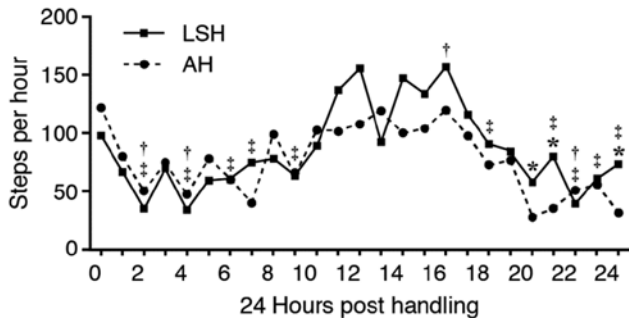


Figure 1. Effect of aggressive (AH) vs. low-stress handling (LSH) of finishing during exercise of up to 1,540 m on the number of steps taken 0 to 24 h post-handling. Angus crossbred steers ($n = 40$; 563 ± 44 kg). For the LSH method, cattle were walked with a lead rider. For the AH method, cattle were run without a lead rider up to the course distance of 1,540 m in 7 to 8 min. *Difference between LSH and AH ($P \leq 0.05$). †Difference between steps at h 0 and the hour indicated for LSH ($P \leq 0.05$). ‡Difference between steps at h 0 and the hour indicated for AH ($P \leq 0.05$).

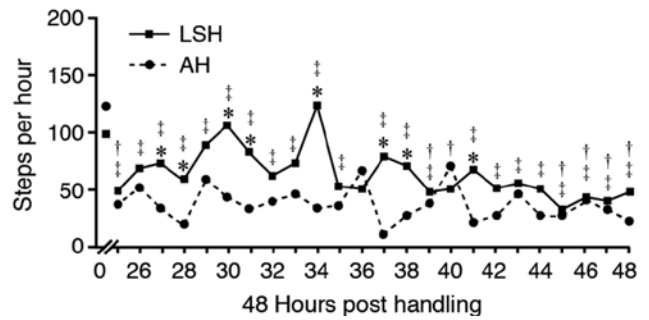


Figure 2. Effect of aggressive (AH) vs. low-stress handling (LSH) of finishing during exercise of up to 1,540 m on the number of steps taken 25 to 48 h post-handling. Angus crossbred steers ($n = 40$; 563 ± 44 kg). For the LSH method, cattle were walked with a lead rider. For the AH method, cattle were run without a lead rider up to the course distance of 1,540 m in 7 to 8 min. *Difference between LSH and AH ($P \leq 0.05$). †Difference between steps at h 0 and the hour indicated for LSH ($P \leq 0.05$). ‡Difference between steps at h 0 and the hour indicated for AH ($P \leq 0.05$).

with the exception of LSH at 1H. Heart rate and RR results closely resembled previously reported results (Kuhlmann et al., 1985; Piguet et al., 1994) with an increase in HR greater in cattle with a greater exercise load.

In this study, BASE LAC concentrations were greater in AH than in LSH cattle, but it is likely of little or no physiologic significance, as the BASE LAC concentration of each treatment group are within the normal physiologic range reported for LAC concentration in cattle on high-grain diets (Burrin and Britton, 1986). Concentrations of LAC in AH cattle closely resembled concentrations and recovery times seen in Hereford calves of lighter weight exercised at a speed of 1.8 m/s (Kuhlmann et al., 1985). This same study also found peak LAC concentrations were greater with an increasing speed of exercise. The results of Kuhlmann et al. (1985) and the distinct lack of change in LAC in LSH cattle in this study illustrate that the speed of handling is an important component of the physiologic response and perhaps may be more important in inducing FCS than the distance the animal moves. The results of this study and previous work in swine and other species show the importance of handling in such a manner as to reduce the possibility of exceeding the anaerobic threshold and incurring possible deleterious effects.

Although RR in this study did not differ between treatments, an increase during exercise was followed by numeric decreases during the recovery period. Respiration rates remained increased during the recovery period compared to BASE, which is consistent with previous research (Piguet et al., 1994). The lack of difference in RR between AH and LSH cattle was possibly due to an increase in tidal volume, which has been reported during exercise in cattle (Piguet et al., 1994).

Cortisol has long been measured as a physiological response marker for stress in many species, including

cattle. In the current study, basal CORT concentrations were greater than those reported in nonstressed cattle and were greater at the end of exercise and during recovery in AH cattle than in LSH cattle (Nikolic et al., 1998). The magnitude of the increase (1.5 to 2 times in the present study) was less than the increase reported in Hereford calves during exercise (Kuhlmann et al., 1985). Increased plasma CORT concentrations have been shown to delay recovery from exhaustive exercise in trout (Pagnotta and Milligan, 1991; Eros and Milligan, 1996). Increased CORT in AH cattle may be a factor in the delayed return of LAC to normal concentrations in these cattle.

In livestock species, substance P has been used as an indicator of physiological response to painful procedures, such as dehorning and castration (Coetzee et al., 2008; Coetzee et al., 2012a, 2012b). This study showed no difference in SUBP between LSH and AH cattle or between NEXH and EXH cattle, but this does not necessarily indicate a lack of pain, as not all studies of painful procedures show a difference in SUBP (Mintline et al., 2014). Also, recent refinements in the assays have led to a change from an ELISA to an RIA method making comparison with results from previous studies that used an ELISA method difficult.

In this study, we induced FCS in cattle with aggressive handling practices. Cattle in this study can be divided into 3 distinct groups: LSH cattle, which showed little physiologic stress and no observed clinical signs of fatigue, AH cattle that completed the course, and EXH cattle that did not complete the course or showed clinical symptoms consistent with the description of FCS. To the authors' knowledge, this is the first description of the use of a handling model to induce FCS in steer cattle near the end of the feeding period.

Exhaustive exercise in this study resulted in greater LAC, BE, and CORT and decreased blood pH, forming

Table 3. Effect of backfat (BF) thickness on selected blood chemistry and vital signs of aggressive (AH) vs. low-stress handling (LSH) of finishing steers during exercise of 1,540 m¹

Item	BF GROUP ⁷	BASE ²		LAP1 ³		LAP2 ⁴		1 h rest ⁵		2 h rest ⁶		P-value by BF interaction
		LSH ⁸	AH ⁹	LSH	AH	LSH	AH	LSH	AH	LSH	AH	
Plasma lactate, mmol/L	THINBF	2.95 ^{a,e}	4.70 ^{a,c}	2.13 ^{a,c}	13.6 ^{b,c}	2.27 ^{a,c}	18.1 ^{b,e}	2.87 ^{a,c}	5.25 ^{c,e}	2.44 ^{a,e}	4.45 ^{a,e}	0.057
	FATBF	3.00 ^{a,e}	3.63 ^{a,e}	2.40 ^{a,e}	20.0 ^{b,e}	2.63 ^{a,e}	27.3 ^{b,e}	2.47 ^{a,e}	9.60 ^{c,f}	2.52 ^{a,e}	3.52 ^{a,e}	
Blood pH	THINBF	7.42 ^{a,e}	7.44 ^{a,e}	7.44 ^{a,e}	7.28 ^{b,e}	7.47 ^{a,e}	7.26 ^{b,e}	7.45 ^{a,e}	7.45 ^{a,e}	7.46 ^{a,e}	7.46 ^e	<0.01
	FATBF	7.42 ^{a,e}	7.42 ^{a,e}	7.45 ^{a,e}	7.21 ^{b,f}	7.47 ^{a,e}	7.10 ^{c,f}	7.47 ^{a,e}	7.25 ^{d,f}	7.45 ^{a,e}	7.44 ^{a,e}	
HCO ₃ ⁻¹⁰	THINBF	29.5 ^{a,e}	28.6 ^{a,e}	29.9 ^{a,e}	19.9 ^{b,e}	29.3 ^{a,e}	15.0 ^{c,e}	29.3 ^{a,e}	27.8 ^{a,e}	30.2 ^{a,e}	30.5 ^{a,e}	<0.01
	FATBF	30.9 ^{a,e}	30.0 ^{a,e}	30.3 ^{a,e}	16.5 ^{b,f}	29.5 ^{a,e}	9.6 ^{c,f}	30.1 ^{a,e}	24.1 ^{d,f}	30.4 ^{a,e}	29.9 ^{a,e}	
Base excess	THINBF	4.9 ^{a,e}	4.7 ^{a,e}	5.9 ^{a,e}	-6.7 ^{b,e}	5.8 ^{a,e}	-11.9 ^{c,e}	6.3 ^{a,e}	4.0 ^{d,e}	6.4 ^{a,e}	6.4 ^{a,e}	<0.01
	FATBF	6.7 ^{a,e}	5.5 ^{a,e}	6.4 ^{a,e}	-11.2 ^{a,f}	5.9 ^{a,e}	-20.0 ^{c,f}	5.4 ^{a,e}	-1.0 ^{d,f}	6.7 ^{a,e}	6.3 ^{a,e}	

^{a-f}Values within rows without a common superscript differ ($P \leq 0.05$).

¹Angus crossbred steers ($n = 40$; 563 ± 44 kg).

²BASE = baseline measurements taken before application of treatment.

³LAP1 = measurements following a distance of 770 m.

⁴LAP2 = measurements following a distance of 1,540 m.

⁵Measurements following a 1 h of rest following the completion of animal handling.

⁶Measurements following a 1 h of rest following the completion of animal handling.

⁷Backfat thickness stratification group. THINBF = 9.1 ± 2.8 mm; FATBF = 13.5 ± 0.5 mm.

⁸Low-stress animal handling methods. Cattle were walked with a lead rider.

⁹Aggressive-animal handling methods. Cattle were ran without a lead rider up to the course distance of 1540 m in 7 to 8 min.

¹⁰HCO₃⁻ = bicarbonate.

a similar pattern of results in both magnitude and duration as has been reported in cattle and in other species (Holmes et al., 1973; Milligan, 1996; Ritter et al., 2005). The difference in P_vO_2 and P_vCO_2 between EXH cattle and NEXH cattle suggest that a possible cause for exhaustion in cattle could be a difference in the efficiency of gas exchange. The decreased P_vCO_2 concentration in EXH cattle compared to that in NEXH cattle is contradictory to reported physiological responses in cattle to greater exercise and tissue oxygen demand (Kuhlmann et al., 1985; Gustin et al., 1988). Kuhlmann et al. (1985) reported that as speed of exercise increased, venous P_vO_2 decreased and P_vCO_2 increased. In the current study, P_vO_2 and P_vCO_2 responded in the opposite manner in AH cattle. However, it should be noted that AH cattle in this study were both heavier and exercised at a greater speed than the cattle studied by Kuhlmann et al. (1985), and these factors could be possible factors in alteration of the response from what has been previously reported. The 4 EXH steers displayed blood gas changes at LAP1 ($P < 0.05$) from BASE compared to NEXH steers in which differences were not shown until LAP2. The earlier changes in blood gas concentrations in EXH cattle compared to NEXH are an indicator that the EXH cattle had some differences in blood gas exchange. Possible hypotheses for the changes in blood gas concentration could be 1) inefficient gas exchange in the lungs of EXH cattle, 2) inefficient gas exchange at the level of muscle tissue, 3) cardiac insufficiency, 4) increased speed of handling compared to previous research, 5) increased BW compared to previous

research, or 6) increased muscle mass. Because the cattle in this study had increased BW and because they were moved at a rate of speed greater than what has been previously reported, it is possible that these 2 variables could have been significant factors in the differences in observations between this study and previous reports. Research in a more controlled environment should be performed to further our understanding of the blood-gas chemistry of cattle of this size. The degree to which this difference in blood gas concentration contributed to the inability of these 4 steers to finish the course is unknown, and data in this study cannot determine the cause of this difference.

The greater CK in EXH cattle at 1H and 2H could originate from greater muscle damage, greater muscle mass, and protein turnover as indicated by the increased LM area of the four EXH cattle, cardiac muscle damage, or any combination of these. The CK concentrations in NEXH cattle are similar to the CK concentrations in LSH cattle but are increased in cattle showing signs of FCS, which is consistent with the findings of FPS in swine.

The statistical differences and trends seen between NEXH and EXH cattle suggest that a small portion of the feedlot cattle population may be at a greater risk for developing detrimental health and welfare problems due to aggressive handling. Research into FPS mitigation strategies led to management changes, such as less aggressive swine handling, greater transportation floor space, and changes in facility design (Ritter et al., 2007; Edwards et al., 2011; Ritter et al., 2012). Animal handling is one of the key components in preventing this multifactorial syn-

Table 4. Effect of aggressive (AH) vs. low-stress handling (LSH) of finishing steers during exercise of up to 1,540 m on standing time and position changes for 48 h post-handling¹

Item	LSH ²	AH ³	SEM	<i>P</i> -value
Standing time, min/h	31.7	44.3	1.10	<0.01
Position changes, changes/h	0.63	0.61	0.20	0.51

¹Angus crossbred steers ($n = 40$; 563 ± 44 kg)

²Low stress animal handling methods. Cattle were walked with a lead rider.

³Aggressive animal handling methods. Cattle were run without a lead rider up to the course distance of 1,540 m in 7 to 8 min.

drome in swine (Ritter et al., 2005; Ritter et al., 2009a). Given the similarity in response to AH in cattle and swine, it is reasonable to conclude that handling is also a factor in the expression of FPS-like clinical signs in cattle and that cattle can undergo FCS. Further research to identify the specific physiologic risk factors that put cattle at greater risk of adverse events is warranted.

The interaction seen between THINBF and FATBF and AH warrants further investigation into the effects of both BF and BW on the severity and duration of the metabolic acidosis induced by AH. Lactic acid concentrations were 50% greater in FATBF cattle than in THINBF cattle. Additionally, it should be noted that blood pH in THINBF cattle did not decrease between LAP1 and LAP2, although LAC concentrations continued to increase, indicating that these cattle were able to compensate for the increased acid load through other means. The degree to which this effect is due to increased BF, BW, or their interaction should be investigated further.

Cattle in the AH group spent more time lying down than cattle in the LSH group, although they did not change standing/lying positions more often. Lying behavior has been shown to be a measure of cattle comfort (Haley et al., 2000). Additionally, restlessness as measured by steps taken and weight shifting has been shown to be a useful indicator of comfort in dairy cattle, which are forced to stand (Chapinal et al., 2011). However, in this study, it seems more likely that the increased time lying down is an indicator of fatigue rather than of increased comfort.

The results of this study support the hypothesis that AH reduces movement in cattle post-handling. However, voluntary reduction in locomotion occurs as early as 2 h from the handling event in both LSH and AH cattle. Reductions in steps taken earlier than 2 h may have been masked by the handling of cattle following the two 1 h rest periods. Also, this study is limited by the lack of a pretreatment BASE for each individual animal and because all comparisons of change over time are compared to TREATHOUR 0 post-handling data.

The reduction in steps is periodic, as there is a period of reduction from 2 to 9 h post-handling for

nearly the entire period from 18 to 48 h post-handling in AH cattle. Cattle in the LSH group also showed a decrease in steps at 2 and 4 h post-handling.

Movement of cattle from their pen to a loading facility for transportation is the final management step of finishing cattle for beef production. It is common for cattle to be located throughout the feedyard, and there may be a distance of 1.6 km to loading facilities in some feedyards. Additionally, cattle may not have been out of the pen for a considerable period before shipment and are sometimes reluctant to leave the pen. It is possible for cattle to run a considerable distance, either voluntarily or forced by an untrained handler.

The average distance that fed cattle are shipped direct to slaughter from feedyards in the United States is 276 ± 15 km (166 mi), which would likely mean an approximately 3-h transit time (USDA-APHIS, 2013). Voluntary reduction of movement in this study began at 2 h post-handling, regardless of handling method, and would coincide with arrival time at an abattoir for many fed cattle. This change in voluntary behavior in both LSH and AH cattle in addition to the arrival time lead to a hypothesis that it is possible that the distance that cattle have to travel to a load out could play a role in the incidence of FCS at abattoirs and should be investigated further.

Swine and cattle suffering from FPS and FCS have been documented to clinically recover if allowed to rest quietly (Anderson et al., 2002; Ritter et al., 2009b; Thomson et al., 2015). The results of this study would suggest that from the standpoint of animal locomotion, following a rest period in cattle showing clinical signs of FCS at abattoirs, a time between 9 and 18 h post-handling could be a time at which cattle might be moved with greater ease. However, cattle that have been handled improperly have markedly reduced movement beyond 18 h post-handling. Additionally, finished cattle that are handled improperly and transported greater than 18 h to slaughter may be at greater risk for mobility problems on arrival at the abattoirs.

Conclusion

This study highlights the need for improved training for animal handling in commercial feeding operations. Aggressive cattle handling produces significant physiological responses, which can be detrimental to some animals. These data and others show that the speed of movement and the aggressiveness of handling affects the physiologic blood chemistry more significantly than does the distance the animals are moved.

Of further importance, we have shown that running cattle 770 m to 1,540 m at a rapid pace can induce clinical signs of FCS. Cattle movements of these distances are commonly found in modern commercial cattle fa-

cilities and highlight the need for proper movement practices of cattle from the pen to the final destination of the cattle within the facility. However, it should be noted that all cattle in this study continued to slaughter without further health and welfare problems.

The beef industry needs to continually improve animal handling practices to ensure that animal well-being is addressed at every phase of beef production from feedyard to the harvest floor. Investigation into these potential risk factors and mitigation strategies should be pursued to further define the management factors that can increase or decrease the risk for FCS.

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