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Effects of ractopamine hydrochloride on growth performance, carcass characteristics, and physiological response to different handling techniques[1,2]

J. A. Hagenmaier,* C. D. Reinhardt,† M. J. Ritter,‡ M. S. Calvo-Lorenzo,‡ G. J. Vogel,‡ C. A. Guthrie,‡ M. G. Siemens,§ K. F. Lechtenberg,# D. J. Rezac,# and D. U. Thomson*[3]

*Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan 66506; †Department of Animal Sciences and Industry, College of Agriculture, Kansas State University, Manhattan 66506; ‡Elanco Animal Health, Greenfield, IN 46140; §Cargill Meat Solutions Corporation, Wichita, KS 67202; and #Midwest Veterinary Services Inc., Oakland, NE 68405

ABSTRACT: Feedlot cattle (n = 128; BW = 549 ± 60 kg) were used to evaluate the effects of ractopamine hydrochloride (RAC) on growth performance, physiological response to handling, and mobility during shipment for slaughter in a study utilizing a split-plot design with a 2 × 2 factorial arrangement of treatments: 1) diet (CON [no β-adrenergic agonist] vs. RAC [400 mg·animal^{-1}·d^{-1} ractopamine hydrochloride for 28 d]) and 2) handling intensity (HI; low-stress handling [LSH; cattle moved at a walking pace with no electric prod use] vs. high-stress handling [HSH; cattle moved at a minimum of a trot and an electric prod applied while in the alley for posthandling restraint and during loading for shipment to the abattoir]). Cattle fed RAC tended to have greater ADG and G:F (P = 0.06), and had greater HCW and LM area (P = 0.04). The HI treatments were applied on the day after the 28-d growth performance period. Blood samples were collected before HI treatment (baseline), after HI treatments (POSTHAND), after transport to the abattoir (POSTTRANS), and during exsanguination at slaughter. A diet × HI interaction (P = 0.01) was observed in the change in cortisol from baseline to POSTTRANS, and there tended ($P \leq 0.07$) to be diet × HI interactions for the change in epinephrine from baseline to POSTHAND and for the change in creatine kinase (CK) from baseline to POSTTRANS. Feeding RAC and HSH both increased the change from baseline to POSTHAND in norepinephrine and pH ($P \leq 0.05$). The HSH cattle also had greater changes from baseline to POSTHAND in blood HCO_3, base excess, partial pressure of CO_2, lactate, cortisol, and glucose ($P \leq 0.01$). Ractopamine and HSH both produced greater increases in CK concentrations from baseline to slaughter ($P < 0.01$). Mobility was not affected by RAC at the feedlot or following an average 6-h lairage ($P \geq 0.43$). This study confirms RAC improves growth performance and suggests metabolic acidosis, a precursor to fatigued cattle syndrome, develops in cattle allowed to trot without the use of a lead rider regardless of RAC administration. Cattle fed RAC displayed altered hormonal responses to handling and transport stress, and the overall proportion of cattle with compromised mobility appears to increase later in the marketing channel. These findings warrant additional research aimed at better understanding the physiological response to stress and protect the welfare of cattle during shipment for slaughter.

Key words: beef cattle, lactate, low-stress handling, ractopamine hydrochloride, welfare

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INTRODUCTION

Ractopamine hydrochloride (**RAC**; Optaflexx; Elanco Animal Health, Greenfield, IN) and zilpaterol hydrochloride (ZIL; Zilmax, Merck Animal Health, Desoto, KS) are Food and Drug Administration (FDA) approved β-adrenergic agonists (βAA) fed to beef cattle at the end of the finishing period to repartition nutrients

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[3]Corresponding author: dthomson@vet.k-state.edu

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towards promotion of lean tissue deposition, thereby increasing ADG and improving feed efficiency (Quinn et al., 2008). Reports of increased mortality rates and difficulty walking at abattoirs in cattle fed βAA have led to dialogues concerning compromised animal welfare due to use of these feed additives. Thomson et al. (2015) reported 2 separate cases in 2013 where a single Holstein steer and approximately 10% of cattle in a large lot of *Bos taurus* steers became nonresponsive to handling cues, sloughed 1 or more hoof walls, and required euthanasia while in lairage. Blood abnormalities in euthanized cattle reported by Thomson et al. (2015) included elevated blood lactate (25.6 mmol/L, reference range: <4 to 5) and creatine kinase (CK; 6,890 U/L, reference range: 159 to 332). A condition exists in swine (fatigued pig syndrome, **FPS**) in which market weight pigs without obvious disease or injury develop metabolic acidosis and have difficulty walking after transportation to abattoirs (Ritter et al., 2009a). Therefore, fatigued cattle syndrome (**FCS**) has been used to describe market weight cattle that develop metabolic acidosis and have difficulty walking when presented to abattoirs (Thomson et al., 2015). Recently, multiple studies (Frese et al., 2016; Hagenmaier et al., 2017) have reported improper handling can induce clinical signs and blood abnormalities similar to those reported by Thomson et al. (2015) for FCS.

Currently, no published studies evaluate the role of βAA administration on the physiological responses to handling and transport for slaughter in contemporary cattle. Therefore, the objective of this study was to determine the effects of RAC and handling intensity on physiological responses to handling and transport in market weight cattle.

MATERIALS AND METHODS

The procedures used in this study were outlined in a protocol with the approval of the Kansas State University Institutional Animal Care and Use Committee (IACUC).

Experimental Design and Treatments

A total of 128 crossbred *Bos taurus* steers and heifers (BW = 549 ± 60 kg) were evaluated in a 2-phase study over a 30-d period in the summer of 2015 at a Nebraska feedlot. Phase I evaluated the effects of feeding RAC for 28 d on growth performance and carcass characteristics. Two diets were utilized in a randomized complete block design: 1) no βAA (**CON**) vs. 2) 400 $mg \cdot animal^{-1} \cdot d^{-1}$ ractopamine hydrochloride (RAC). Phase II occurred the day cattle were slaughtered, and evaluated the effects of RAC and handling intensity (**HI**) on the physiological and behavioral responses to handling and transport. Phase II utilized a split-plot design with a 2 × 2 factorial arrangement to evaluate diet (CON vs. RAC) within HI (low stress vs. high stress), as HI treatments were applied to separate single-sex groups of 8 cattle (4 representing each diet) within a replicate. Low-stress-handled (**LSH**) cattle were moved at a walk around a 750-m rectangular, dirt alley course for a maximum of 2 laps (1,500 m), with 1 handler ahead of the cattle on horseback serving as a lead rider and 2 handlers behind on horseback to prevent cattle from reversing direction. High-stress-handled (**HSH**) cattle were kept at a minimum of a trot around the same 1,500 m course with all 3 handlers behind the cattle on horseback and no lead rider. An electric prod (Miller Manufacturing Co., Glencoe, MN) was applied twice (approximately 1 s per impulse) on the hip of HSH cattle as part of the handling model: once while in the alley before restraint for posthandling sampling and once during loading onto semitrailers at the feedlot The handling model utilized the same stop criteria as Hagenmaier et al. (2017); however, no cattle met the established stop criterion. Therefore, all cattle completed the 1,500-m handling course and were slaughtered the same day.

Treatment Allocation

Phase I. Approximately 1 mo before slaughter, nearly 150 cattle were weighed and examined by a veterinarian, who deemed them eligible for study enrollment based on established inclusion and exclusion criteria for health and lameness. On d 0, the 64 eligible steers and 64 eligible heifers with the narrowest weight range within each sex were selected for enrollment. Extra cattle were no longer used in this study. The 128 experimental cattle were segregated by sex, stratified by BW, assigned uniquely numbered study ear tags, and allocated to 16 identical pens to form 8 single-gender replicates. Diet treatments were randomly assigned to pens within each replicate using the RAND function in Microsoft Excel (Microsoft Corp., Redmond, WA), and then cattle within individual pens were stratified by BW and allocated into 2 groups of 4 animals randomly assigned to HI treatment (Fig. 1). Start date of RAC administration was equally staggered over d 0 and 1, and this served as a blocking factor. Each block consisted of 4 single-gender replicates (2 per sex) for a total of 64 cattle. Ractopamine was fed continuously within each block for 28 d before slaughter, as this is the most common duration utilized by feedlots (Walker et al., 2006; Samuelson et al., 2016).

Phase II. After 28 d of RAC administration, final BW was recorded, and the handling phase was conducted for each block (4 replicates on d 28 and 4 replicates on d 29). Diets were evaluated within HI treatments for each single-gender replicate. This was accomplished by combining adjacent pens to create 2 handling groups (LSH and HSH) of 8 cattle (4

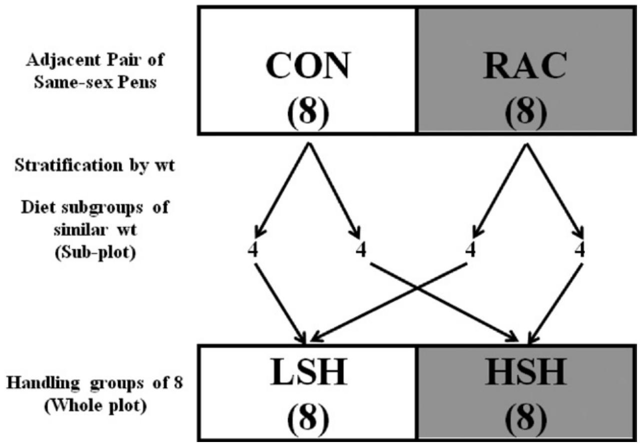

Figure 1. Schematic overview for how pairs of single-sex pens of 8 cattle were stratified by weight and allocated to handling intensity treatments in phase II. Pens of 8 cattle fed 400 $mg \cdot animal^{-1} \cdot d^{-1}$ ractopamine hydrochloride (RAC) or not fed a β-adrenergic agonist (CON) were stratified by weight into subgroups of 4 that were randomly allocated to 1 of 2 handling intensity treatments (LSH or HSH). Handling groups of 8 served as the whole plot (n = 8), and subgroups of 4 representing each diet served as the subplot (n = 16).

CON and 4 RAC) based on the initial allotment to diet and HI treatments (Fig. 1). As a result, each handling group of 8 cattle was single sexed and comprised 4 cattle from both CON and RAC diets to control for variation in HI application over the 1,500-m course.

Animal Housing, Feeding, and Monitoring

Cattle were raised in accordance with the feedlot's standardized operating procedures which were consistent with the Ag Guide (Federation of Animal Science Societies, 2010). Pens were oriented east to west and measured 14.6 m deep × 3.8 m wide with smooth concrete floors. Each pen had a 3.0-m shaded area beginning 3.8 m from the feed bunk and extending over the entire width of the pen. A 3.8-m gate was open to allow each group of cattle access to 2 pens, so that combined pen dimensions were 14.6 × 7.6 m, available pen space and shade area were 13.9 and 2.9 m^2/animal, respectively, and bunk space was 0.95 m/animal. Cattle were provided ad libitum access to water from tanks positioned in the center of the fence partially dividing the 2 communicating pens. Pairs of single-sex pens representing both diets were housed adjacent to each other and combined to form handling replicates. Lastly, pairs of pens alternated between sexes, with heifers beginning at the southernmost end.

Cattle were fed a corn-based 63.2% DM finishing diet formulated to meet or exceed the requirements of growing beef cattle (Table 1; NRC, 2000). Feed bunks were assessed daily beginning at 0600 h by a trained individual who estimated orts to determine the amount of feed needed to be delivered to provide ad libitum access to feed.

Table 1. Ingredient composition and analyzed nutrient content of the finishing ration fed during phase I

Ingredient, % DM basis	Value
Rolled, high-moisture corn	60.7
Wet distillers grain	18.5
Sweet Bran 60[1]	11.5
Corn stover	4.6
Liquid supplement[2]	4.7
Total	100.0
Analyzed nutrient content, % DM basis	
DM	63.2
CP	15.2
TDN	90.3
NDF	18.5
ADF	7.4
Fat	4.6
Ca	0.69
P	0.48
S	0.29

[1]Cargill Inc., Minneapolis, MN.

[2]Formulated to provide each animal with 415 mg monensin, 85 mg tylosin, 167 mg vitamin E, 30,000 IU vitamin A, 3,000 IU vitamin D_3, 360 mg Zn, and 100 mg thiamine.

Health monitoring was performed daily by a veterinarian who recorded abnormal observations by exclusion, and, when required, provided oversight for concomitant therapies. No mortalities occurred for either diet throughout the duration of the study. Six cattle (5 CON, 1 RAC) were treated with 0.22 to 0.45 mg/kg ceftiofur hydrochloride (Excenel RTU; Zoetis, Florham Park, NJ) for infectious pododermatitis while on the study. All 6 cattle responded to treatment, remained in the study, and satisfied the 4-d withdrawal period prior to handling and shipment for slaughter with their cohorts.

Weather data (Table 2) for the growth performance period were obtained from a weather station located approximately 25 km from the feedlot using the National Oceanic and Atmospheric Administration database. On the days cattle were handled and slaughtered, ambient temperature and relative humidity were collected every 10 min using Veriteq data loggers (Vaisala Inc., Boulder, CO) at the feedlot, in the rear compartment of each trailer transporting cattle, and in the lairage area at the abattoir.

Growth Performance (Phase I)

Initial and final BW were recorded for individual animals using a common certified scale (Gallagher, Riverside, MO) during restraint in a hydraulic chute (Daniels Manufacturing, Ainsworth, NE). Dry matter intake was analyzed by dividing daily feed deliveries by the number of cattle in the pen and then multiplying by diet DM (0.632) to estimate daily DMI per animal. After the 28-d feeding period, the pen mean for initial

Table 2. Maximum, minimum, and mean ambient temperature (TA), relative humidity (RH), and temperature humidity index (THI) summarized for phases I and II[1]

	TA, °C			RH, %			THI[2]		
Site	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
Feedlot									
d −0 to d 29[3]	28.7	15.9	22.5	87.2	58.0	74.1	77.7	60.4	70.4
d 28[4]	32.7	13.1	22.9	90.9	34.4	60.0	79.2	55.7	68.2
d 29[4]	32.7	18.9	24.9	93.2	45.1	69.5	81.1	65.7	72.7
Truck[5]									
Group 1	27.2	22.2	23.9	62.2	54.1	58.6	75.1	68.9	71.0
Group 2	30.1	21.0	24.4	70.0	57.2	63.9	79.4	67.6	72.3
Group 3	26.1	21.6	23.6	83.9	61.4	76.3	74.4	69.3	72.2
Group 4	27.7	21.3	24.2	72.4	56.4	68.0	76.1	67.9	72.4
Lairage[6]									
d 28	39.4	24.9	34.2	68.1	32.3	41.3	86.8	73.5	81.6
d 29	35.9	25.8	31.0	71.9	44.8	55.6	85.1	75.1	80.2

[1]Daily weather data during the growth performance period (phase I) were obtained from a weather station located approximately 25 km from the feedlot using the National Oceanic and Atmospheric Administration (NOAA) database, and descriptive statistics were summarized daily. Then, the maximum, minimum, and mean were calculated using the mean over the entire period. Weather data collected on d 28 and 29 (phase II) were collected every 10 min using Veriteq data loggers (Vaisala Inc., Boulder, CO).

[2]THI was calculated using the same equation as that in Mader et al. (2006), where THI = (0.8 × TA) + [(RH × 0.01) × (TA − 14.4)] + 46.4.

[3]RH was not reported in the NOAA database for phase I; therefore, RH was calculated using the equation of Lawrence (2005), where RH = 100 ^ {[(17.625 × TD)/(243.04+TD)]/[(17.625 × TA)/(243.04+TA)]}; TD = dew point temperature.

[4]Data were collected at the feedlot between 0600 and 1300 h.

[5]Data were collected on the trucks from loading at the feedlot until unloading at the abattoir. Groups 1 and 2 (block 1) were transported on d 28, and groups 3 and 4 (block 2) were transported on d 29.

[6]Data were collected in the lairage area at the abattoir between 1200 and 2000 h on d 28 and 29.

and final BW was used for calculation of ADG, and G:F was calculated as a quotient of ADG divided by DMI.

Handling, Transportation, and Lairage Procedures (Phase II)

Cattle were handled and slaughtered using a model similar to that of Hagenmaier et al. (2017), in which cattle were handled for 1,600 m using 2 HI treatments before transport to the abattoir.

Baseline. Cattle were removed from their home pens beginning at 0600 h, sorted into the predetermined HI groups, and handled using a bud box system before being restrained for recording of final BW and baseline measurements with the same scale and hydraulic scale used at enrollment. Baseline procedures were performed following the procedures described by Hagenmaier et al. (2017) and included measurement of vital parameters (rectal temperature [**RT**], heart rate [**HR**], and respiratory rate [**RR**]), venous blood collection, mobility scoring, behavioral scoring, and observations for physical indicators of stress. All cattle were handled in a low-stress manner without electric prod use during baseline procedures, and were allowed a minimum of 1 h rest between baseline restraint and initiation of HI treatments.

Posthandling. Replicates were handled between 0800 and 1200 h based on order of pen location, beginning at the southernmost end, and HI treatment groups within a replicate were handled consecutively to prevent circadian bias. The order of HI treatments was determined randomly for the first replicate and then alternated each subsequent replicate. Immediately after handling, cattle were restrained in the same facility used for baseline measurements, and vital parameters, venous blood collection, and behavioral observations were repeated (POSTHAND). As part of the handling model, HSH cattle received a single, approximately 1-s impulse of an electric prod while in the alley immediately before POSTHAND restraint. After completion of POSTHAND procedures, HI groups within a replicate were kept separate and allowed to rest in concrete-floored pens until loading for transport to the abattoir.

Transport. After completion of POSTHAND procedures for 2 consecutive replicates (32 cattle total; 16 per diet and sex), cattle were loaded by HI treatment within replicates onto 16.2 × 2.6 m aluminum semitrailers. During loading, HSH cattle received another impulse from an electric prod on the hip while ascending the load-out ramp. The HI treatment groups representing a replicate were commingled inside the trailer and shipped together in the deck or belly compartment to prevent confounding HI treatment by trailer compartment. The trailer compartments measured 8.8 × 2.6 m and transport floor space was standardized at 1.5 m^2/animal, which is similar to the deck and belly compartment stocking densities within the industry according to a survey of livestock transport carriers (González et al., 2012a). Cattle were transported approximately 100 km to the abattoir in accordance with the National Cattlemen's Beef Association Beef Quality Assurance guidelines (National Cattlemen's Beef Association, 2007). The mean time cattle spent on the trailer was 1 h 45 min.

Lairage. Upon arrival at the abattoir, cattle were unloaded on a concrete dock and walked to lairage pens with sloping, slated concrete floors to rest until being slaughtered later the same day. Cattle were handled without the use of electric prods and were provided water ad libitum throughout lairage according to the abattoir's standard operating procedures. Mobility scores were recorded for all cattle while they were being moved from the unloading dock to the lairage pens (POSTTRANS). Block 1 cattle were individually restrained in a side chute for POSTTRANS RT measurements, venous blood sampling, and behavioral observations before being returned to lairage pens for housing until slaughter. The mean time cattle spent in lairage was approximately 6 h.

Slaughter. All cattle enrolled in the study passed USDA ante-mortem inspection before being removed from lairage pens and humanely euthanized using a captive bolt gun in accordance with the abattoir's standard operating procedures. At the time of euthanasia, slaughter sequence was recorded using the study ear tags so that blood samples collected during exsanguination could be identified and cross-matched with numbers assigned by the plant for evaluation of carcass characteristics. Carcasses were ribbed between the 12th and 13th ribs, and grading was performed according to the USDA Agricultural Marketing Service standards. The following carcass characteristics were evaluated: HCW, LM area, 12th rib fat thickness, and marbling score. Dressing percentage was calculated by adjusting the final BW for a 4% shrink (BW × 0.96) and dividing the HCW by the adjusted BW.

Mobility Scores and Behavioral Observations

Mobility scoring and behavioral observations were performed at baseline, POSTHAND, and POSTTRANS by trained observers blinded to diet and HI treatments using the same scoring systems and definitions as Hagenmaier et al. (2017). In addition to the previously mentioned time points, a final mobility score was recorded in the lairage pen approximately 1 h before slaughter. To maintain blinding to HI, observers at the feedlot remained in the restraining facility, from which the handling course was not visible. Mobility, temperament, and chute exit scores were each determined using 4-point systems. Mobility scores were assigned as: 1 = normal, walks easily with no apparent lameness or change in gait; 2 = keeps up with normal cattle when the group is walking, but exhibits 1 or more of the following: stiffness, shortened stride, or slight limp; 3 = lags behind normal cattle when the group is walking, and exhibits 1 or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; and 4 = extremely reluctant to move, even when encouraged by handlers (North American Meat Institute [NAMI], 2015). Temperament scores were determined during chute restraint, and defined as 1 = calm, no movement; 2 = restless shifting; 3 = continuous squirming and shaking of the chute; and 4 = rearing, twisting, continuous violent struggle. Finally, chute exit scores were assigned as 1 = walk, 2 = trot, 3 = run, and 4 = jump. Physical indicators of stress were defined as dichotomous outcomes and included open-mouth breathing, vocalization, and muscle tremors.

Blood Collection and Assays

Venous blood samples were collected via jugular venipuncture at baseline, POSTHAND, and POSTTRANS and transferred into 10-mL serum separator, 158-USP lithium-heparin, and K_2EDTA tubes (Becton, Dickinson and Co., Franklin Lakes, NJ). Before centrifugation, whole blood in lithium-heparin tubes was assayed for pH, partial pressure of carbon dioxide (**pCO_2**), partial pressure of oxygen (**pO_2**), base excess, total carbon dioxide (**TCO_2**), and saturated oxygen (**sO_2**) using CG4+ cartridges with the iSTAT Clinical Analyzer system (iSTAT Corp., Princeton, NJ). Mixed venous and arterial blood samples collected during exsanguination were obtained using 50-mL centrifuge tubes and transferred into K_2EDTA and serum separator tubes. Therefore, iSTAT procedures were not performed on these blood samples because of exposure to atmospheric gases during collection. Whole blood in K_2EDTA and lithium-heparin tubes was centrifuged in a 4°C refrigerated centrifuge at 1,400 × *g* for 15 min as soon as feasible following each respective collection. Blood in serum separator tubes was allowed to sit for a minimum of 35 min to allow clot formation and then centrifuged at the same temperature, speed, and duration as blood in K_2EDTA and lithium-heparin tubes. Supernatants from blood samples collected at the feedlot were transferred into cryovials after centrifugation and directly placed in −80°C storage. Cryovials containing supernatants from samples collected at the abattoir were placed on dry ice before being transferred and stored with the remaining samples at −80°C until assays were performed.

Plasma from K_2EDTA tubes was assayed for catecholamines, and serum was assayed for lactate, cortisol, and full chemistry panels. The catecholamines were assayed in duplicate using a commercially available RIA kit (2-CAT RIA, IBL America, Minneapolis, MN). Serum was assayed in singlet for lactate using a Nova

CCX analyzer (Nova CCX analyzer, Nova Biomedical, Waltham, MA) and full serum chemistry panels to analyze potassium, glucose, bicarbonate, and CK using a Cobas c501 analyzer (Roche Diagnostics, Indianapolis, IN). Cortisol concentrations were assayed in duplicate using serum with a solid-phase competitive chemiluminescent immunoassay and an automated analyzer system (IMMULITE 1000 Cortisol, Siemens Medical Solutions Diagnostics, Los Angeles, CA). The intra-assay CV for epinephrine, norepinephrine, and cortisol were 16.5%, 14.7%, and 5.7%, respectively.

Statistical Analysis

Data were analyzed using version 9.3 of SAS (SAS Inst. Inc., Cary, NC).

Phase I. Pen was considered the experimental unit for the growth performance period ($n = 8$ pens per diet), and therefore, the pen mean was calculated and used for analysis of all variables. Continuous variables were analyzed with a linear mixed effects model using the GLIMMIX procedure where diet was included as a fixed effect and block and replicate were included as random effects. Initial BW, final BW, ADG, and G:F analyses were performed on nonadjusted values, whereas dressing percentage was calculated using an adjusted final BW in which the original value was multiplied by 0.96 to adjust for 4% shrinkage.

Phase II. Responses to handling and transportation were analyzed as a split-plot design in which HI was the main plot and diet was the subplot. Handling groups of 8 cattle were considered the experimental unit for the whole plot effect of HI ($n = 8$ groups per HI), and the subgroups of 4 cattle representing each diet were considered the experimental unit for the subplot ($n = 16$ groups per diet). Cattle exhibiting physical signs of stress such as vocalization, muscle tremors, or open-mouth breathing and receiving mobility, temperament, or chute exit scores greater than 1 were considered abnormal events. These data were not normally distributed, and therefore nonparametric analyses were performed using the GENMOD procedure in SAS. The diet × HI interaction could not be tested for multiple variables analyzed with nonparametric models because of zero observed events within diet × HI treatment subclass categories.

Continuous variables were summarized by calculating the mean of the appropriate experimental unit (i.e., group of 8 for HI or subgroup of 4 for diet) and then analyzed with a linear mixed model using the GLIMMIX procedure. The statistical model included the fixed effects of diet, HI, and the diet × HI interaction, and the random effects of replicate and the replicate × HI interaction. The HI × replicate interaction was used as the error term to test for the effects of HI, and the residual error was used as the error term to test for the effects of diet. To normalize the data, CK concentrations were logarithmically transformed for all analyses and back-transformed for reporting purposes. For variables with insignificant diet × HI interactions ($P > 0.05$), the interaction term was sequentially removed, and the reduced model was used for treatment estimates.

The effect of sex was not included in the model because single-sex replicates were utilized. However, variation due to sex is accounted for by including replicate as a random effect in the model. Treatment means were estimated using the LSMEANS statement and compared using two-sided Student's t tests with the PDIFF option. Statistically significant differences were determined by $P \leq 0.05$, and tendencies were declared when $0.06 \leq P \leq 0.10$.

Table 3. Least squares means for the effects of ractopamine hydrochloride (RAC) on growth performance and carcass characteristics of beef cattle

	Diet[1]			
Variable	CON	RAC	SEM[2]	P-value[3]
Growth performance				
Initial BW, kg	549	549	19.0	0.93
Final BW, kg	588	596	24.3	0.11
DMI, kg	8.43	8.62	0.493	0.11
ADG, kg	1.32	1.60	0.193	0.06
G:F, kg:kg	0.15	0.18	0.013	0.06
Carcass characteristics				
HCW, kg	356	363	14.2	0.04
Dressing percentage[4]	63.1	63.4	0.26	0.49
LM area, cm^2	87.9	91.7	2.33	0.04
12th rib fat thickness, cm	1.18	1.11	0.073	0.34
Marbling score[5]	451	455	12.7	0.78

[1]CON: no β-adrenergic agonist; RAC: 400 $mg \cdot animal^{-1} \cdot d^{-1}$ ractopamine hydrochloride for 28 d.

[2]SEM = largest SE in the analysis; $n = 8$ for CON and RAC groups.

[3]Statistical significance was declared for $P \leq 0.05$, and tendencies were declared when $0.06 \leq P \leq 0.10$.

[4]Final BW was adjusted for 4% shrinkage before calculation and statistical analysis of dressing percentage.

[5]Evaluated in the LM between the 12th and 13th ribs; Slight = 300, Small = 400, Modest = 500.

RESULTS

Effects of RAC on Growth Performance and Carcass Characteristics (Phase I)

Cattle fed RAC tended to have greater ADG and G:F ($P = 0.06$; Table 3); however, there was no effect of diet on DMI or final BW ($P = 0.11$). Furthermore, RAC cattle had greater HCW and LM area ($P = 0.04$), whereas no effect of RAC was observed for dressing

Table 4. Effects of ractopamine hydrochloride (RAC) and handling intensity (HI) on percentage of cattle receiving mobility, temperament, and chute exit scores greater than 1 and exhibiting physical indicators of stress at each time point[1]

	Diet[2]			HI[2]		
Variable, %	CON	RAC	P-value[3]	LSH	HSH	*P*-value[3]
Baseline[4] (*n* = 64 per treatment)						
Mobility score[5]	1.6	0.0	1.00	1.6	0.0	1.00
Temperament score	18.8	12.5	0.37	17.1	14.1	0.65
Chute exit score	34.4	28.1	0.53	29.7	32.8	0.75
Vocalization	9.4	6.3	0.53	6.3	9.4	0.53
Muscle tremors[5]	0.0	0.0	1.00	0.0	0.0	1.00
Open-mouth breathing[5]	0.0	0.0	1.00	0.0	0.0	1.00
POSTHAND[4] (*n* = 64 per treatment)						
Mobility score[5]	1.5	4.7	1.00	6.3	0.0	1.00
Temperament score	9.3	7.8	0.76	3.1	14.1	0.03
Chute-exit score	18.8	31.2	0.16	20.3	29.6	0.28
Vocalization[5]	4.7	4.7	1.00	1.6	7.8	0.09
Muscle tremors[5]	0.0	0.0	1.00	0.0	0.0	1.00
Open-mouth breathing[5]	0.0	3.1	1.00	1.6	1.6	1.00
POSTTRANS[4] (*n* = 32 per treatment)						
Mobility score[5,6]	6.3	21.9	0.09	25.0	3.1	0.01
Temperament score	50	46.9	0.88	40.6	56.3	0.37
Chute-exit score	43.8	40.6	0.61	62.5	21.9	0.04
Vocalization[5]	3.1	15.6	0.08	6.3	12.5	0.38
Muscle tremors	21.9	12.5	0.37	21.9	12.5	0.37
Open-mouth breathing[5]	0.0	6.3	1.00	3.1	3.1	1.00
Lairage[4] (*n* = 64 per treatment)						
Mobility score	23.4	17.1	0.43	26.5	14.0	0.11

[1]Mobility, temperament, and chute exit scores were assigned by observers blinded to diet and HI using 4-point systems (see Materials and Methods).

[2]Treatments were assigned in a 2 × 2 factorial arrangement with a split-plot design consisting of the following treatments: 1) diet, with no β-adrenergic agonist (CON) vs. 400 $mg \cdot animal^{-1} \cdot d^{-1}$ ractopamine hydrochloride for 28 d (RAC), and 2) handling intensity (HI) over a 1,500-m course on the day of slaughter: Low-stress handling (LSH) – cattle kept at a walk, vs. High-stress handling (HSH) – cattle kept at a minimum of a trot. The whole plot was HI and diet was the subplot.

[3]Values reported are arithmetic means, whereas the *P*-values signify differences in least squares means for the probability of cattle receiving a score greater than 1 or exhibiting the clinical sign. Statistical significance was declared for $P \leq 0.05$, and tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.

[4]Baseline observations were recorded a minimum of 1 h before HI treatment, and POSTHAND observations were recorded immediately after HI treatment. The POSTTRANS observations were recorded immediately after unloading from semitrailers at the abattoir, and lairage observations were made approximately 1 h before slaughter at the abattoir.

[5]The diet × HI interaction could not be tested due to 0 observed events within at least 1 treatment interaction subclass.

[6]POSTTRANS mobility scores were assigned to both blocks of cattle, whereas other POSTTRANS variables only represent block 1 cattle (*n* = 32 per treatment).

percentage, 12th rib fat thickness, or marbling score ($P \geq 0.34$).

Qualitative Scoring and Physical Indicators of Stress

Mobility Scores. The proportion of cattle with POSTTRANS mobility scores >1 was greater in LSH cattle ($P = 0.01$; Table 4) and tended ($P = 0.09$) to be greater in RAC cattle compared to their LSH and CON cohorts, respectively. On the contrary, no differences were observed between diets or HI on mobility scores at baseline, POSTHAND, or lairage ($P \geq 0.11$).

Behavioral Scoring and Physical Indicators of Stress. During POSTHAND procedures, HSH cattle had more temperament scores >1 ($P = 0.03$; Table 4) and tended to have a greater number of cattle vocalize ($P = 0.09$). Cattle from the LSH treatment had a greater number of POSTTRANS chute exit scores >1 ($P = 0.04$), and POSTTRANS vocalizations tended to be greater in RAC cattle ($P = 0.08$). There was no effect of RAC or HI on open-mouth breathing or muscle tremors at any time point ($P \geq 0.37$).

Physiological Response to Handling and Transportation

Baseline. There were no differences between LSH and HSH cattle at baseline for any vital parameter or blood variable ($P \geq 0.19$; Table 5). However, there appeared to be an effect of diet as CON cattle had greater RR and HR, greater blood lactate, pO_2, sO_2, and epinephrine ($P < 0.05$), and tended to have greater RT

Table 5. Least squares means for the effects of ractopamine hydrochloride (RAC) and handling intensity (HI) on baseline vital parameters and blood variables of beef cattle on the day of slaughter[1]

	Diet[2]				HI[2]			
Variable	CON	RAC	SEM[3]	*P*-value[4]	LSH	HSH	SEM[3]	*P*-value[4]
Weight, kg	588	596	3.1	0.09	590	595	3.0	0.21
Vital parameters								
Respiratory rate,[5] rpm	42.1	40.0	1.33	0.04	41.1	41.0	1.33	0.97
Heart rate,[5] bpm	115.3	105.3	7.83	0.02	109.9	111.8	7.88	0.84
Rectal temperature, °C	39.3	39.1	0.06	0.07	39.2	39.2	0.06	0.56
Blood variables								
Lactate, mmol/L	5.4	4.1	0.39	0.001	4.8	4.7	0.39	0.89
pH	7.42	7.43	0.010	0.19	7.43	7.42	0.010	0.51
HCO_3,[6] mmol/L	23.7	24.6	0.28	<0.01	24.2	24.2	0.28	0.92
pCO_2,[6] mmHg	43.2	44.2	0.68	0.10	43.6	43.8	0.68	0.71
TCO_2,[6, 7] mmol/L	29.3	30.4	0.36	0.02	29.9	29.8	0.36	0.81
pO_2,[6] mmHg	36.7	33.1	1.28	<0.01	35.0	34.8	1.27	0.83
sO_2,[6] %	69.1	64.0	2.10	0.03	67.3	65.7	2.10	0.49
Base excess,[7] mmol/L	3.6	4.8	0.48	0.02	4.34	4.09	0.48	0.61
Epinephrine, pg/mL	428	273	59.3	0.02	335	366	64.6	0.71
Norepinephrine, pg/mL	641	614	75.7	0.59	627	628	78.9	0.99
Cortisol, ng/mL	30.7	29.7	1.89	0.69	31.9	28.5	1.89	0.19
Creatine kinase,[8] U/L	255	232	80.8	0.67	220	268	85.3	0.39
Glucose, mg/dL	101	93	3.8	0.08	98	97	4.0	0.73
Potassium, mmol/L	5.02	5.24	0.068	0.01	5.14	5.12	0.068	0.84

[1]Baseline procedures were performed a minimum of 1 h before HI treatment application.

[2]Treatments were assigned in a 2 × 2 factorial arrangement with a split-plot design consisting of the following treatments: 1) diet, with no β-adrenergic agonist (CON) vs. 400 mg·animal^{-1}·d^{-1} ractopamine hydrochloride for 28 d (RAC), and 2) handling intensity (HI) over a 1,500-m course on the day of slaughter; Low-stress handling (LSH) – cattle kept at a walk, vs. High-stress handling (HSH) – cattle kept at a minimum of a trot. The whole plot was HI and diet was the subplot.

[3]SEM = largest SE in the analysis; n = 16 for CON and RAC groups; n = 8 for LSH and HSH groups.

[4]Statistical significance was declared for $P \leq 0.05$, and tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.

[5]rpm = respirations per minute; bpm = beats per minute.

[6]HCO_3 = bicarbonate; pCO_2 = partial pressure of carbon dioxide; TCO_2 = total carbon dioxide; pO_2 = partial pressure of oxygen; sO_2 = saturated oxygen.

[7]The diet × HI interaction tended ($P \leq 0.07$) to be significant and was included in the final model for treatment estimates.

[8]Statistical analysis was conducted on log-transformed values, and treatment estimates were back-transformed for reporting purposes.

($P = 0.07$) and glucose ($P = 0.08$) than cattle fed RAC. Control cattle also had lower K^+, HCO_3, TCO_2, and base excess ($P < 0.05$) and tended to have lower pCO_2 ($P = 0.10$).

Posthandling. Mean time to complete the 1,500-m handling course for HSH and LSH cattle was 10.0 and 20.5 min, respectively (data not shown). The mean time to complete the course was 15.3 min for cattle from both diets ($P = 1.00$), which was expected because diet represented subplots within HI whole plots to eliminate variation in HI treatment application.

There tended to be a diet × HI interaction on the change in epinephrine concentrations from baseline to POSTHAND ($P = 0.06$; Fig. 2) as RAC cattle had a greater increase than CON cattle for the change in HSH but not LSH cattle. Handling intensity had a profound effect on the change from baseline to POSTHAND measurements for several other variables, as HSH cattle had greater changes from baseline to POSTHAND in HR, lactate, pH, HCO_3, pCO_2, TCO_2, base excess, norepinephrine, cortisol, and glucose compared to LSH cattle ($P \leq 0.01$; Table 6). However, HI did not affect the change from baseline to POSTHAND for RR, RT, pO_2, sO_2, CK, or potassium ($P \geq 0.21$). Compared to CON, cattle fed RAC had greater increases from baseline to POSTHAND in RR and norepinephrine, in addition to greater decreases in pH ($P \leq 0.05$). Otherwise, there was no effect of RAC on the changes from baseline to POSTHAND for the remaining blood variables and vital parameters ($P \geq 0.12$).

Posttransport. There was a diet × HI interaction ($P = 0.01$) on the change in cortisol from baseline to POSTTRANS (Fig. 3), as HSH increased the change in cortisol for RAC but not CON cattle. There tended ($P = 0.07$) to be a similar diet × HI interaction for changes in CK in which HSH only increased the change in CK in RAC cattle (Fig. 4). Changes in HCO_3 ($P = 0.06$) and norepinephrine ($P = 0.07$) concentrations tended to be greater for CON cattle (Table 7), otherwise neither HI nor diet had an effect on the change from baseline to POSTTRANS in RT or the remaining blood variables ($P \geq 0.11$).

Slaughter. Feeding RAC and HSH both increased the overall change in CK from baseline to slaughter ($P < 0.01$; Table 8). There was no effect of diet or HI on any other blood variable measured in samples collected during exsanguination.

DISCUSSION

Beta-adrenergic agonists such as RAC and ZIL function through a mode of action whereby nutrients are diverted away from adipose tissue and towards increased lean tissue accretion through increased lipolysis and protein synthesis and decreased lipogenesis and protein degradation (Mersmann, 1998; Quinn et al., 2008; Strydom et al., 2009). According to a recent survey by Samuelson et al. (2016) encompassing 24 feedlot nutritionists who collectively service over 14 million fed cattle annually, 84.8% of feedlots consulted by those nutritionists administer βAA to their finishing cattle. Of the feedlots utilizing βAA, 95.5% fed RAC, with 28 d being the most common feeding duration, similar to the feeding program utilized during phase I of the current study.

Although this study was not statistically powered to detect differences in performance or carcass characteristics, RAC improved ADG by 21.2% and feed efficiency by 20%, increased HCW by 7 kg, and increased LM area by 4 cm^2. These findings are in alignment with the βAA meta-analysis performed by Lean et al. (2014), which included over 50 comparisons involving RAC vs. no βAA and concluded RAC consistently improves growth performance and leads to greater HCW and LM area. Given this, the performance results of the present study suggest cattle displayed the expected response to RAC administration.

Thomson et al. (2015) reported an event which occurred in 2013 where cattle that had been fed ZIL were visually distressed, had difficulties walking, sloughed hoof walls and had to be euthanized while in lairage at the abattoir. In response to these events and other coeval reports from abattoirs suggesting an increased prevalence of cattle without obvious disease or injury becoming reluctant to move, a major U.S. packer decided to stop accepting cattle fed ZIL. Shortly thereafter, Merck Animal Health (Desoto, KS) announced a self-imposed suspension of ZIL sales in the U.S. and Canadian markets to investigate the matter (Lyles and Calvo-Lorenzo, 2014). Although similar events have not been reported in cattle fed RAC, the prospect of losing βAA to improve feed utilization and meat yield in food animals poses a significant threat to the advancement of production agriculture (Lyles and Calvo-Lorenzo, 2014). Furthermore, this condition, termed FCS within the beef industry

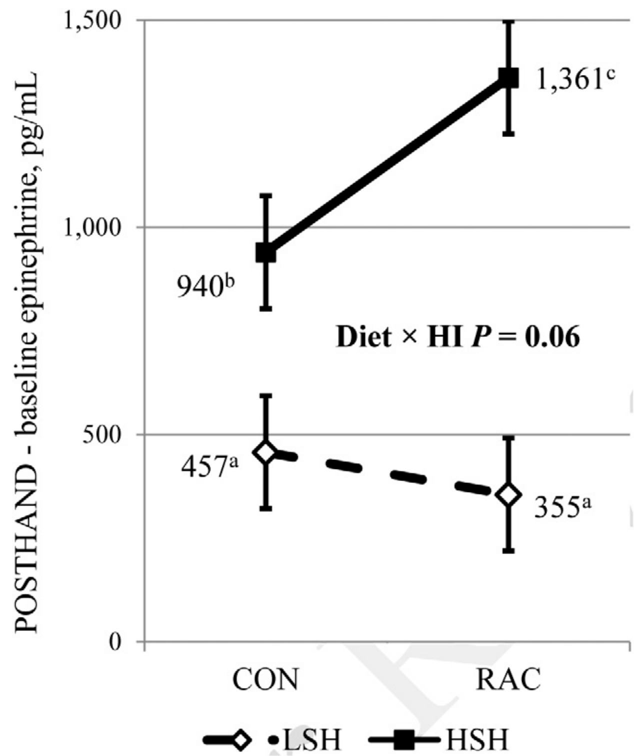

Figure 2. Diet × handling intensity (HI) interaction for the change in epinephrine concentrations from baseline to POSTHAND. Treatments were assigned in a 2 × 2 factorial arrangement with a split-plot design consisting of the following treatments: 1) Diet: CON – no β-agonist vs. RAC – 400 mg·animal-1·d-1 ractopamine hydrochloride for 28 d, and 2) Handling intensity (HI) over a 1,500 m course on the day of slaughter: Low-stress handling (LSH) – cattle kept at a walk, vs. High-stress handling (HSH) – cattle kept at a minimum of a trot. The whole plot was HI, and diet was the subplot. Samples were obtained a minimum of 1 h before HI treatment (baseline) and immediately after completion of the handling course (POSTHAND). [a,b]Means without common superscripts differ ($P \leq 0.05$).

(Thomson et al., 2015), is believed to be multifactorial, and other factors proposed to play a role include heat stress, aggressive handling, heavy muscling, increasing slaughter weights, subclinical laminitis, long-haul transport, and temperature and duration of lairage (Boyd et al., 2015; Thomson et al., 2015). Although anecdotal reports implicate the use of βAA, there are no data to either confirm or refute this supposition.

Shortly after the approval of RAC in swine, reports surfaced proposing increased rates of nonambulatory pigs, and a caution statement was added to the U.S. Paylean label (FDA, 2002). Research conducted in swine demonstrated that metabolic acidosis and elevated stress hormones are major determinants for the vast majority of nonambulatory pigs classified as fatigued without obvious disease or injury (Anderson et al., 2002; Ritter et al., 2009a). Fatigued pigs display signs of acute stress such as open-mouth breathing, skin discoloration, and muscle tremors, and are in a state of metabolic acidosis indicated by elevated blood lactate and decreased pH (Benjamin et al., 2001; Anderson et al., 2002; Ivers et al., 2002). Dead and nonambulatory

Table 6. Least squares means for the effects of ractopamine hydrochloride (RAC) and handling intensity (HI) on the change in physiological measurements and blood variables of beef cattle from baseline to after HI treatments (POSTHAND) on the day of slaughter[1]

	Diet[2]				HI[2]			
Variable	CON	RAC	SEM[3]	*P*-value[4]	LSH	HSH	SEM[3]	*P*-value[4]
Vital parameters								
Respiratory rate,[5] rpm	9.8	12.7	1.85	0.02	10.0	12.5	1.99	0.21
Heart rate,[5] bpm	17.3	17.8	7.49	0.89	6.5	28.6	8.01	0.01
Rectal temperature, °C	0.54	0.64	0.074	0.30	0.55	0.63	0.080	0.51
Blood variables								
Lactate, mmol/L	2.1	3.0	0.75	0.26	−1.7	6.8	0.77	<0.0001
pH	0.01	−0.02	0.017	0.04	0.06	−0.07	0.019	<0.001
HCO_3,[6] mmol/L	−2.1	−2.9	0.76	0.19	1.4	−6.4	0.81	<0.0001
pCO_2,[6] mmHg	−6.0	−6.1	0.81	0.83	−4.4	−7.7	0.85	<0.01
TCO_2,[6] mmol/L	−3.0	−4.0	0.82	0.14	1.2	−8.2	0.92	<0.0001
pO_2,[6] mmHg	1.1	3.6	1.25	0.15	1.4	3.2	1.33	0.37
sO_2,[6] %	2.3	3.7	2.14	0.61	4.5	1.5	2.24	0.38
Base excess, mmol/L	−2.6	−4.1	1.04	0.12	2.2	−8.9	1.14	<0.0001
Epinephrine,[7] pg/mL	699	858	103.9	0.23	406	1151	103.9	<0.001
Norepinephrine, pg/mL	522	716	108.4	0.05	346	892	109.6	<0.001
Cortisol, ng/mL	10.3	11.6	1.50	0.54	3.7	18.2	1.50	<0.001
Creatine kinase,[8] U/L	267	307	133.2	0.68	258	317	112.3	0.62
Glucose, mg/dL	47	55	6.7	0.42	−2	104	6.7	<0.0001
Potassium, mmol/L	−0.03	−0.01	0.096	0.82	−0.04	0.00	0.096	0.75

[1]Change = POSTHAND value − baseline value.

[2]Treatments were assigned in a 2 × 2 factorial arrangement with a split-plot design consisting of the following treatments: 1) diet, with no β-adrenergic agonist (CON) vs. 400 $mg \cdot animal^{-1} \cdot d^{-1}$ ractopamine hydrochloride for 28 d (RAC), and 2) handling intensity (HI) over a 1,500-m course on the day of slaughter; Low-stress handling (LSH) – cattle kept at a walk, vs. High-stress handling (HSH) – cattle kept at a minimum of a trot. The whole plot was HI and diet was the subplot.

[3]SEM = largest SE in the analysis; n = 16 for CON and RAC groups; n = 8 for LSH and HSH groups.

[4]Statistical significance was declared for $P \leq 0.05$, and tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.

[5]rpm = respirations per minute; bpm = beats per minute.

[6]HCO_3 = bicarbonate; pCO_2 = partial pressure of carbon dioxide; TCO_2 = total carbon dioxide; pO_2 = partial pressure of oxygen; sO_2 = saturated oxygen.

[7]The diet × HI interaction tended to be significant for epinephrine (P = 0.06; Fig. 2) and was included in the final statistical model.

[8]Statistical analysis was conducted on log-transformed values, and treatment estimates were back-transformed for reporting purposes.

pigs due to FPS are a multifactorial issue involving, but not limited to, improper handling, inappropriate trailer stocking densities, and poorly designed facilities where pigs are required to travel far distances and ascend steep loading ramps (Benjamin et al., 2001; Anderson et al., 2002; Ritter et al., 2009b). Additionally, studies have suggested the effect of RAC on physiological responses to handling can be dependent on handling methods (James et al., 2013). Because of the similar clinical presentations and serum biochemical abnormalities of FPS and FCS, this study was designed to examine the effects of RAC and HI on the stress responses and mobility status of cattle throughout the marketing channel from the feedlot to the abattoir.

Generally speaking, impaired mobility can be attributed to either fatigue or lameness. For that reason, NAMI recently adopted a new mobility scoring system designed to evaluate fatigued cattle (NAMI, 2015), and compliment traditional scoring systems such as the Zinpro Step-Up Locomotion Scoring System primarily used for determining the grade of lameness (Zinpro, 2014). This new scoring system takes into account signs of fatigue such as reluctance to move, inability to keep up with contemporaries, and responsiveness to handlers. A June 2016 industry report by Elanco Animal Health assessing mobility at arrival to the abattoir on over 200,000 U.S. market cattle with the same scoring system as our study revealed that 8.5% of cattle received a score of 2 or greater (M. Genho, Elanco Animal Health, Greenfield, IN, personal communication). In the current study, 7.1% of cattle had mobility scores of 2 or greater at POSTTRANS, which increased to 20.3% after lairage.

The findings of the current study demonstrate feeding RAC did not adversely affect mobility at the feedlot or after 6 h of lairage at the abattoir. Although no published studies have reported mobility scores in cattle fed RAC compared to cattle not fed βAA, our findings are supported by others who found ZIL did not impact cattle mobility at the feedlot (Bernhard et al., 2014; Burson, 2014; Boyd et al., 2015). Still, it is important to note that mobility scores worsened after transportation and lairage regardless of treatment, which is in

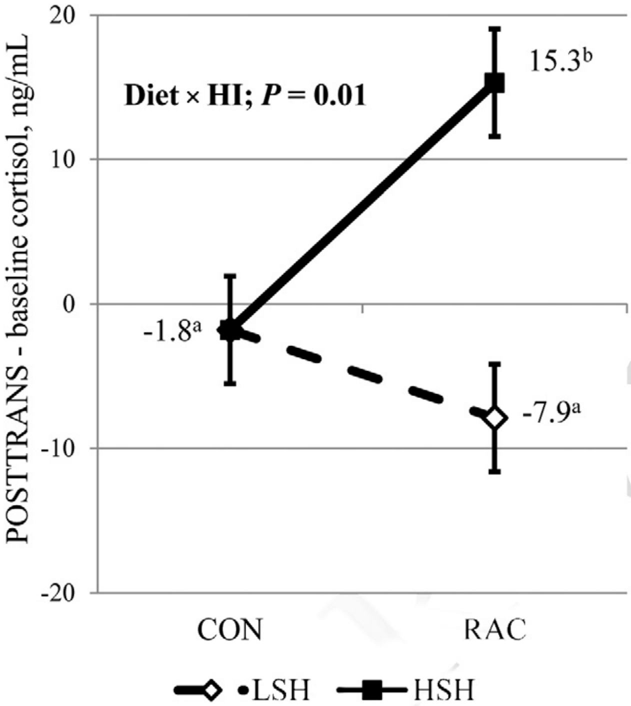

Figure 3. Diet × handling intensity (HI) interaction for the change in cortisol concentrations from baseline to POSTTRANS. Treatments were assigned in a 2 × 2 factorial arrangement with a split-plot design consisting of the following treatments: 1) Diet: CON – no β-agonist vs. RAC – 400 mg·animal-1·d-1 ractopamine hydrochloride for 28 d, and 2) Handling intensity (HI) over a 1,500 m course on the day of slaughter: Low-stress handling (LSH) – cattle kept at a walk, vs. High-stress handling (HSH) – cattle kept at a minimum of a trot. The whole plot was HI, and diet was the subplot. Samples were obtained a minimum of 1 h before HI treatment and immediately after approximately 100 km transport to the abattoir (POSTTRANS). [a,b]Means without common superscripts differ ($P \le 0.05$).

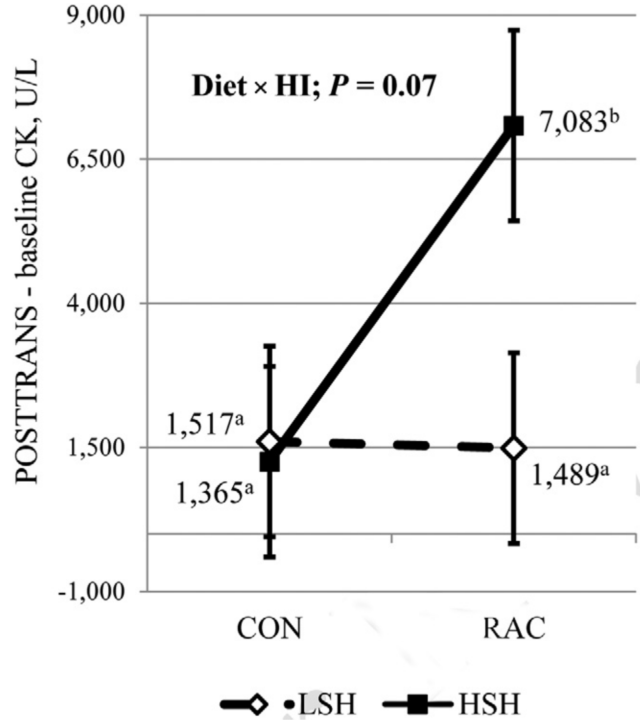

Figure 4. Diet × handling intensity (HI) interaction for the change in creatine kinase (CK) concentrations from baseline to POSTTRANS. Treatments were assigned in a 2 × 2 factorial arrangement with a split-plot design consisting of the following treatments: 1) Diet: CON – no β-agonist vs. RAC – 400 mg·animal-1·d-1 ractopamine hydrochloride for 28 d, and 2) Handling intensity (HI) over a 1,500 m course on the day of slaughter: Low-stress handling (LSH) – cattle kept at a walk, vs. High-stress handling (HSH) – cattle kept at a minimum of a trot. The whole plot was HI, and diet was the subplot. The whole plot was HI, and diet was the subplot. Samples were obtained a minimum of 1 h before HI treatment and immediately after approximately 100-km transport to the abattoir (POSTTRANS). [a,b]Means without common superscripts differ ($P \le 0.05$).

agreement with multiple recent studies that reported the proportion of cattle with compromised mobility increased with progression through the marketing channel (Boyd et al., 2015; Hagenmaier et al., 2017).

The reason why prevalence of abnormal mobility scores was greater in LSH cattle after transport in the current study is not fully understood. These cattle were screened for lameness at enrollment and before HI treatment; therefore, changes in mobility are likely due to fatigue or an injury acquired during transport to the abattoir. The former seems counterintuitive as one can assume fatigue would have been greater in cattle handled aggressively, yet no appreciable injuries were observed during mobility scoring at the abattoir and so this must be considered. Not only does this stimulate one to consider other sources of preslaughter stress at the feedlot which might predispose cattle to fatigue, but it also leads one to speculate that additional factors exist during transport and at the abattoir that contribute to an animal's willingness to respond to handling. In addition to long-haul transport and hot weather, González et al. (2012b) reported inappropriately high stocking densities in the deck compartment of trailers increased the prevalence of nonambulatory cattle, although this should not have been a factor in our study as cattle from each HI were commingled and consequently allotted the same amount of trailer space. Another element worthy of consideration is that multiple cattle with abnormal mobility scores during unloading at the abattoir did not have abnormal mobility scores after lairage. This may be indicative that subsets of cattle initially balked because of the unfamiliarity of slated concrete floors in the lairage area when, indeed, they had no mobility issue. Or, perhaps just as likely, the cattle were experiencing fatigue that was acute in nature and able to be overcome when cattle were allowed to rest in the lairage pens. In addition to HI and βAA, an array of other factors exists that warrants investigation because of their potential to impact mobility, including BW, musculoskeletal confirmation (genetics), subclinical disease (foot rot, laminitis, etc.), injury, and transport and lairage conditions. The impact of BW is of particular interest as the average live wt of fed cattle at slaughter increased 150 pounds from 1999 to 2015 (USDA, 2000 and 2016); however, it was not evaluated in the study being reported herein and will not be discussed further.

Table 7. Least squares means for the effects of ractopamine hydrochloride (RAC) and handling intensity (HI) on the change in rectal temperature and blood variables of beef cattle from baseline to after transport to the abattoir (POSTTRANS) on study d 28[1]

	Diet[2]				HI[2]			
Variable	CON	RAC	SEM[3]	*P*-value[4]	LSH	HSH	SEM[3]	*P*-value[4]
Vital parameters								
Rectal temperature, °C	0.37	0.64	0.250	0.31	0.38	0.62	0.250	0.41
Blood variables								
Lactate, mmol/L	1.6	2.4	0.95	0.27	0.8	3.2	1.10	0.16
pH	0.02	0.02	0.017	0.94	0.02	0.02	0.018	0.95
HCO_3,[5] mmol/L	2.3	1.2	0.51	0.06	2.2	1.2	0.64	0.34
pCO_2,[5] mmHg	−1.2	−2.4	1.29	0.50	−1.4	−2.2	1.29	0.62
TCO_2,[5] mmol/L	0.7	0.0	0.53	0.20	0.9	−0.2	0.62	0.26
pO_2,[5] mmHg	−4.9	−3.0	1.08	0.24	−4.9	−3.0	1.08	0.31
sO_2,[5] %	−6.2	−4.3	2.13	0.53	−7.2	−3.3	2.13	0.29
Base excess, mmol/L	1.0	0.4	0.66	0.30	1.3	0.1	0.81	0.37
Epinephrine, pg/mL	730	588	128.6	0.46	600	718	128.6	0.56
Norepinephrine, pg/mL	657	484	175.4	0.07	480	661	175.4	0.11
Cortisol,[6] ng/mL	−1.8	3.7	3.42	0.14	−4.9	6.7	3.42	0.07
Creatine kinase,[6,7] U/L	1,439	3,247	1,974.1	0.07	1,503	3,109	2,007.2	0.21
Glucose, mg/dL	9	12	6.4	0.62	7	14	6.4	0.28
Potassium, mmol/L	−0.21	−0.30	0.160	0.63	−0.19	−0.31	0.160	0.58

[1]Change = POSTTRANS value – baseline value.

[2]Treatments were assigned in a 2 × 2 factorial arrangement with a split-plot design consisting of the following treatments: 1) diet, with no β-adrenergic agonist (CON) vs. 400 $mg \cdot animal^{-1} \cdot d^{-1}$ ractopamine hydrochloride for 28 d (RAC), and 2) handling intensity (HI) over a 1,500-m course on the day of slaughterLow-stress handling (LSH) – cattle kept at a walk, vs. High-stress handling (HSH) – cattle kept at a minimum of a trot. The whole plot was HI and diet was the subplot.

[3]SEM = largest SE in the analysis; $n = 8$ for CON and RAC groups; $n = 4$ for LSH and HSH groups.

[4]Statistical significance was declared for $P \leq 0.05$, and tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.

[5]HCO_3 = bicarbonate; pCO_2 = partial pressure of carbon dioxide; TCO_2 = total carbon dioxide; pO_2 = partial pressure of oxygen; sO_2 = saturated oxygen.

[6]The diet × HI interaction was significant for cortisol ($P = 0.01$; Fig. 3) and tended to be significant for creatine kinase ($P = 0.07$; Fig. 4) and was therefore included in the final statistical model.

[7]Statistical analysis was conducted on log-transformed values, and treatment estimates were back-transformed for reporting purposes.

Although slight differences were present at baseline, our findings suggest feeding RAC for 28 d does not notably alter resting vital parameters and blood variables. The values reported at baseline were within normal reference ranges reported for cattle and comparable to 2 other recent studies in which blood was collected via jugular venipuncture from feedlot cattle that were either fed RAC (Hagenmaier et al., 2017) or no βAA (Frese et al., 2016). Furthermore, multiple studies have reported no differences in lactate concentrations or other acid-base measurements in cattle fed RAC for 28 d or ZIL for 20 d compared to cattle not fed a βAA (Abney et al., 2007; Van Bibber-Krueger et al., 2015; Hales et al., 2016). Hales et al. (2016) reported findings similar to our study in which cattle fed ZIL had decreased blood lactate and glucose concentrations, and they speculated reductions in glucose can be attributed to βAA causing a shift in metabolic substrate utilization from glucose to fatty acids in peripheral tissues as described by Eisemann et al. (1988). On the other hand, transient metabolic alterations characterized by greater blood lactate and glucose concentrations have been reported during the early phases of clenbuterol administration in young calves, suggesting skeletal muscle glycogenolysis is mediated by β-adrenoreceptors, although changes are diminished relatively quickly because of altered receptor affinity and density (Blum and Flueckiger, 1988; Eisemann et al., 1988; Bruckmaier and Blum, 1992). Reinhardt et al. (2014) observed that transient decreases in DMI after initiation of ZIL administration in beef cattle is most prominent during hot summer months, and speculate this may be related to metabolic alterations leading to accumulation of blood lactate. Nonetheless, the differences observed in these blood parameters in the current study were minor, and their biological significance is ambiguous.

Reports from packers indicate the frequency of lots with mobility-impaired cattle varies greatly across feedlots, regardless of βAA administration, and this warrants further investigation into various other factors occurring at the feedlot, particularly cattle handling (D. U. Thomson, Kansas State University, Manhattan, personal communication). The HSH cattle in the present study developed metabolic acidosis characterized by

Table 8. Least squares means for the effects of ractopamine hydrochloride (RAC) and handling intensity (HI) on the overall change in blood variables of beef cattle from baseline to slaughter blood collections[1]

	Diet[2]				HI[2]			
Variable	CON	RAC	SEM[3]	*P*-value[4]	LSH	HSH	SEM[3]	*P*-value[4]
Lactate, mmol/L	6.3	7.0	0.52	0.12	6.3	6.9	0.52	0.16
HCO_3,[5] mmol/L	2.3	2.8	0.31	0.19	2.7	2.3	0.32	0.29
Epinephrine, pg/mL	7,569	7,571	321.4	0.99	7,502	7,638	321.4	0.63
Norepinephrine, pg/mL	8,036	8,004	446.0	0.96	7,703	8,337	446.0	0.35
Cortisol, ng/mL	20.0	21.9	2.21	0.56	18.6	23.3	2.21	0.17
Creatine kinase,[6] U/L	2,157	4,077	1,278.1	<0.001	1,838	4,787	1,208.1	<0.01
Glucose, mg/dL	74	62	9.0	0.36	75	61	9.0	0.33
Potassium, mmol/L	2.13	1.94	0.150	0.21	2.1	1.9	0.150	0.30

[1]Change = slaughter value − baseline value.

[2]Treatments were assigned in a 2 × 2 factorial arrangement with a split-plot design consisting of the following treatments: 1) diet, with no β-adrenergic agonist (CON) vs. 400 mg·animal^{-1}·d^{-1} ractopamine hydrochloride for 28 d (RAC), and 2) handling intensity (HI) over a 1,500-m course on the day of slaughter: Low-stress handling (LSH) – cattle kept at a walk, vs. High-stress handling (HSH) – cattle kept at a minimum of a trot. The whole plot was HI and diet was the subplot.

[3]SEM = largest SE in the analysis; $n = 16$ for CON and RAC groups; $n = 8$ for LSH and HSH groups.

[4]Statistical significance was declared for $P \leq 0.05$, and tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.

[5]HCO_3 = bicarbonate.

[6]Statistical analysis was conducted on log-transformed values, and treatment estimates were back-transformed for reporting purposes.

elevations in blood lactate and decreases in pH, HCO_3, and pCO_2, which is consistent with other studies in cattle where aggressive handling has been shown to cause development of metabolic acidosis (Holmes et al., 1972; Frese et al., 2016; Hagenmaier et al., 2017). Compared to recent studies conducted by Hagenmaier et al. (2017) and Frese et al. (2016) in which HSH cattle were handled at comparable speeds, the degree of acidosis experienced in the current study was less severe considering lower lactate concentrations and the pH being closer to physiological normal. This variability in severity of acidosis following handling stress could be attributed to several factors, including, but not limited to, differences in degree of finish, as cattle with greater back fat thickness have been shown to have lower blood pH compared to cattle with less back fat for up to 1 h after aggressive handling (Frese et al., 2016).

Catecholamines increased more in HSH cattle at POSTHAND, which supports previous research in which stress due to increased workloads elevated both epinephrine and norepinephrine concentrations in cattle (Blum and Eichinger, 1988; Blum and Flueckiger, 1988; Hagenmaier et al., 2017). Changes from baseline were relatively similar for epinephrine and norepinephrine at each time point, which aligns with previous research suggesting equal increases in release of each catecholamine by the adrenal medulla after stress (Lefcourt et al., 1986; Rulofson et al., 1988). Each stress hormone had the greatest increase from baseline concentrations at slaughter, which has been previously described in cattle euthanized by captive bolt (Rulofson et al., 1988; Hagenmaier et al., 2017).

There is a lack of research reporting the effects of RAC on the physiological responses to handling and transport in feedlot cattle. In the current study, RAC cattle had larger increases in norepinephrine at POSTHAND, and diet × HI interactions were noted for the change in epinephrine and cortisol from baseline to POSTHAND and POSTTRANS, respectively. This relationship between βAA and alterations in the profile of stress hormones has not been reported in cattle to these authors' knowledge and warrants further investigation. Swine studies have reported increased epinephrine concentrations after the administration of RAC and suggest the effects of RAC on stress responses are dependent on handling whereby pigs fed RAC show larger stress responses to aggressive handling (James et al., 2013). Peterson et al. (2015) noted that RAC increased epinephrine when pigs were fed 7.5 but not 5.0 mg/kg RAC, indicating the effect of RAC on response to stress may be dose dependent. Both downregulation and desensitization of β receptors have been proposed to ensue after βAA usage, which may consequently lead to upregulation of catecholamine production to overcome decreased receptor population and affinity and maintain sympathetic tone (Bruckmaier and Blum, 1992; Marchant-Forde et al., 2003). Previous research suggests long-term exposure to βAA leads to desensitization through reduced levels of mRNA encoding for the β-adrenergic receptor (Hausdorff et al., 1990). Such reductions in mRNA have been proposed to be related to receptor instability, attributable to either increased cyclic adenosine monophosphate (**cAMP**) elicited by βAA or a secondary pathway induced by βAA involving protein kinase A and G proteins, but independent of cAMP (Hadcock et al., 1989). These changes in catecholamine responses may also be partly attributed to binding of the orally administered βAA to receptors, leading to fewer available receptor sites for endogenous catecholamines, thereby increasing free catecholamine concentrations in plasma, although this is purely speculation.

Creatine kinase is an enzyme released during rhabdomyolysis of striated myocytes that has been identified as a potential indicator of metabolic stress associated with βAA administration and nonambulatory cattle at abattoirs (Loneragan et al., 2014; Thomson et al., 2015). Similar to our study, Frese et al. (2016) and Hagenmaier et al. (2017) reported HI did not affect CK immediately after handling. The larger increases in CK from baseline to slaughter in cattle fed RAC in the current study was not particularly surprising, as similar results have been reported in cattle fed ZIL (FDA, 2006; Fuller et al., 2014). The diet × HI interaction for the change from baseline to POSTTRANS is of particular interest and suggests that aggressive handling combined with transport may be more harmful and cause greater degrees of muscle breakdown in cattle fed RAC than in those not fed a βAA. Although different species, Athayde et al. (2013) and Rocha et al. (2013) have reported increased CK in pigs fed RAC compared to pigs not fed βAA, and speculate RAC increased CK concentrations because of microlesions causing enlarged muscle fibril diameters and greater stimulation for release of the enzyme. In addition to this, the authors of the present study speculate muscle hypertrophy from RAC administration could lead to subsequent stretching and thinning of the sarcolemma, resulting in a greater degree of CK release. Other studies in beef cattle have reported similar findings in which CK concentrations were elevated significantly after transport, likely attributable to muscle fatigue and trauma from maintaining stance and shifting inside the trailer (Warriss et al., 1995; Buckham Sporer et al., 2008; Hagenmaier et al., 2017). The greater increase in CK concentration observed in HSH cattle from baseline to slaughter relative to LSH cattle is likely a delayed reflection of greater extents of rhabdomyolysis sustained during handling. Physiologically, this can be explained as CK is released into the interstitial space for lymphatic recycling and systemic absorption before reaching peak concentrations in the blood approximately 6 h after muscle insult (Brancaccio et al., 2007; Hagenmaier et al., 2017).

In conclusion, this study confirms the advantages of feeding RAC in regard to growth performance and the negative implications of aggressive handling without the use of a lead rider with respect to physiological and metabolic responses in beef cattle, regardless of whether or not they had been fed βAA. The risk of fed cattle having impaired mobility appears to be greatest following transport and while in lairage at the abattoir, which warrants further investigation.

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