INVESTIGATION INTO AN ONGOING DILEMMA: UNDEFINED WELFARE IMPLICATIONS CHALLENGING THE USE OF β-ADRENERGIC AGONISTS IN BEEF PRODUCTION

by

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B.S., Kansas State University, 2014
D.V.M., Kansas State University, 2016

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine/Pathobiology
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Abstract

Beta-adrenergic agonists (βAA) are administered during the final weeks of the beef production system to improve efficiency and increase meat yield. Welfare concerns linked to the administration of βAA have garnered significant attention in recent years due to anecdotal reports of increased mortality during βAA feeding periods and cattle without obvious disease or injury having difficulty walking at abattoirs being overrepresented in cattle fed βAA. Thomson et al. (2015) reported 2 events where cattle were distressed, became non-responsive to handling, sloughed hoof walls and were euthanized while in lairage at the abattoir. Consistent blood abnormalities in euthanized cattle included elevated blood lactate (25.6 mmol/L; ref. range: < 4-5) and creatine kinase (CK; 6,890 U/L, ref. range: 159- 332). Although no causal relationship had been established, dialogues among groups of packers, animal scientists, and welfare experts implicating the βAA zilpaterol hydrochloride (**ZIL**; Zilmax®, Merck Animal Health, Desoto, KS) as one possible etiology resulted in a major beef packer announcing plans to stop accepting cattle fed ZIL. Consequently, Merck announced a self-imposed suspension of ZIL sales in U.S. and Canadian markets until further research could be conducted to investigate the manner. Utilization of technologies such as βAA are imperative to meeting the demands of a growing world population and verdicts regarding such technologies, including their impact on animal welfare, should be based on scientific merit. The first objective of this research was to evaluate the effect of shade on performance and animal well-being in cattle fed ZIL. The second objective was to characterize the clinical description and hematological profile of fatigued cattle presented to abattoirs. The third objective was to evaluate the effects of handling intensity during shipment for slaughter in cattle fed a βAA. The fourth objective was to evaluate the effects of βAA administration on performance and physiological response to different handling intensities

during shipping for slaughter. Shade provision reduced open-mouth breathing and increased dry matter intake and dressing percentage. Fatigued cattle observed at abattoirs had increased respiratory rates and muscle tremors, although blood parameters were relatively normal compared to their cohorts. Metabolic acidosis, a precursor for Fatigued Cattle Syndrome, was observed in cattle exposed to aggressive handling regardless of βAA status. This research confirms the improved growth performance of cattle fed βAA and highlights the improvement of animal welfare through shade provision and low-stress handling in heavy-weight feedlot cattle.

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List of Abbreviations

ADG – Average daily gain
AT – Ambient temperature
βAA – Beta adrenergic agonist
bpm – Beats per minute
BW – Body weight
cAMP – Cyclic adenosine monophosphate
CK – Creatine kinase
CON – Control (no beta agonist)
d – Day(s)
DM – Dry matter
DMI – Dry matter intake
DOF – Days on feed
FATG - Fatigued
FDA – Food and Drug Administration
FPS – Fatigued Pig Syndrome
FCS- Fatigued Cattle Syndrome
g – G-force
G:F – Gain to feed ratio
GP – Glycolytic potential
h – Hour(s)
HCO_3 - bicarbonate
HCW – Hot carcass weight
HI- Handling intensity
HR – Heart rate
HSH – High-stress handling
LM – Longissimus muscle
LSH – Low-stress handling
m - Meter
min - Minute
OMB – Open-mouth breathing
pCO_2 – Partial pressure carbon dioxide
pO_2 – Partial pressure oxygen
PFT – Pen floor temperature
POSTHAND – Post-handling
POSTTRANS – Post-transport
RAC – Ractopamine hydrochloride
RH – relative humidity
rpm – Respirations per minute
SM – Semimembranosus muscle
sO_2 – Saturated oxygen
TCO_2 – Total carbon dioxide
THI – Temperature humidity index
THI_{CAT} – Temperature humidity index category
THI_{MAX} – Maximum temperature humidity index
USDA – United States Department of Agriculture
ZIL – Zilpaterol hydrochloride

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Dedication

This dissertation is dedicated to my grandparents, H.V. “Smitty” and Marian Smith, both of whom passed away during the fall of 2015. There are so many memories their grandchildren have of spending time with them, including fishing in the Pouder Canyon of the Rocky Mountains and enjoying vanilla milkshakes with home-made French fries following a long day of grade school. I will never forget the encouragement they provided me when my academic endeavors felt over-whelming, and the research summarized within this dissertation would not have been possible without their love and support. We are all deeply saddened they are no longer with us, but also comforted knowing they are together in heaven.

Preface

Chapters 2, 3, 4 and 5 in this dissertation entitled “Effect of shade on animal welfare, growth performance and carcass characteristics in large pens of beef cattle fed a beta agonist in a commercial feedlot”, “Clinical presentation and hematological profile of cattle with decreased mobility at a commercial abattoir”, “Effect of handling intensity at the time of transport to slaughter on physiological response and carcass characteristics in beef cattle fed ractopamine hydrochloride” and “Effects of feeding ractopamine hydrochloride on growth performance, carcass characteristics and physiological response to different handling techniques” were prepared for publication in the *Journal of Animal Science.* Accordingly, the text, tables and figures within these chapters are formatted in alignment with the guidelines specified by the journal.

Chapter 1 - Justification for Research Reported

Performance Advantages of β-adrenergic agonist (βAA) used in Beef Production

According to a July 2015 report by United Nations Department of Economic and Social Affairs, the world population is projected to increase nearly 25 % to 9.7 billion people by 2050. Adoption and discovery of current and new technologies are essential to increasing the efficiency of modern food production systems and enable their ability to produce a wholesome, nutritious food supply large enough to meet the demands of this growing population, which is projected to increase meat, milk and egg requirements by 70% (Capper and Hayes, 2012a). For every 454×10^6 kg of beef produced in the U.S., Capper and Hayes (2012a) estimated nearly 400,000 more cattle and over 20 billion more liters of water would be required in order to offset potential decreases in efficiency from the removal of growth-enhancing technologies such as anabolic steroids, in-feed ionophores, βAA and in-feed hormones from the production system. To produce the same amount of beef annually as the U.S. produced in 2012, it would require 10 million more cattle in the national herd, 3 million more cattle fed to harvest, 81 million more tons of feed, 17 million more of land to grow that feed and graze, and 138 billion more liters of water (Capper and Hayes, 2012b).

Ractopamine hydrochloride (RAC; Optaflexx®, Elanco Animal Health, Greenfield, IN; Actogain 45®, Zoetis Animal Health, Florham Park NJ) and zilpaterol hydrochloride (ZIL; Zilmax®, Merck Animal Health, Desoto, KS) are FDA approved β-adrenergic agonists (βAA) fed to beef cattle at the end of the feeding period to repartition nutrients and promote lean tissue deposition, thereby increasing ADG and improving feed efficiency (Quinn et al., 2008; Strydom et al., 2009). According to one of the nation's largest beef packers in 2012, an estimated 70 to

80% of the cattle killed in the U.S. were being administered 1 of these 2 agents (Cargill, 2013). Samuelson et al. (2016) reports 84.8% of feedlots consulted by surveyed nutritionists use βAA in their finishing cattle. Ractopamine hydrochloride is labelled to be fed in the complete feed at the rate of 8.2 to 24.6 g/ton to target 70 – 430 mg·animal^{-1}·d^{-1} on a 90% DM basis for 28 – 42 d for increased weight gain and improved feed efficiency (FDA, 2002). Additionally, RAC can be fed in a minimum of 1 lb top dress at the rate of 40 – 400 mg·animal^{-1}·d^{-1} for the same duration as the complete feed. Although RAC can be fed for up to 42 d, it is most commonly fed for 28 d (Walker et al., 2006). Zilpaterol is more potent than RAC and was originally approved to be fed at an inclusion rate of 6.8 g/ton of feed in order to provide 60 – 90 mg·animal^{-1}·d^{-1} on a 90% DM basis (8.3 mg/kg of DM). In 2014, a supplemental, more flexible label was approved for ZIL which allows ZIL to be fed in a component feed to target 60 mg·animal^{-1}·d^{-1}. Similar to RAC, ZIL can be fed for up to 40 d; however, it is most commonly administered for 20 d and requires a 3 d withdrawal period.

Although relatively new compared to other technologies utilized by beef producers, extensive research focused on βAA has been conducted and confirms their use as a tool to consistently enhance growth performance efficiency. Numerous studies have shown RAC and ZIL improve growth performance and HCW in beef cattle (Aveñdano-Reyes et al., 2006; Walker et al., 2006; Winterholler et al., 2007). Moreover, Lean et al. (2014) performed a meta-analysis using data from over 50 comparisons for each agent and concluded both RAC and ZIL consistently and significantly improved feed utilization and increased weight gain, hot carcass weight and longissimus muscle area. Traditionally, the repartition nutrients from adipose tissue to increased lean tissue accretion in ZIL considered to be more potent than RAC and, correspondingly, increases in HCW and dressing percentage have been shown to be amplified in

cattle and sheep administered ZIL vs. RAC (López-Carlos et al., 2010; Scramlin et al., 2010). Still, the advantages in growth performance and meat yield do not come without a trade-off. Although more severe in ZIL, both βAA have been shown to decrease meat tenderness measured by Warner-Bartzler Shear Force (Aveñdano-Reyes et al., 2006; Scramlin et al., 2010) . However consumer sensory data suggest this should not be of concern (Hilton et al., 2009) and the benefits of growth performance significantly outweigh negligible differences in carcass quality.

βAA Mechanism of Action

Epinephrine and norepinephrine are naturally occurring βAA and bind β-adrenoceptors on almost every type of mammalian cell (Mersmann, 1998). Mersmann (1998) previously elucidated the cascade concerning βAA mechanism of action. Briefly, activation of the Gs protein subunit occurs due to βAA/β-receptor coupling followed by the α-subunit of the Gs protein activating adenylyl cyclase which facilitates production of cyclic adenosine monophosphate (cAMP). cAMP then binds the regulatory subunit of protein kinase A, enabling the release of the catalytic subunit for phosphorylation of a number of intracellular proteins including the rate-limiting enzyme involved in adipocyte triacylglycerol degradation: hormone sensitive lipase. Finally, the cAMP response element binding protein (CREB) becomes phosphorylated by protein kinase A and binds to a cAMP response element in the regulatory part of a gene and gene transcription. Phosphorylation increases the transcriptional activity of the CREB, providing the mechanism for β-AR agonist-mediated transcription of a number of genes in the mammalian cell (Mersmann, 1998). In addition to activation of enzymes due to phosphorylation, other enzymes included the rate-limiting enzyme for long-chain fatty acid biosynthesis become inactivated.

In a more general sense, synthetic βAA such as RAC and ZIL are analogous to naturally occurring βAA and function as a repartitioning agent through a mode of action whereby nutrients are diverted away from adipose tissue production and towards increased lean tissue accretion through increased lipolysis and protein synthesis, and decreased lipogenesis and protein degradation (Bell et al., 1998; Mersmann, 1998; Quinn et al., 2008; Strydom et al., 2009). The effect of specific βAA on growth performance vary across livestock species, and is likely due physiological potential for increases in growth or different receptor types, densities, responses or affinity in target tissues (Mersmann et al., 1998).

There are 3 β –adrenoceptor sub-types (β_1, β_2, β_3), and distribution of these subtypes varies depending on the species and specific tissues both between and within species. According to Sillence and Matthews (1994), predominately β_2 are present on bovine skeletal muscle and adipocytes. This seems intuitive, as ZIL (β_2 adrenoceptor agonist) has been demonstrated to produce more profound effects on muscle accretion and decreased fat deposition than RAC (β_1 agonist) in cattle (Scramlin et al., 2010). Skeletal muscle glycogenolysis also appears to be mediated by β-adrenoceptors in beef cattle (Bruckmaier and Blum, 1992). During periods of limited oxygen availability, anaerobic metabolism will convert pyruvate to lactate, which is removed via the hematogenous system and explains elevated blood lactate concentrations during early phases of βAA administration such as reported with clenbuterol (Blum and Flueckiger, 1988; Bruckmaier and Blum, 1992).

Challenges Restricting the Adoption of βAA in Beef Production

The adoption of βAA usage within the beef industry has not without come scrutiny. Although no supporting scientific evidence exists and no causal relationships have been

established, suspected human health risk regarding consumption of meat from livestock administered βAA has caused China and Russia to ban imports of meat from animals fed approved products such as RAC and ZIL (Center et al., 2014). The Russian decision to no longer accept meat produced with RAC has been estimated to cost the U. S. $500 million annually (Center for Food Safety, 2013). Maximum residue limits established by the United Nations' Food and Agriculture Organization (FAO) and World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) have been accepted by the Codex Alimentarius Commission, the international, who establishes international standards for trade. This fact combined with the scale of economic losses absorbed by the U.S. due to lost trade have led to many people encouraging the U.S. to bring the issue to the World Trade Organization dispute settlement body (Centner et al., 2014).

Almost certainly, a majority of hesitations concerning adoption of RAC and ZIL in livestock systems and refusal to import meat from animal fed βAA are tied to the adverse effects observed in humans associated with βAA treatments, most typically in cases of asthma where bronchodilation is desired, and the potential for human abuse (Kuiper et al., 1998). Adverse effects reported involve interactions with myocardial β_1 receptors resulting in palpitation, and skeletal and central nervous system β_2 receptors evident by muscle tremors and headaches (Drennan, 1994; Kuiper et al., 1998). Furthermore, high dosages βAA has been associated with increased mortality in asthma patients, although it is unclear whether a causal relationship exists between βAA and mortality or greater doses and duration of βAA are merely associated with severe cases of asthma (Suissa et al., 1994). The only study performed in humans investigating possible toxicological effects of RAC showed no adverse effects (WHO, 2004), albeit the sample size was relatively small. Clearly, there is a need for more research to understand the potential, if

any, of adverse reactions from human consumption of meat fed with these feed additives. Currently, discussions concerning international disparities over global trading of meat produced with βAA continue to be ongoing and lack of progression in regards to international disputes over acceptance of meat from animals fed βAA have led to some major meat producing companies to move away from use of this feed technology (Center et al., 2014).

More recently, anecdotal reports claiming feedlot mortality, inability to cope with heat stress, and difficulties walking are overrepresented in cattle fed βAA have added fuel to the international βAA dilemma and led to speculations of compromised animal welfare in cattle fed βAA garnering more attention (Loneragan et al., 2014; Thomson et al., 2015). Undeniably, such issues pose a significant threat to the welfare and productivity of beef cattle, however described issues remain to be undefined and scientific evidence to quantify and validate these claims is lacking. Indeed, such anecdotal reports have potential to negatively influence consumer perceptions about food animal agriculture, which ultimately lead to major impacts on industry acceptance and adoption of technologies such as βAA.

Accounts from cattle feeders have suggested increased feedlot mortality rates during periods of βAA administration, most prominent in hot summer months. Soon after, Loneragan et al. (2014) concluded RAC and ZIL increased feedlot mortality in cattle, especially during the hot summer months. The role of temporality on the association was consistent across multiple data sets and suggests a significant seasonal factor exists that might exacerbate the effect of feeding βAA on mortality, most likely the thermal heat index (THI). In their conclusions, Loneragan et al. addressed the need for further research, development of effective mitigation strategies to prevent heat stress in cattle fed βAA during warm environmental conditions, and advocated for a "broad and inclusive" dialogue exploring other growth promoting technologies in addition to

βAA and potential for unidentified compromises of welfare of food animals. Vogel et al. (2015) summarized 9 years of feedlot close-out records and reported that mortality rates during the last 30 days on feed (when βAA are typically fed at feedlots) remained constant and did not increase from 2005 through 2014, which coincides with the introduction and adoption of RAC and ZIL into the North American beef industry. However, no controlled experiments have been reported to either refute or confirm the proposition that feedlot mortality is increased with the use of βAA.

In addition to the prospect of undefined differences in feedlot mortality, recent reports of beef packers suggesting cattle fed ZIL having difficulty walking or being reluctant to move upon presentation to abattoirs has also raised animal welfare concerns related to βAA administration. Thomson et al. (2015) reported 2 cases which occurred during the summer of 2013 where various cattle fed ZIL or not fed a βAA developed characteristic mobility problems soon after arrival at an abattoir. Cattle appeared normal and were moved to concrete floored lairage pens upon arrival. While in lairage, many cattle without obvious disease or trauma became reluctant to move or had difficulty walking, and severely affected cattle became non-ambulatory. Animals sloughing hoof walls were immediately euthanized, however other animals reluctant to move were able to past ante-mortem inspection after being allowed to rest overnight. Euthanized cattle had various serological abnormal and indicators of stress including elevated blood lactate and creatine kinase concentrations, tachypnea with an abdominal component, muscle tremors, and reluctance to move. Furthermore, some cattle sloughed one or more hoof walls while in lairage pens at the abattoir and were euthanized, while other cattle recovered after being rested overnight. Histopathologic evaluation of the hooves were not consistent with the changes typically observed due to carbohydrate-induced laminitis, and alternative etiologies that can cause sloughing of hoof walls such as selenium toxicity and ergot toxicosis were investigated

and ruled out. Because of this, the authors hypothesize the pathogenesis of these foot lesions differs from the previously mentioned etiologies.

Although no scientific basis, initial emotionally-driven dialogues and anecdotal reports implicating administration of βAA as one possible etiology continue to gain traction within the feedlot industry. Such anecdotal accounts combined with additional reports of fatigued and downed cattle at the time of delivery to abattoirs generated enough momentum and raised such an industry concern that it led to a major U.S. packer announcing they would no longer accept cattle fed ZIL. Consequently, during August of 2013, Merck Animal Health voluntarily announced a self-imposed suspension of Zilmax® sales in U.S. and Canadian markets until research could be conducted to investigate the matter. Since then, a supplemental label has been approved for Zilmax® with an added caution label due to the potency of the product and suspected associations that reads "CAUTION: Not to be fed to cattle in excess of 90 mg/head/day in complete feed. If pen consumption of complete feed exceeds 26.5 lb./head/day (90 percent dry matter basis), zilpaterol should not be fed in complete feed" (FDA, 2014).

Collectively, the speculation of potential human health risks and consequential restrictions in international trade, concerns of compromised animal welfare, and packer decisions to no longer accept cattle fed ZIL allow one to conclude the adoption of βAA has faced significant resistance. Although different circumstances, agriculture faces similar situations with other technologies which improve food production efficiency such as genetically modified organisms (GMO), steroid implants, recombinant bovine somatotropin (rBST), and antibiotics administered in the feed (Schroeder and Tonsor, 2011). The latter of these technologies has received so much attention that improved growth performance are no longer label indications for use of medically-important antibiotics, therefore making their use for that purpose illegal (FDA

Guidance #213, 2013). Comparisons drawn to weigh the advantages of modern technologies used in conventional food animal production vs. possible human health and animal welfare concern should be based on sound scientific merit, as losing the privilege to utilize these tools would erase years of research and improvements in efficiency in food production, thereby posing a significant risk to the advancement and sustainability of food animal agriculture (Lyles and Calvo-Lorenzo, 2014).

Fatigued Pig Syndrome

Ractopamine hydrochloride was first FDA approved for swine in 1999 (Paylean®, Elanco Animal Health; Greenfield, IN), prior to the approval of Optaflexx® for cattle in 2003. Shortly after the approval of RAC in swine at doses of 4.5 to 18 g/ton, reports surfaced of increased rates of non-ambulatory pigs. Marchant-Forde et al. (2003) conducted a study in finishing hogs and showed that feeding 10 ppm RAC was associated with pigs becoming more hyper or alert, greater circulating catecholamines, and resistance to handling. These findings along with other research and reports from the field eventually led to the manufacturer of the reducing the recommended dose and adding a precautionary statement states "CAUTION: Ractopamine may increase the number of injured and/or fatigued pigs during marketing" (FDA, 2002). Non-ambulatory pigs are defined as pigs that are unable to walk or keep up with contemporaries during movements at the packing plant, and Fatigued Pig Syndrome has been used to describe this condition (Anderson et al., 2002; Ritter et al. 2005). Research conducted in swine has demonstrated metabolic acidosis and elevated stress hormones are major determinants for the vast majority of non-ambulatory pigs classified as fatigued (Anderson et al., 2002; Ritter et al., 2009b).

Fatigued pigs display an assortment of signs of acute stress such as open-mouth breathing, skin discoloration, and muscle tremors and have elevated blood lactate and decreased pH and HCO_3, characteristic of metabolic acidosis (Benjamin et al., 2001; Anderson et al., 2002; Ivers et al., 2002, Ritter et al., 2005). Anderson et al. (2002) reported fatigued non-ambulatory pigs had greater blood lactate and CK concentrations compared to their cohorts, similar to the affected cattle being reported by Thomson et al. (2015). Research conducted in swine has identified fatigued non-ambulatory pigs are a culmination of a multi-factorial issue including improper handling with use of an electric prod, genetics (porcine stress syndrome), inappropriate trailer stocking densities, unsuitable facility designs and environmental factors such as heat stress (Ritter et al., 2009a).

Studies have suggested the effects of RAC on stress responses of finishing swine are dependent upon handling whereby stress responses to aggressive handling are more profound in pigs fed RAC (James et al., 2013; Peterson et al., 2015) and characterized by increased epinephrine concentrations (Marchant-Forde et al, 2003; Puls et al., 2015; Peterson et al., 2015). Peterson et al. (2015) reported epinephrine was increased when pigs were fed 7.5 mg/kg but not 5.0 mg/kg RAC, indicating that the effect of RAC may be dose dependent. Both down-regulation and desensitization of β-receptors have been proposed to ensue following βAA usage, which may consequently lead to up-regulation of catecholamine production in order to overcome decreased receptor population and affinity and maintain sympathetic tone (Bruckmaier and Blum, 1992; Marchant-Forde et al., 2003).

Conclusions

It is difficult to refute that βAA and other technologies which improve efficiency of modern agriculture will play a key role in undertaking the challenge of providing a safe, wholesome food supply capable of nourishing a growing world population. Consumers are further removed from agriculture today than ever before and gather information from numerous sources to form opinions concerning food production, illustrating the importance of scientific investigation to refute or confirm anecdotal claims concerning welfare implications of beef cattle fed βAA, not only to be responsible stewards of these technologies, but also to appropriately address questions regarding anecdotal claims as these as have great potential to influence consumer perception of food safety and wholesomeness. Current issues lead one to conclude that research is warranted to more thoroughly understand the effect βAA usage on beef cattle welfare during periods when heat stress is likely. Therefore, the first objective of this research was to evaluate the effect of shade on performance and animal well-being in cattle fed ZIL in large lots of cattle during the final weeks of the feeding period. Furthermore, the similarities in clinical signs and serum biochemical abnormalities of cattle reported to have difficulty walking and pigs affected with FPS make it plausible a similar condition exists in cattle. Inherently, Fatigued Cattle Syndrome has been used to describe this condition in cattle, and research is needed to characterize the clinical signs and identify contributing factors in order to develop mitigation strategies and protect the welfare of beef cattle presented to abattoirs. Accordingly, the other objects of the research summarized in the report herein were to characterize the clinical description and hematological profile of fatigued cattle presented to abattoirs, evaluate the effects of handling intensity during shipment for slaughter in cattle fed a RAC, and evaluate the effects of βAA administration on performance and physiological response to different handling intensities during shipment for slaughter.

Chapter 2 - Effect of shade on animal welfare, growth performance and carcass characteristics in large pens of beef cattle fed a beta agonist in a commercial setting

J. A. Hagenmaier, C. D. Reinhardt, S. J. Bartle, D. U. Thomson

ABSTRACT

Feedlot cattle (n = 1,395; BW = 568 ± 43 kg) were used to evaluate the effects of shade on animal welfare, growth performance, and carcass quality during the summer of 2013 in a Kansas commercial feedlot. Seven lots of predominately black steers and heifers (4 and 3, respectively) visually determined to be approaching the final month on feed were identified, randomly gate-sorted and allocated to pens located across the feed alley from each other to receive 1 of 2 treatments: 1) Shade (mean shade area = 1.5 m^2/ animal) or 2) No shade. Shade was provided using a 13 ounce polyethylene fabric and pens were oriented northwest to southeast. The mean starting date was June 13 and the mean DOF for lots while on the study was 38 d. Cattle were fed a 77.67% DM steam-flaked corn-based diet and had *ad libitum* access to water throughout the duration of the trial. Zilpaterol hydrochloride (**ZIL**) was included in the finishing ration at an inclusion rate of 8.3 mg/kg of DM for the last 20 d on feed with a 3 d withdrawal period. Pen floor temperatures (**PFT**) were measured using an infrared thermometer and prevalence of cattle open-mouth breathing (**OMB**) was recorded on a pen basis. In addition to shade treatment, the effect of temperature humidity index (**THI**) on PFT and OMB was analyzed by classifying days as either "Alert" (THI < 79) or "Danger" (THI > 79). On the day of slaughter, pens within a replicate were kept separate through all stages of the marketing channel from loading at the feedlot until stunning at the plant. Pen served as the experimental unit for all

measurements. There was a THI × shade treatment interaction for PFT and OMB ($P < 0.001$) where days classified as "Danger" increased PFT and prevalence of OMB compared to "Alert" days in unshaded but not shaded cattle. Shaded cattle had greater DMI ($P = 0.01$); however, unshaded cattle had greater G:F ($P = 0.05$) and therefore no differences were observed in ADG ($P = 0.39$). Shaded cattle had greater dressing percentage ($P = 0.01$), although HCW, LM area, fat thickness, marbling score, and quality grade did not differ between treatments ($P > 0.05$). Heat stress, a significant animal welfare concern and cause of reduced performance in feedlot cattle during the final phase of the feeding period, was alleviated in shaded cattle and illustrates the importance of shade provision as one tool to protect the welfare and increase feed consumption in large pens of feedlot cattle during hot summer months.

Key words: beef cattle, growth performance, heat stress, shade, welfare

INTRODUCTION

In 2015 alone, over 7.2 million commercial cattle were slaughtered during the summer months (USDA, 2016). As a result, large numbers of finished cattle are exposed to Midwest summer conditions where heat stress could occur and be potentially devastating with regards to death loss and decreased performance (Hubbard et al., 1999; Mader, 2014). Over a decade ago, St.-Pierre (2003) estimated heat stress in finished beef cattle cost the U.S. approximately $282 million, or roughly 1.5% gross income per animal, annually due to decreased performance and death loss.

Loneragan et al. (2014) reported increased mortality rates in cattle fed the β-adrenergic agonists (**βAA**) zilpaterol hydrochloride and ractopamine hydrochloride were most prominent in hot summer months. These findings, combined with anecdotal reports of decreased mobility in

cattle fed βAA, have led to scrutiny of βAA use and growing welfare concerns in cattle fed βAA, particularly their ability to cope with heat stress. Samuelson et al. (2016) reports 84.8% of feedlots consulted by surveyed nutritionists use βAA in their finishing cattle. Yet, Samuelson et al. (2016) and Simroth et al. (2016) both report only 17% of feedlots provide shade in finishing pens. Together, these findings indicate an opportunity for future shade implementation and highlight the need for current research to better appreciate the effects of shade on animal welfare and performance in cattle fed βAA.

No studies have evaluated the effect of providing shade under commercial management conditions where entire lots of nearly finished feedlot cattle are staged into shaded pens during summer months and fed βAA during final weeks on feed before slaughter. Therefore, the objective of this study was to evaluate the effects of shade provision during hot summer months on the welfare, growth performance and carcass characteristics in large lots of short-fed cattle fed βAA at a Kansas commercial feedlot.

MATERIALS AND METHODS

All animal handling and treatment procedures involved in conducting this study were in compliance with the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching (FASS, 2010).

Cattle and Experimental Design

A total of 1,395 (BW = 568 ± 43 kg) predominately black-hided steers and heifers from 7 lots (4 and 3, respectively; mean = 200 animals) with similar phenotypic characteristics that were visually determined to be approaching the final month on feed were enrolled in a completely

randomized design during the summer of 2013. Animals within a lot had been fed and housed together (mean DOF = 89 d) in dirt floored pens in accordance with the feedlot's standard operating procedures. Upon arrival and before enrollment on the study, incoming lots of cattle were treated for internal parasites (Synanthic®, Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO), external parasites (Noromectin®, Norbrook Inc., Lenexa, KS), and vaccinated against clostridial toxins (Ultrabac 7®, Zoetis Animal Health, Florham Park, NJ), infectious bovine rhinotracheitis, parainfluenza-3 virus, bovine respiratory syncytial virus, and bovine viral diarrhea virus type I and II (Pyramid 5®, Boehringer Ingelheim Vetmedica Inc.). Lots classified as "high-risk" were identified by the feedlot and received a similar vaccine that incorporated *Mannheimia haemolytica* toxoid in addition to the viral antigens (Pyramid 5 + Presponse®, Boehringer Ingelheim Vetmedica Inc.). Before study inclusion, all 7 lots received a single steroid implant by 1 of 4 scenarios determined by the feedlot based on arrival weight, degree of finish and projected harvest dates: 1) Revalor 200® (200 mg trenbolone acetate and 20 mg estradiol 17β; Merck Animal Health, Desoto, KS) during arrival processing, 2) Revalor 200® approximately 120 d before slaughter 3) Revalor S® (120 mg trenbolone acetate and 24 mg estradiol 17β; Merck Animal Health) during arrival processing, or 4) Revalor S® approximately 90 d before slaughter.

Approximately 1 month before projected slaughter dates, lots of cattle identified for enrollment were independently removed from their home pen and walked to nearby holding pens to be randomly gate-sorted by groups of 5 into 2 smaller groups of similar number (mean n = 100 animals per group). The resulting smaller groups were then randomly assigned to pens to receive 1 of 2 treatments: 1) Shade or 2) No shade. Pen served as the experimental unit and resulting pairs of pens from a single lot served as a replicate (n = 7 replications). The mean study

enrollment date, slaughter date, and DOF across replicates were June 13, July 21, and 38 d, respectively.

Housing and Shade Design

Pens within a replicate were located directly across the feed alley from each other and oriented northwest to southeast so that feed bunks were located on the southernmost end for shaded pens and vice versa for non-shaded pens. Furthermore, a 5% slope was maintained from the feed bunk within each pen housing experimental cattle according to the feedlot's standard operating procedures. Shade was provided using a 12.2 × 13.7 m structure with 13 ounce polyethylene product (Fig. 2-1; Cattle Cabana®, Accu-Steel Inc., Templeton, IA) and structures were positioned along both fence lines so that shade was shared between adjacent pens. A 257 L water tank (Johnson Concrete Products, Hastings, NE) was positioned within each fence line so that cattle had access to water while under shade, and was in the same location for unshaded pens. The mean shade area, pen space, and linear bunk space over all pens in the study was 1.5 m^2, 36.2 m^2, and 0.55 m per animal, respectively.

Animal Feeding and Monitoring

Before enrollment on the experiment, all cattle were fed using the same diet step-up program until maintained on a 77.67 % DM steam flaked corn-based finishing diet formulated to meet or exceed the requirements of growing cattle (Table 2-1; NRC, 2000). Zilpaterol hydrochloride (**ZIL**; Zilmax®, Merck Animal Health) was included in the diet according to label instructions at an inclusion rate of 8.3 mg/kg DM for 20 d followed by a 3 d withdrawal period. Start date of ZIL was determined so that lots met the withdrawal period by the Monday of the week cattle would be shipped for slaughter. In other words, commencement of ZIL

administration occurred on a Saturday and the last day cattle received ZIL was on the Thursday 20 d later for all lots. Accordingly, cattle had met the withdrawal period and were eligible for shipment to slaughter the following Monday. Feed bunks were assessed daily beginning at 0500 h by a trained individual who would estimate orts and consumption to determine the amount of feed to be delivered over 2 daily feedings to provide *ad libitum* access to feed. Feed deliveries were performed at the same times for shaded and unshaded pens due to their close proximity and to avoid circadian bias. Water was available *ad libitum* throughout the duration of the study.

Health observations were performed once daily in accordance with the feedlot's standard operating procedures by trained pen riders. Concomitant therapy was under the direction of veterinarians. Only 1 animal that received medical treatment failed to meet the established withdrawal time and was therefore unable to be shipped to slaughter with penmates. Post-mortem reports were obtained from the feedlot's individual-animal based health management software to calculate the incidence density of mortality.

Heat Stress Observations

All pens housing experimental cattle were observed and the prevalence of cattle with open-mouth breathing (**OMB**) was determined between 1500 and 1700 h. Pens within a replicate were observed consecutively to avoid circadian or environmental bias. Open-mouth breathing was defined as the lower jaw being held open exposing the tongue and panting characterized by increased inhalation and exhalation effort (Fig. 2-2; Johnson et al., 2010). Due to availability of personnel, observations to determine prevalence of OMB was only performed on a subset of days (n = 29). The prevalence of cattle OMB was determined by dividing the number of cattle OMB by the total number of cattle in the pen on that day. Immediately after OMB observations

for an individual pen housing experimental cattle, pen floor temperatures (**PFT**) were measured in dirt-floored shaded (shade treatment pens only) and unshaded areas within that pen. Pen floor temperatures were measured on all but 4 OMB observation days (n = 25) using a Fluke 62 Mini Infrared Thermometer (Fluke Corporation, Everett, WA). Temperature of unshaded areas was measured in both shaded and unshaded pens to account for variation across pens. However, only temperatures from unshaded pens were used to represent unshaded areas in the statistical analysis because the variation in temperature between pens was less than 1% of the mean temperature for unshaded pens (data not shown).

Growth Performance and Carcass Characteristics

Initial and final BW were obtained using the same certified scale at the feedlot. Initial pen weights for each treatment were obtained during sorting into shaded or unshaded pens and average individual animal weight was calculated by dividing the overall pen weight by the number of cattle in the pen. Final BW was calculated using the same methods during weigh-out on the mornings cattle were shipped for slaughter. Daily feed deliveries were multiplied by 0.7767 to adjust for DM content and divided by daily pen counts to determine DMI per animal on a pen basis. Then, the mean over the entire study for each respective pen within a replicate was used for analysis.

Data were provided by the abattoir for analysis of carcass characteristics. Quality grades and HCW were reported for all replicates; whereas LM area, marbling score and 12th rib fat depth were only available for 4 replicates due to differences in marketing strategies in those lots of cattle. Dressing percentages were also reported by the abattoir for all replicates and calculated

by dividing the HCW by the final BW measured at the feedlot multiplied by 0.96 to account for 4% shrink.

Weather Data

Hourly weather data were collected continually throughout the duration of the study at a National Oceanic and Atmospheric Administration (NOAA) weather station located approximately 1.6 km from the feedlot. The temperature humidity index (**THI**) was calculated using the same equation as Mader et al. (2006) where THI = (0.8 × TA) + [(RH × 0.01) × (TA – 14.4)] + 46.4; **TA** = Ambient Temperature; **RH** = Relative Humidity. Weather data are summarized over the entire study period and for each replicate independently (Table 2-2).

Statistical Analysis

The incidence density for mortality was calculated for each shade treatment using an exact denominator and is reported for incidence of mortalities per 1,000 animal d. For all remaining variables, data were analyzed using the GLIMMIX procedure in version 9.3 of SAS (SAS Inst. Inc., Cary, NC) and pen was considered the experimental unit (n = 7 replications). To evaluate the effect of THI, pen-level OMB prevalence and PFT measurements were bucketed into 1 of 2 THI categories ($\mathbf{THI_{CAT}}$) based on the maximum THI ($\mathbf{THI_{MAX}}$) on individual observation days. Categories were formed in accordance with the Livestock Weather Safety Index (LWSI) taxonomy for heat stress reported by Mader et al. (2006) so that days with THI_{MAX} > 79 were classified as a "Danger" and days with THI_{MAX} < 79 were classified as "Alert". As a result, the model for PFT and OMB included the fixed effects of shade treatment, THI_{CAT} and the shade treatment × THI_{CAT} interaction, and replicate was included as a random effect.

Growth performance and carcass data were analyzed with a linear mixed effects model using the GLIMMIX procedure with the fixed effect of shade treatment and a random intercept with replicate identified as the subject to account for the clustering of random effects. Prevalence of cattle USDA grading choice or greater at slaughter were calculated for each pen and used for analysis. In addition to overall growth performance, ADG and G:F were analyzed using a carcass-adjusted final BW calculated by dividing the HCW by the common dressing percentage of 65.23% (the mean dressing percentage over all replicates included on the study) to account for possible differences in gut-fill during weigh-out and allow for more accurate evaluation of cattle growth.

The DMI and the change in DMI were evaluated between shade treatments from before initiation of ZIL through d 2 to 9 after commencement of ZIL administration **(post-ZIL**) using procedures similar to Reinhardt et al. (2014) with day post-ZIL was treated as a repeated measure using an autoregressive covariance structure. Dry matter intake before initiation of ZIL (**baseline**) was calculated using the mean DMI for 5 d before beginning ZIL. One replicate began ZIL after only 1 d on study; therefore baseline DMI was retrospectively calculated using the consumption before enrollment on study for that replicate.

The effect of sex was not included in the model and will not be discussed in the results section because single sex replicates were used. However, the variation due to sex is accounted by accounting for replicate within the statistical models. Treatment means were estimated using the LSMEANS statement and compared using the PDIFF option in SAS. Statistical differences were determined by $P \leq 0.05$ and tendencies were declared when $0.06 \leq P \leq 0.10$.

RESULTS AND DISCUSSION

No cattle deaths were deemed to be the direct result from heat stress throughout the duration of the study. When accounting for all mortalities (shaded = 6; unshaded = 2) that occurred while lots were on study, the incidence density for shaded and unshaded cattle was 0.215 and 0.079 cases per 1,000 animal d, respectively. It is interesting to note that 4 of the 6 mortality cases in shaded cattle occurred in a single lot of heifers and all 4 were diagnosed as atypical interstitial pneumonia (AIP) by a consulting veterinarian. Yet, the unshaded pen representing the same lot had no AIP related deaths. Loneragan et al. (2001) reported that AIP was more likely to occur in heifers, yet the etiology of AIP is poorly understood. Of particular interest in regards to our study, one factor suggested to play a role in the AIP pathogenesis is irritation of the airway epithelium secondary to inhalation of dust (Woolums et al., 2015). To prevent heat stress, which also appears to be related to development of AIP, providing shade seems intuitive for reducing the incidence of this disease. Yet, other possible outcomes associated with providing shade such as cattle crowding into confined areas to compete for shade and associated decreases in ventilation and their effects on AIP frequency have not been extensively studied and merit further consideration.

There was a shade treatment × THI_{CAT} interaction for both PFT and OMB ($P < 0.0001$), whereby days classified as "Danger" increased PFT and prevalence of OMB compared to "Alert" days in unshaded but not shaded cattle (Fig. 2-3 and 2-4). Eigenberg et al. (2009) reported polyethylene material effectively reduced predicted heat stress by decreasing solar radiation and ambient temperature. Sullivan et al. (2011) reported similar results where shade reduced panting scores during periods of high heat loads (heat load index > 86) in Angus yearling heifers in Australia; however, panting scores were not decreased by providing shade in

periods when the heat load was less significant (heat load index < 86). Likewise, Gaughan et al. (2010) and Blaine et al. (2011) reported that panting was directly related to thermal loads and decreased by providing shade to feedlot cattle. Furthermore, our findings are in agreement with Valtorta et al. (1997) who measured concrete floor temperatures at a dairy facility using an infrared thermometer and reported shade reduced floor temperature by nearly 20°C.

The heat load cattle experience is dependent on 2 sources: 1) metabolic heat from tissue metabolism and digestive fermentation, and 2) environmental heat (Sullivan et al., 2011; NRC, 2016). Cattle and other species have an effective thermoneutral zone dependent on numerous environmental, animal, and management factors whereby thermoregulation and physiological homeostasis are maintained without necessary adaptation to increase or decrease heat production. However, temperatures above this range in cattle require the animal to expend more energy dissipating heat to thermoregulate through evaporative processes including sweating and respiration (Mader, 2003; NRC, 2016). Heat stress results from a culmination of numerous human, animal, dietary and climatic factors and occurs when the ability to dissipate heat through these evaporative processes becomes overwhelmed by the collective heat load experienced from metabolic and environmental sources (Robertshaw et al. 2006; Sullivan et al., 2011; Gaughan and Mader, 2014). Factors shown to affect the predisposition to heat stress include, but are not limited to, energy content of diets, feeding patterns, phenotypic characteristics, previous history of pneumonia, pen floor design (i.e. availability to access shade, water, and wind), solar radiation, ambient temperature and relative humidity (Brown-Brandl et al., 2006a; Mader et al., 2006; 2010a). Cattle begin open-mouth breathing during heat stress to increase tidal volume to more effectively dissipate heat loads that exceed the ability of primary evaporative cooling mechanisms such as sweating, increased respiratory rate, or “through the nose” panting

(Robertshaw, 2006; Sullivan et al., 2011; Gaughan and Mader, 2014). It has been proposed a slight increase in respiration rate and effort or both can increase maintenance energy expenditure by 7% and in cases of severe, labored, open-mouth breathing the maintenance requirement can be increased by 11 to 25% (NRC, 1981).

The effect of shade on growth performance and carcass characteristics in feedlot cattle has been studied extensively; however, the outcomes reported are inconsistent. In the current study, shaded cattle had greater DMI ($P < 0.01$, Table 2-3); however, unshaded cattle had greater G:F ($P = 0.05$) and as a result ADG and final BW did not differ between treatments ($P > 0.05$). On the other hand, and similar to ADG, G:F did not differ between treatments ($P > 0.05$) when analyzing growth performance using a carcass adjusted final BW. In similar climatic conditions as those experienced by the cattle on the current study, Mitlöhner et al. (2001, 2002) reported increased feed consumption in *Bos taurus* feedlot cattle provided shade in the Texas panhandle. However, those studies also reported improved ADG reflecting the increase in DMI in shaded cattle, which differs from the current study. Conversely, Boyd et al. (2015) reported DMI in feedlot cattle is unaffected by shade in Nebraska with environmental conditions similar to the current study. Similarly, Bond and Laster (1975) also reported shade had no effect on DMI in bulls fed high-roughage diets in Nebraska. Others have speculated shade did not benefit growth performance of cattle in certain situations due to an ability to acclimate to heat and compensate so effectively where the benefits of shade provision are diminished (Mader et al., 1999a; Brown-Brandl et al., 2006b). For instance, Mader et al. (1999a) speculated compensatory responses to heat could explain their findings in Angus × Hereford crossbred cattle fed in pens with wind barriers where feed conversion was improved during 0 through 56 DOF in shaded compared to

unshaded cattle; yet, the opposite was true during the last 22 DOF as unshaded cattle tended to have greater G:F.

Relative to the large amount of research conducted to evaluate the effect of shade in feedlot cattle, only a small number of studies have included cattle fed βAA. From the research reported, Boyd et al. (2015) concluded shade did not improve growth performance in cattle fed ZIL for 21 d in Nebraska, whereas Barajas et al. (2009) reported shade increased ADG by 8.8% and improved G:F by 6.1% in cattle fed ZIL for 30 d at the end of a 248 d trial in bull calves northwest Mexico. In a study where ZIL was fed to predominantly Bonsmara crossbred steers and bulls in South Africa for 35 d, Blaine et al. (2011) found shade increased ADG, and although measured at the pen level and therefore not statistically analyzed, DMI was roughly 0.2 kg/animal more per day for shaded animals and G:F was improved by 7.2%.

It is well-accepted that greater heat loads lead to decreased DMI (Hahn, 1999), and the fluctuations in DMI and THI_{MAX} appear to be closely related on the current study (Fig. 2-5). Peaks in THI_{MAX} are followed by decreases in DMI (Fig. 2-5) for both shaded and unshaded cattle, suggesting DMI does not decrease on the day of the heat event, but rather on the subsequent days due to carry-over heat loads. This is expected, as feed delivery would have been completed and a majority of feeding time occurs before the heat of the day in commercial feedlots. Furthermore, others have suggested decreased DMI on days subsequent to heat stress indicates failure of cattle to adequately dissipate heat overnight when THI remains high (Mader et al., 2006; Gaughan et al., 2008). A minimum nightly THI value greater than 70 has been proposed to be a strong indicator that cattle were not able to effectively cool down overnight and could be indicative of inadequate heat abatement achieved overnight to reduce or prevent heat stress the following day (Mader et al., 1999a, 2006, 2010b). When adequate heat abatement fails

to occur overnight, cattle continue to pant to utilize cooler morning temperatures and increased body to environment temperature gradients to dissipate residual heat from the previous day (Brosh et al., 1998; Gaughan and Mader, 2014). Gaughan and Mader (2014) proposed panting within 1 to 2 h from sunrise is likely due to the animal trying to recover and compensate from heat experienced the previous day. Mader and Kreikemeier (2006) reported heat stressed cattle may prepare for impending heat loads by overcompensating and reducing body temperatures further than expected during the morning. Collectively, findings from previous research combined with the DMI fluctuations observed in concert with changes in THI on the current study further support the use of tools such as THI and the Heat Load Index (Gaughan et al., 2008) to predict heat periods of heat stress.

Reinhardt et al. (2014) analyzed feed intakes from more than 1,500 pens from 3 different commercial Kansas feedlots and reported reduced DMI shortly after initiation of ZIL administration, especially during summer months. The findings of the current study support this, as DMI was lower and the decrease in DMI was greater in unshaded compared to shaded cattle when pooled across d 2 through 9 ($P < 0.01$; Fig. 2-6 and 2-7), although the shade treatment × post-ZIL day interaction was not significant ($P > 0.05$). Certainly, limitations of the current study are low number of replications and the inability to extend baseline DMI calculations over a longer period to account for possible trends in DMI before ZIL initiation. Still, our findings suggest shade mitigated the decrease in DMI after ZIL administration, which warrants further discussion. Although the exact mechanism for the decrease in DMI after commencement of ZIL is not completely understood, Reinhardt et al. (2014) proposed βAA-induced metabolic alterations leading to increased glycogenolysis and lactate production, hepatic preference of propionate from grain-fed diets as the substrate for gluconeogenesis, and consequent build-up of

lactate concentrations resulting in secondary intake depression as one possible cause. Moreover, the original Zilmax® label called for inclusion based on g/ton only, resulting in DMI as the lone determinant of actual amount of ZIL consumed. Given this fact, it is foreseeable that doses of ZIL greater than approved ranges occur in subsets of cattle and decreased DMI after initiation of ZIL could manifest as a dose-dependent side effect not observed in pre-approval research (Reinhardt et al., 2014; Frese, 2015).

The growth performance advantages achieved through use of βAA such as ZIL appear to be achieved through decreased protein degradation and increased protein accretion (Mersmann, 1998; Quinn et al., 2008; Strydom et al., 2009). Increased protein accretion results in increased metabolic heat production (Chwalibog et al., 1996). Therefore, it is conceivable such increases in metabolic heat predispose certain cattle to heat stress. To cope with added heat loads, Taylor et al. (1969) and Beatty et al. (2006) reported cattle increase respiratory rates and hyperventilate, resulting in respiratory alkalosis. Bruckmaier and Blum (1992) reported a more potent βAA (clenbuterol) increased lactate and glucose concentrations immediately after administration and speculate skeletal muscle glycogenolysis appears to be mediated by β-adrenoreceptors. Because of this, Reinhardt et al. (2014) speculated that if ZIL has similar effects as clenbuterol thereby increasing lactate production, simultaneous commencement of ZIL administration with heat stress could amplify the degree of respiratory alkalosis cattle endure and present as voluntary reductions in feed consumption.

Shaded cattle had greater dressing percentages ($P = 0.01$, Table 2-3); otherwise there were no effects of shade treatment for the remaining carcass characteristics ($P > 0.05$). Similar to growth performance, the benefits of shade on carcass traits reported in the literature are varying. Barajas et al. (2009) reported 3.6 m^2 shade increased dressing percentage by 3-fold times greater

than it did on our study and also led to increased HCW, although the cattle in that study were mixed *Bos taurus* and *Bos indicus* bull calves and the duration of shade provision on their study was almost 8-fold longer. Blaine et al. (2011) reported that providing 2.9 m^2 shade increased HCW by around 9 kg over a 35 d ZIL feeding period. Mitlöhner et al. (2001) reported shade increased HCW and fat thickness in Charolais heifers. Our findings differ from Boyd et al. (2015) who reported shade did not improve dressing percentage in cattle fed ZIL for 21 d or not fed a βAA, although our findings are in agreement as shade also did not increase HCW, LM area, fat thickness, or marbling scores on their study. Mader et al. (1999a) reported shade had no effect on dressing percentage, fat thickness and marbling score in Angus × Hereford crossbred steers provided 2.65 m^2 shade area compared to unshaded cattle. Furthermore, our findings support several studies where shade provision did not improve carcass quality grades of beef cattle (Mitlöhner et al., 2001; Gaughan et al., 2010; Boyd et al., 2015). Mitlöhner et al. (2002) reported shade increased the percent of Angus cross and Charolais cattle grading USDA Choice, although marbling score did not differ and therefore they report the difference was attributed to a reduced prevalence of dark cutting carcasses. There were only 2 dark cutting carcasses on the current study and both were shaded cattle from a single lot. One could reason that some dark-cutting carcasses may be associated with chronic heat stress leading to depletion of muscle glycogen, as glycogen is the main determinate of post-mortem decreases in carcass pH and prevention of dark-cutting carcasses (Scanga et al., 1997), although these findings have not been reported consistently in the literature.

More research is needed which evaluates the ideal amount of shade required by feedlot cattle to improve performance in a cost-effective manner for modern feedlots. The amount of shade space provided in our study (1.5 m^2/animal) was considerably less than other studies

(Mader et al., 1999a; Sullivan et al., 2011). Early research conducted in beef cattle in California suggested an adequate shade area per animal around 5.5 m^2 (Bond et al., 1958), however this is not practical given modern feedlot pen designs and stocking densities. Garrett et al. (1962) found that providing 4.6 vs. 2.5 m^2 did not improve growth performance in Hereford steers in California. Likewise, Sullivan et al. (2011) evaluated the effects of different available shade space (2.0, 3.3, and 4.7 m^2/animal) in short-fed cattle similar to the lots on our study and found that providing more than 2.0 m^2 does not offer added growth performance advantages, although panting scores were improved with greater shade availability. More recently, Boyd et al. (2015) found no advantages in growth performance or carcass characteristics when cattle fed ZIL were provided 3.0 m^2 of shade. According to a recent survey conducted by Simroth et al. (2016), only 7 of 42 (17%) feedlots provided shade. Furthermore, only 1 feedlot provided more than 2.3 m^2 shade area per animal. Based on our field experiences and the findings of Simroth et al. (2016), the scenario presented in the current study where cattle were provided 1.5 m^2 shade area is typical of situations feedlot managers face in regards to decision-making with large lots of heavy cattle prone to heat stress and a limited number of pens with shade due to the cost of implementation. As a result, feedlot managers may be inclined to stage lots in a manner that maximizes number of animals with availability to shade as long as bunk space and pen size are adequate.

Cattle fed diets with greater energy concentrations to maximize gain in feedlot settings generate greater amounts of metabolic heat compared to lower-energy, roughage-based diets in other phases of the beef production systems and are therefore at greater risk to be adversely affected by heat stress (Mader et al., 1999b). In addition to dietary contributions to heat load, a majority of feedlot cattle harvested have black hides (McKeith et al., 2012; Corah, 2016) and are

raised in arid environments without the ability to access to naturally occurring shade or bodies of water to help dissipate heat. Indeed, the decision for feedlot managers of whether or not to invest in shade structures is dependent upon many factors, particularly recent heat loads and associated death and performance losses. Furthermore, the time required to achieve breakeven on the cost of constructing shades is difficult to predict as weather conditions and the net benefits from shade will likely vary greatly from year to year. For instance, the feedlot described herein may have observed more profound advantages in cattle performance and positive returns on investment during 2011 where the mean daily-high THI was greater than 79 for each of the summer months (Table 2-4), whereas none of the months had a mean daily-high THI greater than 79 during 2013 while the current study was being conducted.

In conclusion, the findings of this study indicate shade decreased the prevalence of open-mouth breathing suggesting that heat stress was visually alleviated. Additionally, provision of shade increased feed consumption, the ultimate driver of feedlot performance, but did not lead to increased gains during the mild summer of 2013. Together, these findings advocate shade as an effective tool to protect the welfare of beef cattle at feedlots and suggest even short-term access to shade may offer performance advantages in large lots of cattle during hot summer conditions at the end of the feeding period.

Table 2-1. Ingredient composition (percent DM basis) and analyzed nutrient content of the finishing diet fed throughout the duration of study period.

Ingredient	% DM basis
Steam flaked corn, %	52.5
Steam flaked wheat, %	24.3
Alfalfa, %	8.6
Finish Premix[1], %	8.1
Liquid Supplement[2], %	2.8
Tallow, %	2.5
Microingredients[3], %	1.2
Total	100 %
Analyzed nutrient content, DM basis	
DM, %	77.67
CP, %	16.1
CF, %	4.9
aNDF, %	13.7
Ca, %	0.82
P, %	0.37

[1]Formulated to contain (DM basis): 21.4% sunflower meal, 19.4% milo dried distillers grains, 17.3% wheat middlings, 14.5% ground calcitic limestone (38% Ca), 11.1% dolomitic limestone, 4.3% urea, 3.2% salt, 2.9% vitamin/trace mineral supplement (XF Beef #15, Xtra Factors, Pratt, KS), 2.1% potassium chloride premix (50% K), 2.1% ammonium sulfate, 1.3% magnesium oxide, 0.20% zinc sulfate premix (35.5% Zn), 0.17% zinc polysaccharide premix (22% Zn), 0.02% copper chloride premix (54% Cu) and 0.01% selenium premix (0.3% Se).

[2]Formulated to contain 45% CP on a 100% DM basis using 66.9% corn condensed distillers solubles, 29.2% cornsteep, and 3.9% urea.

[3]Formulated to provide each animal a target daily-dose of 50,000 IU vitamin A, vitamin 5,000 IU vitamin D_3, 50 mg direct-fed microbial (Micro-Cell Gold, Lallemand Animal Nutrition, Milwaukee, WI), 70 mg zilpaterol hydrochloride (Zilmax®, Merck Animal Health, Desoto, KS), 425 mg monensin (Rumensin, Elanco Animal Health, Greenfield, IN), and 90 mg tylosin (Tylan 100, Elanco Animal Health). Additionally, the finishing ration for heifers included melengestrol acetate at a target dosage of 0.50 mg/animal.

Table 2-2. Maximum, minimum, and mean daily ambient temperature (TA), relative humidity (RH) and temperature humidity index (THI) summarized for each replicate and over the entire study period.[1]

Item	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Study Period
Ambient temperature, °C								
Maximum	32.4	33.4	33.1	33.0	32.6	32.0	31.6	31.9
Minimum	18.3	19.2	19.4	19.5	19.1	19.4	19.9	19.1
Mean	25.2	26.2	26.1	26.0	25.7	25.4	25.4	25.3
Relative humidity, %								
Maximum	83.8	79.4	80.8	82.7	82.9	86.8	89.4	86.3
Minimum	29.1	26.6	28.8	29.7	30.2	34.7	40.3	35.0
Mean	55.9	51.7	54.1	55.7	55.9	60.9	78.4	60.5
Temperature humidity index[2]								
Maximum	78.0	78.6	78.5	78.6	78.2	78.2	78.4	78.1
Minimum	64.1	65.3	65.7	66.0	65.3	66.0	66.9	65.5
Mean	71.6	72.6	72.5	72.6	72.2	72.3	72.9	72.2
Date Enrolled	June 7	June 10	June 10	June 11	June 7	June 14	July 5	June 13
Date slaughtered	July 3	July 11	July 18	July 23	July 22	August 6	August 8	July 21
DOF	26	31	38	42	45	53	34	38

[1]Hourly weather data was collected continually by a weather station located approximately 1.6 km from the feedlot and accessed through the online National Oceanic and Atmospheric Administration (NOAA) database.
[2]Calculated using the equation from Mader et al. (2006) where $THI = (0.8 \times TA) + [(RH \times 0.01) \times (TA - 14.4)] + 46.4$

Table 2-3. Least squared means for the effects of shade provision on growth performance and carcass characteristics of beef cattle.[1]

	Treatment			
Variable	Shaded	Unshaded	SEM	*P*-value[2]
No. of pens[3]	7	7	-	-
No. cattle per pen	100	100	-	-
Growth performance				
Initial BW, kg	570	568	16.8	0.59
Final BW, kg	644	644	22.0	0.98
DMI, kg	10.8	10.5	0.34	< 0.01
ADG, kg	1.94	1.97	0.100	0.39
G:F, kg:kg	0.18	0.19	0.007	0.05
Carcass adjusted growth performance				
caFinal BW, kg[4]	645	642	21.6	0.30
caADG, kg[4]	1.98	1.90	0.109	0.33
caG:F, kg:kg[4]	0.18	0.18	0.008	0.62
Carcass characteristics				
HCW, kg	404	402	13.6	0.31
Dressing percentage[5]	65.41	65.05	0.002	0.01
LM area, cm[2, 6, 7]	95.5	93.2	2.37	0.13
12th rib fat thickness, cm[6]	1.38	1.34	0.093	0.34
Marbling score[6, 8]	441	433	12.2	0.36
Choice or greater, %	72.3	67.0	0.02	0.12

[1]Large lots of predominately black steers or heifers (4 and 3, respectively) visually determined to be approaching the final month on feed were identified and randomly gate-sorted and allocated to pens located across the feed alley from each other to receive 1 of 2 treatments: 1) Shaded or 2) Unshaded.
[2]Statistical significance was declared for $P \leq 0.05$ and tendencies were declared when $0.06 \leq P \leq 0.10$.
[3]Pen was considered the experimental unit
[4]Growth performance estimates were based on a carcass-adjusted final BW using a common dressing percentage of 65.23% to remove potential differences in gut fill and allow for more accurate evaluation of growth.
[5]Final BW was adjusted for 4% shrinkage prior to calculation and statistical analysis of dressing percentage
[6]Data were analyzed on only 4 lots, these variables were not reported for the remaining lots (n = 3) due to differences in marketing strategies in those lots of cattle.
[7]Measured between the 12th and 13th ribs.
[8]Evaluated in the longissimus dorsi m. between the 12th and 13th ribs; Traces = 200, Slight = 300, Small = 400, Modest = 500, Moderate = 600, Slightly Abundant = 700.

Table 2-4. Five yr perspective of the mean daily-high for ambient temperature (AT) and temperature humidity index (THI) during the summer months at the feedlot used in the current study and the Livestock Weather Safety Index heat stress classifications based on temperature humidity index (THI).[1]

Item	2011	2012	2013	2014	2015
Ambient Temp., °C					
June	34.9	32.8	31.2	29.2	31.1
July	38.6	37.1	31.7	29.5	33.0
August	35.6	32.2	30.5	33.1	30.7
THI[1]					
June	79.4	77.9	77.0	72.5	74.0
July	82.4	81.0	77.8	72.3	75.0
August	80.8	76.9	77.7	75.7	72.8

Code	**THI**
Normal	THI ≤ 74
Alert	74 < THI < 79
Danger	79 ≤ THI < 84
Emergency	84 ≤ THI

[1]Data was obtained using a weather station located approximately 1.6 km from the feedlot and accessed through the online National Oceanic and Atmospheric Administration (NOAA) database. Temperature humidity index was calculated using the same equation as Mader et al. (2006) where THI = (0.8 × TA) + [(RH × 0.01) × (TA – 14.4)] + 46.4; TA = Ambient Temperature; RH = Relative Humidity.

Figure 2-1 Shade Structures. Shade was provided using 13 ounce polyethylene product (Cattle Cabana®, Accu-Steel Inc., Templeton, IA) and structures were positioned along both fence lines so that shade was shared between adjacent pens. Water tanks (Johnson Concrete Products, Hastings, NE) were positioned within the fence line so that cattle had access to water while under shade.

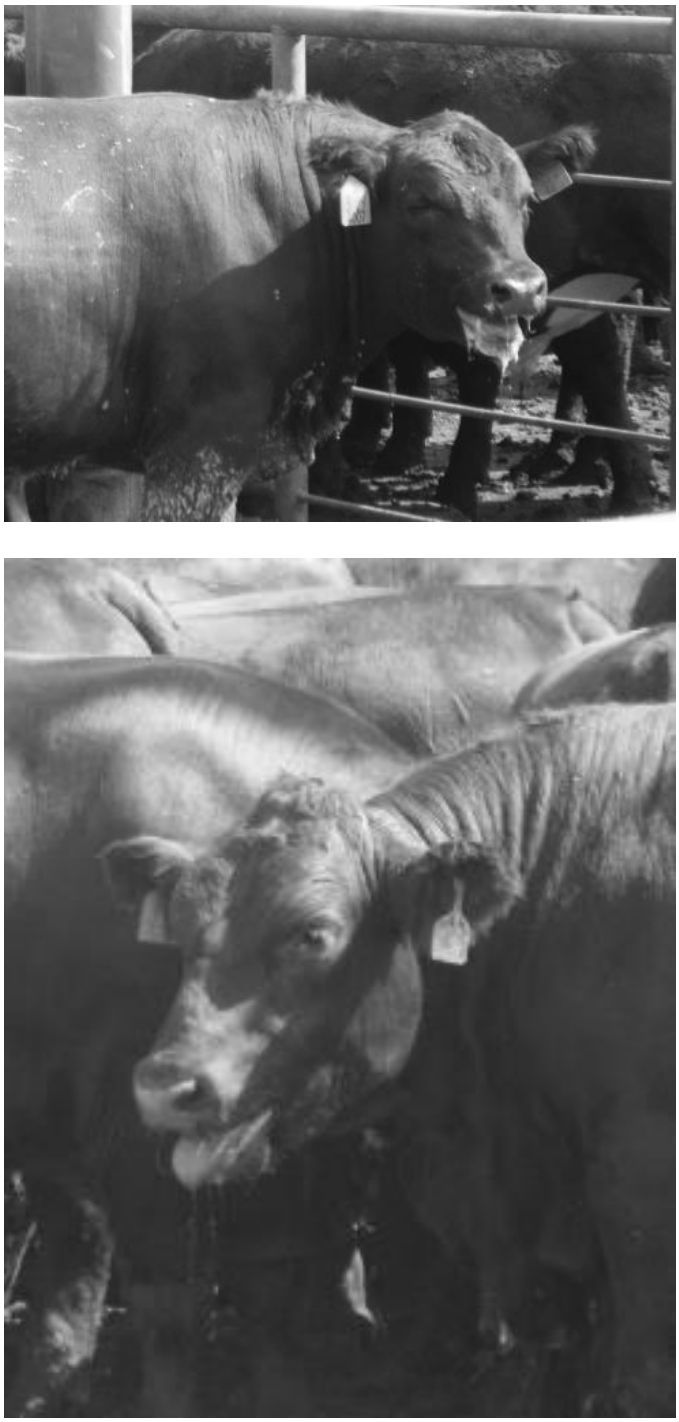

Figure 2-2 Examples of open-mouth breathing. Open-mouth breathing was defined as the lower jaw being held open exposing the tongue and panting characterized by increased inhalation and exhalation effort with flank involvement (Johnson et al., 2010).

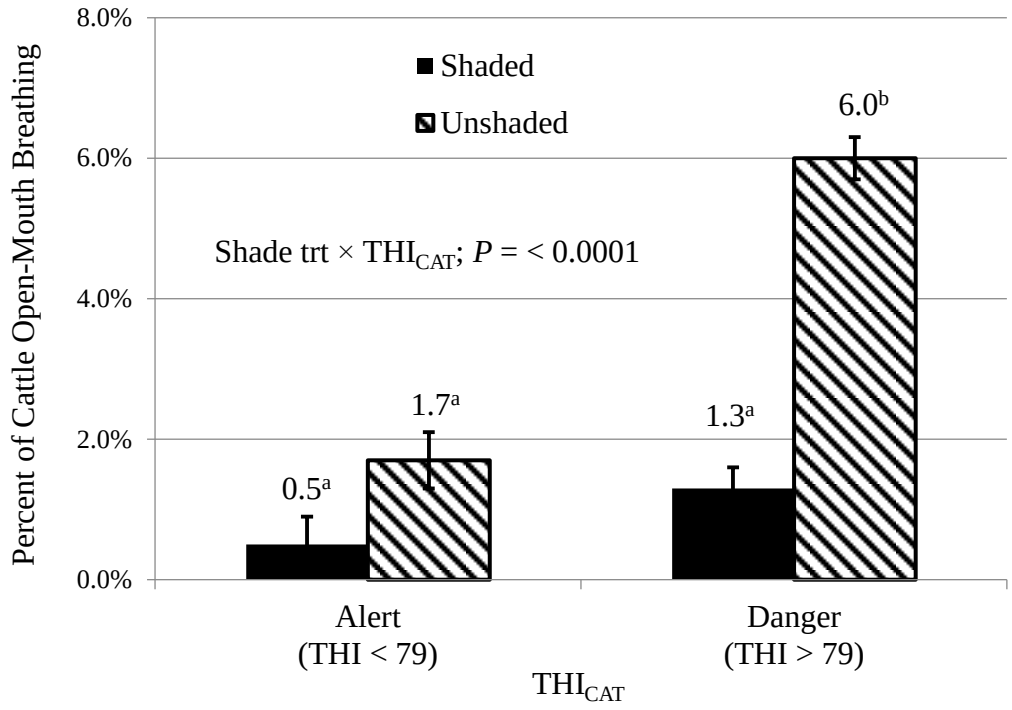

Figure 2-3. Graphical representation of the pen prevalence of cattle open-mouth breathing (OMB) with access to shade (mean = 1.5 m^2 per animal) or no access to shade on days when the temperature humidity index category (THI_{CAT}) were classified as "Alert" (THI < 79) or "Danger" (THI > 79). All pens housing experimental cattle were observed and the number of cattle with open-mouth breathing (**OMB**) was determined between 1500 and 1700 h (n = 29 d). There was a shade treatment × THI_{CAT} interaction (P < 0.0001). Means without a common lowercase letter (a-b) differ (P < 0.0001). The THI was calculated using the same equation as Mader et al. (2006) where THI = (0.8 × TA) + [(RH × 0.01) × (TA – 14.4)] + 46.4; TA = Ambient Temperature; RH = Relative Humidity.

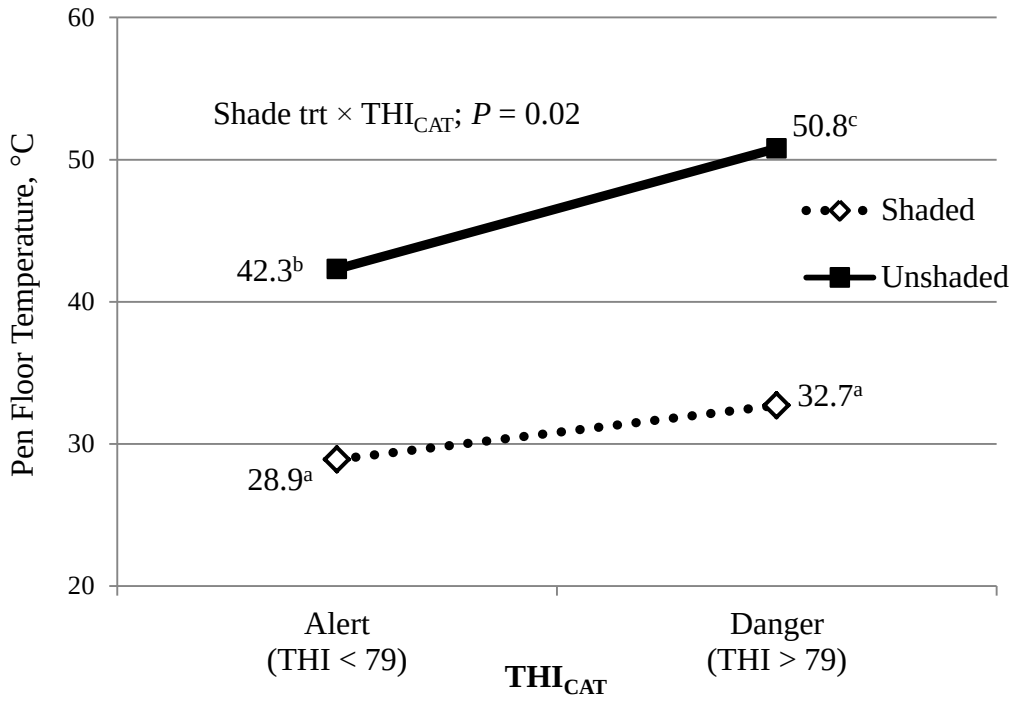

Figure 2-4. Graphical representation of the interaction between shade treatment and the temperature humidity index category (THI_{CAT}) on pen floor temperatures on days when the THI were classified as "Alert" (THI < 79) or "Danger" (THI > 79). Pen floor temperatures (**PFT**) were measured using an infrared thermometer on heat all but 4 open-mouth breathing observation days (n = 25). Means without a common lowercase letter (a-b) differ ($P < 0.05$). The THI was calculated using the same equation as Mader et al. (2006) where THI = (0.8 × TA) + [(RH × 0.01) × (TA – 14.4)] + 46.4; TA = Ambient Temperature; RH = Relative Humidity.

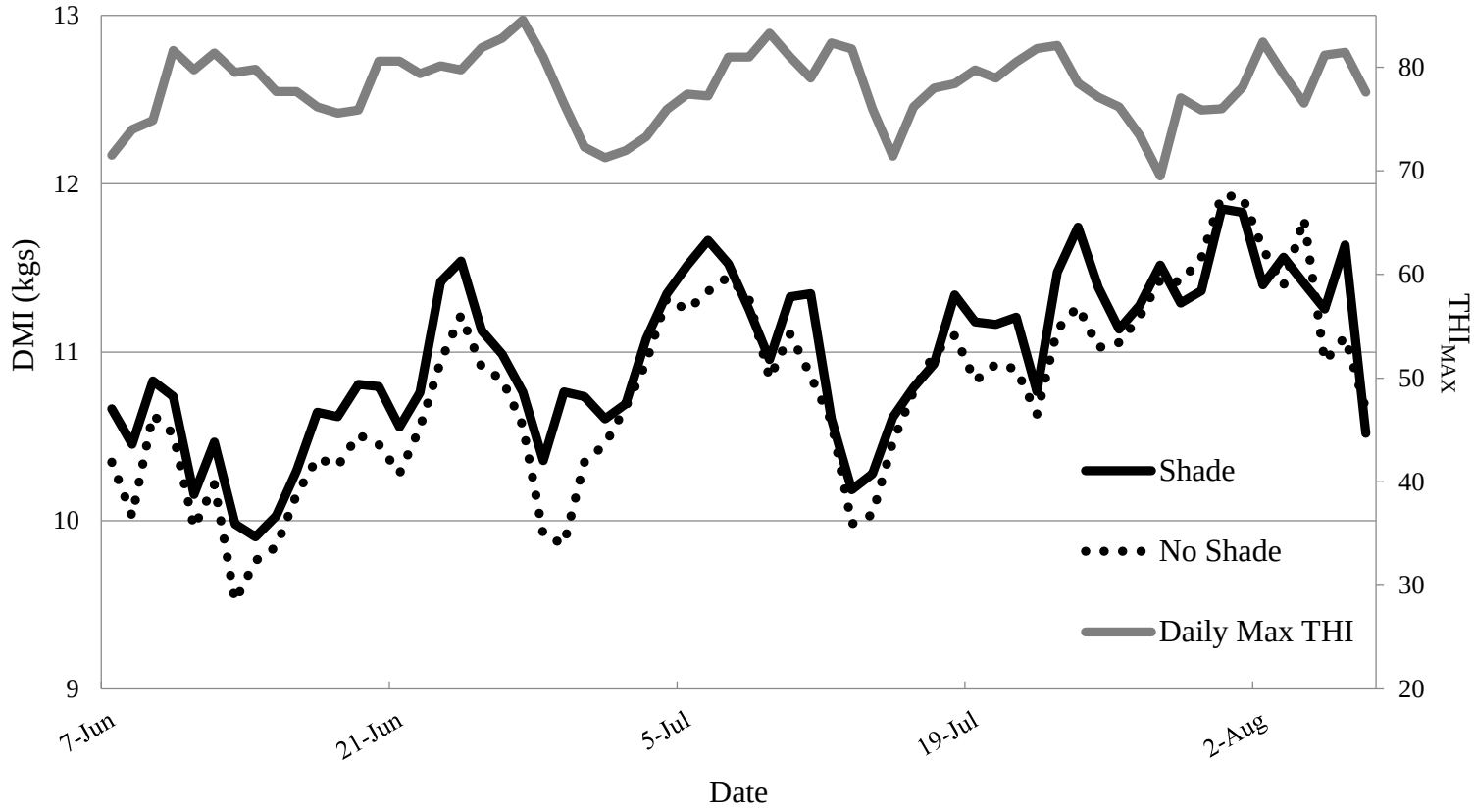

Figure 2-5. Graphical representation of mean daily DMI for shaded vs. unshaded pens and maximum temperature humidity index (THI_{MAX}) throughout the entire study period. Daily DMI was calculated by taking the mean DMI across all pens on each shade treatment at that time. The THI was calculated using the same equation as Mader et al. (2006) where THI = (0.8 × TA) + [(RH × 0.01) × (TA – 14.4)] + 46.4; TA = Ambient Temperature; RH = Relative Humidity.

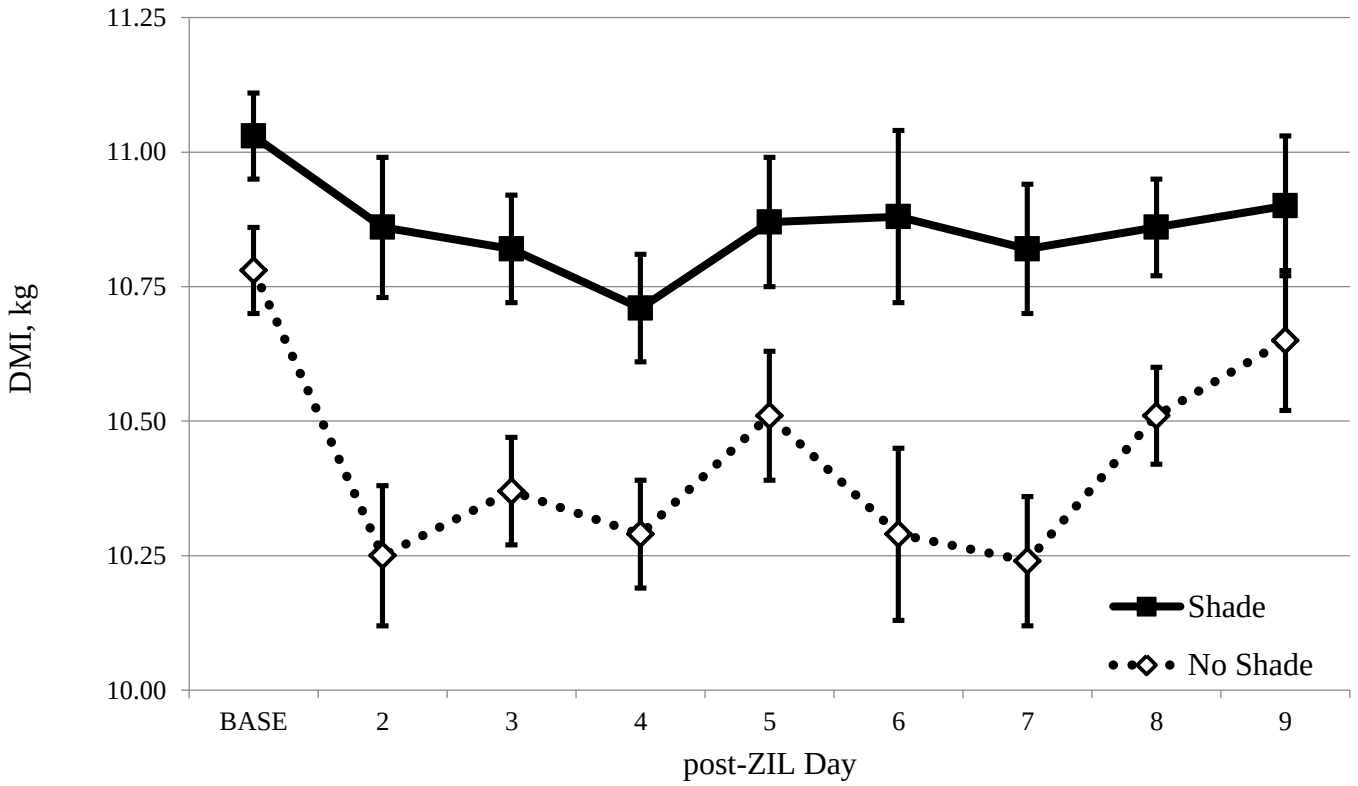

Figure 2-6. Mean daily DMI following initiation of zilpaterol hydrochloride (ZIL) administration by day of zilpaterol feeding (post-ZIL day) and shade treatment. Shaded cattle had greater (P = 0.01) pooled mean DMI over d 2 – 9 following initiation of ZIL administration, however the shade × day interaction was insignificant (P = 0.38). Baseline DMI (BASE) was determined by calculating the mean DMI for the 5 d prior to the initiation of ZIL administration.

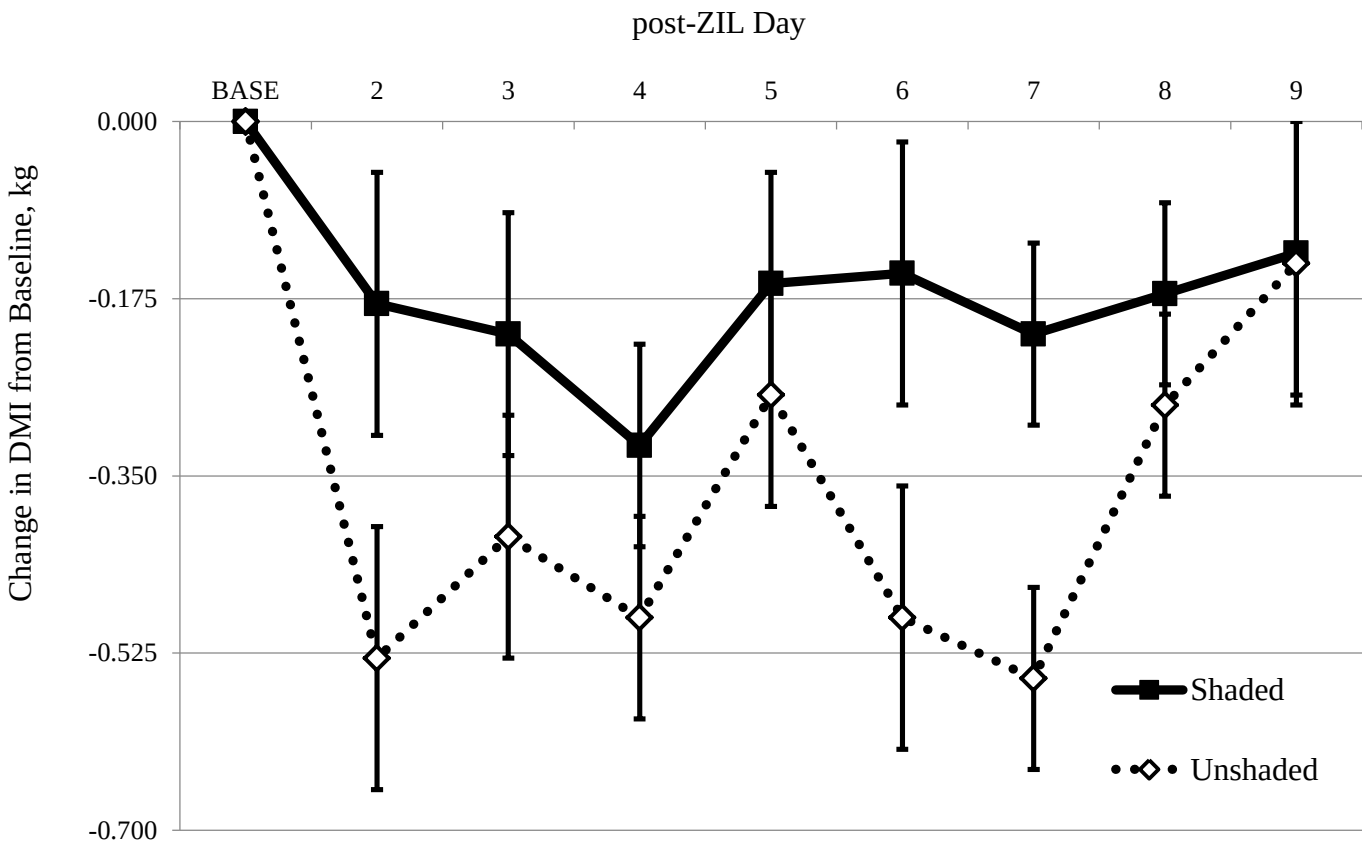

Figure 2-7. Mean change in daily DMI from baseline following initiation of zilpaterol hydrochloride (ZIL) administration by day of zilpaterol feeding and shade treatment. Shaded cattle had smaller (P = 0.01) pooled decreases in DMI over d 2 – 9 following initiation of ZIL administration, however the shade × day interaction on the decrease in DMI was insignificant (P = 0.95). Baseline DMI (BASE) was determined by calculating the mean DMI for the 5 d prior to the initiation of ZIL administration.

Chapter 3 - Clinical presentation and hematological profile of cattle with decreased mobility at a commercial abattoir

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INTRODUCTION

The welfare of market cattle is a priority for the beef industry and has gained considerable attention in recent years due to anecdotal reports of decreased mobility in market weight cattle presented to abattoirs. Thomson et al. (2015) reported 2 cases which occurred during the summer of 2013 where cattle developed characteristic mobility problems soon after arrival at an abattoir. Affected cattle had various clinical signs and hematological abnormalities including tachypnea with an abdominal component, muscle tremors, reluctance to move, and elevated blood lactate and creatine kinase (**CK**) concentrations. Furthermore, some cattle sloughed one or more hoof walls while in lairage pens at the abattoir and were euthanized, while other cattle recovered after being rested overnight.

Although no controlled investigations had been performed and initial dialogues were purely based on speculation, anecdotal reports suggesting these mobility issues were overrepresented in cattle fed the beta-adrenergic agonist (**βAA**) zilpaterol hydrochloride (**ZIL**; Zilmax®, Merck Animal Health, Desoto, KS) and ractopamine hydrochloride (**RAC**; Optaflexx®; Elanco Animal Health, Greenfield, IN) continued to circulate. Eventually, such anecdotal accounts combined with additional reports of fatigued and downed cattle at the time of delivery to abattoirs generated enough momentum and raised such an industry concern that it led to a major U.S. packer announcing they would no longer accept cattle fed ZIL. Consequently, Merck

Animal Health voluntarily removed ZIL from U.S. and Canadian markets until research could be conducted to investigate the matter. Although anecdotal dialogues suggesting mobility issues were over-represented in cattle fed ZIL, this condition is likely multifactorial and strategies to prevent this condition are needed to protect the welfare of animals presented for slaughter (Thomson et al., 2015). It should be noted that βAA fed to cattle has been associated with increased death loss late in the feeding period (Loneragan et al., 2014) and these losses are greater in the summer months indicating that higher thermal heat load in cattle is potentially part of the problem, which is also consistent with mobility issues being reported by packers.

Undoubtedly, the use of technologies such as βAA will play a key role in improving the efficiency of food production in order to meet the demands of a growing global population and risk of losing the βAA technology to improve feed utilization in food animals poses a significant threat to the advancement of food animal agriculture (Lyles and Calvo-Lorenzo, 2014). Although it is generally agreed upon by individuals involved in food production that decisions regarding usage of βAA and other technologies should be based on scientific merit, anecdotal claims cannot be ignored when they create opportunities for consumer misconceptions. With that being said, although events such as those reported by Thomson et al. (2015) appear to be relatively rare, it is imperative that the beef industry thoroughly investigate this manner to identify and mitigate potential contributing factors to aid in the prevention of repeated cases.

Thomson et al. (2015) proposed the issues being observed in beef cattle at abattoirs may be representative of varying degrees of a currently undefined health problem in cattle with clinical characteristics similar to those displayed by swine with Fatigued Pig Syndrome (FPS; Ritter et al., 2005). With the exception of the sloughed hoof walls, the clinical signs and serum biochemical abnormalities observed in affected cattle were similar to those observed FPS-

affected hogs (Ivers et al., 2002, Anderson et al., 2002; Ritter et al., 2005). Fatigued Pig Syndrome (FPS) was described in the swine industry during the early 2000's following investigations of a sudden increase in transport losses (Ritter et al., 2009a). A pig suffering from FPS is characterized as a pig without obvious injury or disease which refuses to walk or becomes recumbent and exhibits physical signs of stress such as vocalization, blotchy skin, and muscle tremors at any one of the respective stages of the marketing channel from loading at the farm to stunning at the plant (Ritter et al., 2005). Elevated stress hormones and development of metabolic acidosis characterized by increased blood lactate and decreased pH were consistent in pigs affected by FPS and seem to be major contributing factors in pigs developing this condition (Anderson et al., 2002; Ivers et al., 2002; Ritter et al., 2005). Because of this, it has been proposed FPS has a multi-factorial etiology and is a consequence of acute, stress-induced acidosis or physical exhaustion due to chronic stress and depletion of muscle glycogen stores (Ritter et al., 2005). Research conducted in swine have identified aggressive handling with electric prods, long handling distances, genetics (porcine stress syndrome), heat stress and inadequate transport space as additive factors that contribute to the incidence of FPS (Anderson et al., 2002; Ivers et al., 2002; Ritter et al., 2009b). It is suspected that a similar phenomenon exists in heavy finished cattle that undergo stress during handling and transport to the packing facility.

Recognized stressors cattle can experience during shipment for slaughter include extreme heat loads, aggressive handling during movement from home pens to load-out facilities and during loading onto livestock trailers, long duration standing on concrete-floored holding pens, suboptimal transport space, and long haul transport. González et al. (2012a) reported transporting cattle for slaughter during periods of high heat loads (> 30 °C), long durations of

transport (> 30 h) and inappropriate stocking densities in the belly and deck compartments caused increased transport loss. According to Annual Livestock Summaries, slaughter weights of cattle have increased 150 lbs from 1999 to 2015 (USDA, 2000 and 2016), which can be attributed to multiple factors including economic signals increasing DOF, genetics, and growing use of growth-promoting technologies such as βAA. Holmes et al. (1973) reported double-muscled cattle were more susceptible to stress and development of metabolic acidosis than normally muscled cattle characterized by excitability, difficulty handling and increased lactate production following epinephrine injections and exercise.

Interestingly, Ashmore and Doerr (1971) predicted that continued selection for muscling, with no regards to type of muscle being selected, would result in cattle having increased proportion of glycolytic α White fibers, and therefore more susceptible to metabolic acidosis and stress at the time of slaughter. Given the size and scale of modern feedlots, it is not uncommon for cattle to have to travel long distances from their home pen to load out facilities (Frese et al., 2016). Hagenmaier et al. (2017b) recently demonstrated trotting finished beef cattle prior to shipping to the abattoir increases blood lactate and causes significant breakdown of muscle indicated by increased CK concentrations measured at exsanguination, illustrating how failing to provide a lead rider and allowing heavy cattle to voluntarily run could pose a significant threat to the ability of cattle to cope with other stressors endured during transport in periods of hot weather.

Although different species, the changes in serological chemistry and clinical presentation of affected cattle such as those reported by Thomson et al. (2015) are strikingly similar to FPS-affected swine, suggesting a similar phenomenon may exist in cattle that undergo stress during handling and transport to the packing facility. However, there are no controlled experiments

reported characterizing the clinical presentations and blood profiles of slow-moving cattle at abattoirs directly within the production system. Therefore, the objective of this experiment was to define normal blood parameters in beef cattle following transportation and identify the clinical signs and blood abnormalities in cattle with decreased mobility at a commercial abattoir.

MATERIALS AND METHODS

The procedures used in these experiments were performed in cooperation with Cargill Meat Solutions Corporation (Wichita, KS) and were outlined in a protocol with the approval of the Kansas State University Institutional Animal Care and Use Committee.

Experiment 1

Experimental Design and Definitions. Beef steers (n = 60) were used in a cross sectional study. Blood samples were collected on a single day during exsanguination at a commercial abattoir from 20 cattle fed ractopamine hydrochloride (**RAC**; Optaflexx®; Elanco Animal Health, Greenfield, IN), zilpaterol hydrochloride (**ZIL**; Zilmax®, Merck Animal Health, Desoto, KS), or not fed βAA (**Natural**) as identified to the abattoir. Samples were collected by placing a PVC pipe containing clotting tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) under the carcass during exsanguination. Following collection, blood samples were placed on ice, transported approximately 4 h and centrifuged at 2,000 × g for 15 min. Serum supernatants were harvested, placed in -80°C storage until assays could be performed for lactate and creatine kinase (**CK**) concentrations.

Experiment 2

Experimental Design and Definitions. Beef cattle (n = 34) were enrolled in a case-control observational study conducted through collaboration with a commercial abattoir (Cargill Meat Solutions, Dodge City, KS) during the summer months of 2014. Animals were observed in the unloading and lairage areas at the abattoir and could be identified for enrollment in the study at any point from the time of unloading from the trailer until restraint for captive bolt euthanasia. Animals were enrolled as 1 of 2 mobility statuses by a trained observer: 1) Slow-mover/Non-ambulatory (**abnormal**) or 2) Normal cohort (**normal**). Slow-moving cattle were enrolled based on meeting 4 of the following 5 criteria: 1) Animal was reluctant to move and did not have obvious disease or injury, 2) Animal did not keep up with contemporaries, 3) Animal had shortened strides and appeared stiff, 4) Animal displayed nervous system abnormalities such as muscle tremors defined by muscular fasciculation of the trunk or extremities (Johnson et al., 2010), and 5) Animal exhibited physical indicators of stress such as increased respiratory rate or vocalization. Animals considered non-ambulatory were identified and deemed so by packing plant personnel. In cases where cattle became non-ambulatory (n = 2), euthanasia was performed by plant personnel using a captive bolt gun in accordance with the abattoirs standard operating procedures and venous blood samples were immediately collected. For all enrolled abnormal animals, a normal animal was subsequently selected and restrained in the side-chute for venous blood collection and measurements based on meeting the following 4 criteria: 1) The animal was a cohort to the abnormal animal, 2) There were no signs of obvious disease or injury present, 3) The animal was able to travel without difficultly determined by responsiveness to handling cues and hind feet placement being approximately in the same location as the front feet with each

stride, and 4) Phenotypical characteristics such as sex, hide color, and BW were the same as the abnormal animal.

Cattle Observation. Cattle were observed between of 0300 and 1800 h. Observations and enrollment occurred in the following situations: 1) during unloading from livestock trailers, 2) during movement from lairage pens to the center alley prior leading to the restrainer, and 3) any time an animal without obvious injury or disease became non-ambulatory during lairage. As a result, an animal could be identified as abnormal at 4 possible locations including the unloading dock, the alley from the unloading dock to the lairage pens, inside the lairage pens, and within the center alley leading to the restrainer.

Clinical Presentation. Physical signs of stress and other clinical descriptors were recorded for each animal at the time of enrollment for each animal included in the study. There was no limit to the number of descriptors an individual animal could possess, and descriptors included: 1) reluctance to move, 2) shortened strides, 3) unable to keep up with contemporaries 4) increased respiratory rate (≥ 100 respirations per min), 5) open-mouth breathing, 6) vocalization, 7) muscle tremors and 8) recumbent and unable to rise.

Blood Sampling and Physiological Measurements. Cattle were restrained in a side-chute in the lairage area for venous blood sampling and physiological measurements. Blood samples were collected from the jugular or tail vein using 10 mL uncoated clotting tubes with the Vacutainer collection system (Becton, Dickinson, and Company, Franklin Lakes, NJ). The samples were allowed to clot for at least 30 minutes following collection and then centrifuged at 2,000 × *g* for 15 min. Following centrifugation, serum was harvested and transferred into 5mL cryovials to be placed on ice for transportation to -80°C storage until assays were performed.

Respiratory rate (**RR**) was measured by a veterinarian thru flank visualization for 30 sec and multiplied by 2 to calculate respirations per min (rpm). Rectal temperature (**RT**) was measured in °F using a digital thermometer (GLA Agricultural Products, San Luis Obispo, CA) and converted to °C for the remainder of this report.

Assays

Serum was assayed for lactate, cortisol and full chemistry panels at the Kansas State Veterinary Diagnostic Laboratory (Manhattan, KS). Lactate was assayed using a Nova CCX analyzer (Nova CCX analyzer, Nova Biomedical, Waltham, MA). Full serum chemistry panels were performed to analyze potassium, glucose, bicarbonate (**HCO_3**) and CK using a Cobas c501 analyzer (Roche Diagnostics, Indianapolis, IN). Additionally, 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 cortisol was 3.6%.

Statistical Analysis

Data were analyzed using PROC GLIMMIX in SAS Version 9.3 (SAS Inst. Inc., Cary, NC). For experiment 1, the model included the fixed effects of βAA status and the random effect of source (feedlot). For experiment 2, sample size calculations were using lactate concentrations observed in experiment 1 with the POWER procedure in SAS. Calculations were based on achieving a power of 0.80 to detect mean group differences in a two-sided t-test with $\alpha = 0.05$. Within SAS, lactate concentrations were estimated to be 10 and 12 mmol/L for NORM and abnormal cattle, respectively, and standard deviation was estimated to be 2.0 mmol/L. The power

calculation required 17 pairs of animals to detect statistical significance with a 2.0 mmol/L difference between treatment group means.

Respiratory rate, rectal temperature, and blood parameters were analyzed with a linear mixed effects model using the GLIMMIX procedure. Mobility status (abnormal vs normal) was included as a fixed effect, and sample pair and day were included as random effects in the statistical model. Prevalence of abnormal cattle observed at each location was not normally distributed and therefore non-parametric analyses were performed using the GENMOD procedure in SAS. Physical signs of stress were analyzed using $\chi 2$ tests within the FREQ procedure. For prevalence of muscle tremors, vocalization, and recumbency, $\chi 2$ test were not valid due to the high event rates of "0" and 50% of the cells having expected counts less than 5, and for those variables frequency distributions were analyzed using the Fisher's Exact Test within the FREQ procedure.

Creatine kinase concentrations were logarithmically transformed and back transformed for reporting purposes in both experiments. For both experiments, comparisons between the least square means of factor level comparisons were computed using the PDIFF option of the LSMEANS statement. Statistically significant differences between estimates were determined by P-value ≤ 0.05 and tendencies were declared when $0.06 \leq P \leq 0.10$.

RESULTS AND DISCUSSION

Experiment 1

Exsanguination blood lactate concentrations were greater in natural cattle compared to cattle fed ZIL ($P \leq 0.05$), however there was no difference in RAC vs. natural or ZIL vs. RAC

cattle ($P > 0.05$). Hagenmaier et al. (2017b) reported similar lactate concentrations at the feedlot and during exsanguination in feedlot cattle fed RAC for 28 d or not fed βAA. Walker et al. (2006) and Van-Bibber-Krueger et al. (2015) also reported similar results where lactate concentrations at the feedlot did not differ compared to cattle not fed a βAA following a 28 d RAC and 20 d ZIL feeding period, respectively. Additionally, Abney et al., 2007 measured blood pH, pCO_2 and HCO_3 weekly in cattle being fed 200 mg RAC for 28 d vs. cattle not receiving βAA and reported no difference at any interval. Burson (2014) reported similar findings where blood was analyzed on d 5, 10, 15 and 20 of ZIL administration, although lactate itself was not measured in the latter 2 studies. Although different species, our findings are also consistent with studies conducted in swine which have demonstrated RAC did not increase lactate concentrations in blood collected following transportation to the slaughter facility or collected during exsanguination (Athayde et al., 2013; Puls et al., 2015).

Creatine kinase is an enzyme released during myocardial and skeletal muscle rhabdomyolysis and has been identified as a potential indicator of metabolic stress associated with βAA administration in cattle (Loneragan et al., 2014). Creatine kinase concentrations were greater in ZIL and RAC cattle compared to natural cattle ($P \leq 0.05$). Similar findings have been reported when cattle were fed ZIL 1.5X and 10X approved dosages (FDA, 2006). Furthermore, Hagenmaier et al. (2017b) reported greater increases in CK concentrations in cattle fed RAC vs. not fed a βAA from the feedlot to exsanguination at the abattoir, although CK was not different prior to transportation to the abattoir alone. Likewise, Burson (2014) found CK concentrations measured at the feedlot were not increased in cattle fed ZIL. Athayde et al. (2013) and Rocha et al. (2013) have reported increased CK in blood collected at exsanguination from swine fed RAC. It has been speculated that RAC increases CK concentrations in swine due to microlesions

causing enlarged muscle fibril diameters and stimulation of CK release (Athayde et al., 2013). Furthermore, we speculate RAC administration causes myofibril hypertrophy and, consequently, results in subsequent stretching and thinning of the sarcolemma allowing CK to permeate, leach into the interstitial space and eventually reach systemic circulation.

A major limitation of this study is the fact that βAA status is confounded with feedlot. Certainly, some degree of muscle fatigue and trauma is unavoidable during the process of sending cattle for slaughter. Several studies have reported large increases in CK following transportation to the abattoir in beef cattle (Warriss et al., 1995, Lambert et al., 2000, Buckham Sporer et al., 2008; Hagenmaier et al., 2017a). Additionally, Lambert et al. (2000) reported 24 h in lairage also increased CK concentrations, indicating both transport and lairage are significant sources of muscle fatigue or trauma or both.

Experiment 2

Tables 3-2 and 3-3 outline the location where abnormal cattle were observed and prevalence of individual clinical descriptors within each mobility status. More abnormal cattle were identified during handling from the loading dock to the lairage pen than at any other location (Table 3-2). This differs from Boyd et al. (2016) who reported that, in general, mobility continued to decrease following lairage on concrete slated floors and mobility scores were worse as cattle were being moved to the restrainer for stunning. With that being said, it is conceivable that some slow-moving cattle were not detected during movement from the lairage pen to the restrainer since the ability to visualize cattle and individually analyze their gait was significantly reduced and cattle had a relatively short distance to walk.

Unsurprisingly, abnormal cattle had a greater prevalence of cattle with shortened-strides, unable to keep up with contemporaries, and that became reluctant to move, consistent with our case definition ($P < 0.0001$; Table 3-3). Furthermore, abnormal cattle had greater respiratory rates ($P < 0.01$, Tables 3-3 and 3-4) and tended to have a greater number of cattle exhibiting muscle tremors ($P = 0.10$), however vocalization, open-mouth breathing and rectal temperature did not differ based on mobility status ($P > 0.05$; Tables 3-3 and 3-4). Hagenmaier et al. (2017a) reported that aggressive handling increased prevalence of muscle tremors at the feedlot, however this difference was not observed during lairage following transportation to the abattoir. Furthermore, muscle tremors, increased respiratory rates and open-mouth breathing are common acute signs of stress exhibited by swine affected with FPS.

Glucose concentrations tended ($P = 0.09$) to be greater in normal cattle, otherwise mobility status had no effect on blood variables ($P > 0.05$; Table 3-4). Glucose concentrations in the present study were similar to concentrations reported by Probst et al. (2014) and Hagenmaier et al. (2017b) in beef cattle measured at the abattoir. Creatine kinase concentrations were much lower in the present study than reported by Hagenmaier et al. (2017b) who collected blood samples both at the feedlot and then again following transport. Although we cannot be certain about the conditions at the feedlot and during transport prior to arrival at the abattoir for the cattle in the present study, it is hard to imagine they were individually restrained in a chute which may contribute to the greater CK concentrations observed by Hagenmaier et al. (2017b). Furthermore, CK concentrations observed in both of our experiments were most similar to baseline concentrations in the studies by Hagenmaier et al. (2017a, 2017b). Moreover, multiple studies have shown that transportation increases CK concentrations to similar levels as those observed in the cattle in this study (Warriss et al., 1995; Lambert et al., 2000; Hagenmaier et al.,

2017a). Cortisol concentrations were roughly 60% greater in cattle in this study compared to the values reported by Hagenmaier et al. (2017b) where venous blood was collected from feedlot cattle upon arrival to the abattoir. Cortisol concentrations in cattle have been reported to decrease following repeated handling and venipuncture (Hopster et al., 1999; Andrade et al., 2001), which may explain this large difference as the cattle reported in Hagenmaier et al. (2017b) had been handled over a 1,500 m course and restrained multiple times in a hydraulic chute at the feedlot prior to transport to the abattoir.

Two animals included in the study were euthanized in the lairage area and their data are reported individually in Table 3-5. One animal became non-ambulatory shortly after unloading from the trailer, and the other animal was the only slow-mover identified while being moved from the lairage pen to the center alley (Table 3-2). By study definition, neither non-ambulatory animal had an identifiable injury; although it is possible the animal identified on the unloading dock may have endured some sort of traumatic injury during transportation. Both non-ambulatory animals had substantially elevated creatine kinase concentrations compared with to the rest of the cattle enrolled in the study. Thomson et al. (2015) also reported CK concentrations were markedly elevated in non-ambulatory beef cattle at the abattoir. However, lactate concentrations were also increased well above normal values (mean = 25.6 mmol/L, ref. range 2 – 4 mmol/L) in non-ambulatory cattle those cattle; whereas lactate concentrations in non-ambulatory cattle in this study were substantially lower (Table 3-5). Similar to Thomson et al. (2015), studies conducted in swine have also reported substantially increased CK and lactate concentrations in downer pigs (Anderson et al., 2002). The cause for the extreme elevations in creatine kinase concentrations reported by Thomson et al. (2015) and in the non-ambulatory cattle in our study suggests unknown sources of severe muscle trauma or fatigue and warrants

further investigation. Possible identifiable critical control points include weigh-outs, loading at the feedlot, and available space during transport as these tend to be periods when cattle are handled improperly and contained in close quarters, and therefore the risk for muscle-crushing injuries is more likely.

One limitation of this study was the inability to achieve our desired sample size, as we only enrolled 17 pairs which was one-third of the desired sample size. Although their scoring system had not been described at the time of data collection, the enrollment criteria for slow-moving cattle in this study closely resembles the definitions of mobility scores 3 and 4 using the new 1-4 scoring system adopted by the North American Meat Institute (NAMI) where: 1 = normal, walks easily with no apparent lameness or change in gait; 2 = keeps up with normal cattle when the group is walking, exhibits 1 or more of the following: stiffness, shortened stride, or slight limp; 3 = lags behind normal cattle when the group is walking, exhibits 1 or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = extremely reluctant to move, even when encouraged by handlers. Keeping that in mind, recent data provided by Elanco Animal Health (Greenfield, IN) summarizing a 12 month mobility assessment of over 200,000 cattle presenting to abattoirs using the NAMI scoring system suggests only 0.65% of cattle receive a score of 3 or 4. Based on our observations, we speculate the prevalence is even smaller at the abattoir where data collection occurred in the current study. Further investigation is warranted to determine if prevalence of slow-moving cattle significantly differs across different abattoirs.

Most cattle having difficulties walking were observed during handling from the unloading dock to the lairage pen suggesting certain cattle may be exposed to stressors prior to and during transportation from the feedlot to the abattoir. This study had a limited sample size,

and differences in blood parameters based on mobility status of cattle presenting to abattoirs were not detected. Slow-moving cattle had greater respiratory rates and prevalence of muscle tremors, physical indicators of stress that have been reported in FPS and cattle such as those described by Thomson et al (2015). Additional research is needed to measure the prevalence of cattle presented to abattoirs with impaired mobility and characterize the clinical presentation and hematological profiles to better understand the pathophysiology of Fatigued Cattle Syndrome in order to develop mitigation strategies and protect the welfare of beef cattle presented to abattoirs.

Table 3-1. Least squared means for the effects of β-adrenergic agonist (βAA) status on blood lactate and creatine kinase concentrations at exsanguination.[1]

	βAA Status[1]			
Variable	Natural	RAC	ZIL	SEM
No. of observations[2]	20	20	20	-
Lactate, mmol/L	13.1[a]	12.0[a,b]	11.5[b]	0.48
Creatine kinase, U/L[3]	490[a]	667[b]	688[b]	45.0

[1]Blood samples were collected at exsanguination in cattle fed ractopamine hydrochloride (RAC), zilpaterol hydrochloride (ZIL), or not fed a βAA as identified to the abattoir.
[2]No. of observations = number of experimental units used to calculate treatment means. In this case, experimental unit = individual animal.
[3]Statistical analysis was conducted on log transformed values and treatment estimates were back-transformed for reporting purposes.
[a,b]Means without a common superscript are statistically different ($P \leq 0.05$).

Table 3-2. Location of enrollment observations for slow-moving/non-ambulatory (ABNORMAL) cattle.[1,2]

(n = 17 cattle)	**Unloading dock**	**Alley from unloading dock to lairage pen**	**Lairage pen**	**Alley from lairage pen to center alley**
No. (%)	4 (24%)[a]	14 (82%)[b]	3 (18%)[a]	1 (6%)[a]

[1]Animals could be observed at multiple locations for determination of mobility status.

[2]Each individual animal was assigned 1 of 2 mobility statuses: 1) Slow-mover/non-ambulatory (ABNORMAL) or 2) Normal Cohort (NORM). Slow-moving cattle were enrolled based on meeting 4 of the following 5 criteria: 1) Animal was reluctant to move and did not have obvious disease or injury, 2) Animal did not keep up with contemporaries, 3) Animal had shortened strides and appeared stiff, 4) Animal displayed nervous system abnormalities such as muscle tremors defined by muscular fasciculation of the trunk or extremities (Johnson et al., 2010), and 5) Animal exhibited physical indicators of stress such as increased respiratory rate or vocalization. Animals considered non-ambulatory were identified and deemed so by packing plant personnel.

[3]For all enrolled abnormal animals, a normal animal was subsequently selected and restrained in the side-chute for venous blood collection and measurements based on meeting the following 4 criteria: 1) The animal was a cohort to the abnormal animal, 2) There were no signs of obvious disease or injury present, 3) The animal was able to travel without difficultly determined hind feet placement being approximately in the same location as the front feet with each stride, and 4) The animal had the same phenotypical characteristics such as sex, hide color, and BW as the abnormal animal.

[a,b]Means without a common superscript are significantly different ($P \leq 0.05$).

Table 3-3. Count and prevalence of physical signs of stress within each mobility status (n = 17 per mobility status).[1]

Reluctant to move			Vocalization			Shortened Strides			Recumbent		
ABNORMAL[2]	NORMAL[3]	*P*-value[4]	ABNORMAL[2]	NORMAL[3]	*P*-value[4]	ABNORMAL[2]	NORMAL[3]	*P*-value[4]	ABNORMAL[2]	NORMAL[3]	*P*-value[4]
16 (94%)	0 (0%)	< 0.0001	1 (6%)	1 (6%)	1.0	15 (88%)	0 (0%)	< 0.0001	1 (6%)	0 (0%)	1.0

Muscle tremors			Increased respiratory rate (>100 bpm)			Open-mouth breathing			Unable to keep up with contemporaries		
ABNORMAL[2]	NORMAL[3]	*P*-value[4]	ABNORMAL[2]	NORMAL[3]	*P*-value[4]	ABNORMAL[2]	NORMAL[3]	*P*-value[4]	ABNORMAL[2]	NORMAL[3]	*P*-value[4]
4 (24%)	0 (0%)	0.10	10 (59%)	3 (18%)	0.01	0 (0%)	0 (0%)	1.0	15 (88%)	1 (6%)	< 0.0001

[1]There was no limit to the number of physical indicators of stress an animal could possess.
[2]Each individual animal was assigned 1 of 2 mobility statuses: 1) Slow-mover/non-ambulatory (ABNORMAL) or 2) Normal Cohort (NORM). Slow-moving cattle were enrolled based on meeting 4 of the following 5 criteria: 1) Animal was reluctant to move and did not have obvious disease or injury, 2) Animal did not keep up with contemporaries, 3) Animal had shortened strides and appeared stiff, 4) Animal displayed nervous system abnormalities such as muscle tremors defined by muscular fasciculation of the trunk or extremities (Johnson et al., 2010), and 5) Animal exhibited physical indicators of stress such as increased respiratory rate or vocalization. Animals considered non-ambulatory were identified and deemed so by packing plant personnel.
[3]For all enrolled abnormal animals, a normal animal was subsequently selected and restrained in the side-chute for venous blood collection and measurements based on meeting the following 4 criteria: 1) The animal was a cohort to the abnormal animal, 2) There were no signs of obvious disease or injury present, 3) The animal was able to travel without difficultly determined hind feet placement being approximately in the same location as the front feet with each stride, and 4) The animal had the same phenotypical characteristics such as sex, hide color, and BW as the abnormal animal.

Table 3-4. Least squared means for the effects of mobility status on physiological measurements and blood variables.[1]

	Mobility Status[2]			
Variable	ABNORMAL	NORMAL	SEM	*P*-value
No. of observations[3]	17	17	-	-
Respiratory rate, rpm[4]	94.6	77.3	7.51	< 0.01
Rectal temperature, ºC	40.0	39.9	0.06	0.62
Lactate, mmol/L	4.6	5.8	0.71	0.23
HCO_3, mmol/L[4]	21.3	21.2	0.89	0.91
Cortisol, ng/mL	50.9	44.4	4.44	0.31
Creatine kinase, U/L[5]	572	318	1,479	0.15
Glucose, mg/dL	98.2	112.7	7.37	0.09

[1]Blood collection and physiological measurement procedures were completed in a side-chute in the lairage area at the abattoir.
[2]Each individual animal was assigned 1 of 2 mobility statuses: 1) Slow-mover/non-ambulatory (ABNORMAL) or 2) Normal Cohort (NORM). Slow-moving cattle were enrolled based on meeting 4 of the following 5 criteria: 1) Animal was reluctant to move and did not have obvious disease or injury, 2) Animal did not keep up with contemporaries, 3) Animal had shortened strides and appeared stiff, 4) Animal displayed nervous system abnormalities such as muscle tremors defined by muscular fasciculation of the trunk or extremities (Johnson et al., 2010), and 5) Animal exhibited physical indicators of stress such as increased respiratory rate or vocalization. Animals considered non-ambulatory were identified and deemed so by packing plant personnel. For all enrolled abnormal animals, a normal animal was subsequently selected and restrained in the side-chute for venous blood collection and measurements based on meeting the following 4 criteria: 1) The animal was a cohort to the abnormal animal, 2) There were no signs of obvious disease or injury present, 3) The animal was able to travel without difficultly determined hind feet placement being approximately in the same location as the front feet with each stride, and 4) The animal had the same phenotypical characteristics such as sex, hide color, and BW as the abnormal animal.
[3]No. of observations = number of experimental units used to calculate treatment means. In this case, experimental unit = individual animal.
[4]rpm = respirations per min; HCO_3 = bicarbonate
[5]Statistical analysis was conducted on log transformed values and treatment estimates were back-transformed for reporting purposes.

Table 3-5. Blood parameters for non-ambulatory animals and differences from the mean of all abnormal animals.[1]

Variable	Animal 1	Animal 2
Location observed	Unloading dock	Center alley
Lactate, mmol/L	8.0 (+ 3.4)	1.9 (- 2.7)
HCO_3, mmol/L[2]	21.9 (+ 0.6)	20.1 (- 1.2)
Cortisol, ng/mL	45.1 (- 5.8)	55.6 (+ 4.7)
Creatine kinase, U/L	50,080 (+ 49,508)	10,610 (- 10,038)
Glucose, mg/dL	80 (- 18.2)	91 (- 8.2)

[1]Each individual animal was assigned 1 of 2 mobility statuses: 1) Slow-mover/non-ambulatory (ABNORMAL) or 2) Normal Cohort (NORM). Slow-moving cattle were enrolled based on meeting 4 of the following 5 criteria: 1) Animal was reluctant to move and did not have obvious disease or injury, 2) Animal did not keep up with contemporaries, 3) Animal had shortened strides and appeared stiff, 4) Animal displayed nervous system abnormalities such as muscle tremors defined by muscular fasciculation of the trunk or extremities (Johnson et al., 2010), and 5) Animal exhibited physical indicators of stress such as increased respiratory rate or vocalization. Animals deemed non-ambulatory were identified and deemed so by packing plant personnel.
[2]HCO_3 = bicarbonate

Chapter 4 - Effect of handling intensity at the time of transport for slaughter on physiological response and carcass characteristics in beef cattle fed ractopamine hydrochloride

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ABSTRACT

The effects of handling intensity on the physiological response and carcass characteristics of feedlot cattle fed ractopamine hydrochloride were evaluated at the time of transport to slaughter. Eighty steers (BW = 668 ± 36 kg) representing 10 lots of similar breed, frame size and degree of finish were blocked by lot, stratified by weight and randomly assigned to 1 of 2 handling intensities (**HI**) over a 1,600 m dirt alley course: 1) Low-stress handling (**LSH**) or 2) High-stress handling (**HSH**). For the LSH treatment, 4 penmates were kept at a walk with the use of a lead rider. For the HSH treatment, 4 penmates were kept at a minimum of a trot and received 2 applications of an electric prod (approximately 1 s per impulse) at 2 separate instances: first in the alley before post-handling sampling, and again during loading for transportation to the abattoir. Behavioral observations and physical indicators of stress were recorded a minimum of 1 h before handling (**baseline**), immediately after handling (**POSTHAND**) and while in lairage after a 200 km transport to the abattoir. Vital parameters were recorded at baseline and POSTHAND. Venous blood samples were collected via jugular venipuncture at baseline and POSTHAND, and mixed arterial and venous blood samples were collected during exsanguination at slaughter. Muscle tremors tended to be more prevalent in HSH cattle at

POSTHAND (P = 0.10). The HSH cattle tended to have greater POSTHAND heart rate (P = 0.08); however, there was no effect of HI on POSTHAND respiration rate or rectal temperature ($P \geq 0.34$). The HSH cattle had greater lactate, epinephrine, norepinephrine, cortisol and glucose concentrations at POSTHAND ($P \leq 0.02$). Additionally, HSH cattle had lower POSTHAND blood pH, bicarbonate, base excess and partial pressure carbon dioxide ($P < 0.0001$). Bicarbonate concentrations were greater in HSH cattle at slaughter (P = 0.05); however, there were no differences between HI treatments for the remaining blood variables ($P \geq 0.11$). Concentrations of stress hormones and CK were significantly greater at slaughter relative to baseline and POSTHAND for both LSH and HSH cattle ($P < 0.001$). These findings suggest cattle trotted without a lead rider develop metabolic acidosis, and illustrates the importance of low-stress handling at the time of transport for slaughter. Further research is warranted to develop strategies to mitigate stress at the time of transport and ensure the welfare of beef cattle presented to abattoirs.

Key words: beef cattle, beta-adrenergic agonist, low-stress handling, welfare

INTRODUCTION

Recent reports of cattle having difficulty walking at abattoirs have garnered significant attention and are an important animal welfare concern. Thomson et al. (2015) reported 2 events where cattle with elevated blood lactate and creatine kinase (CK) concentrations became non-responsive to handling and sloughed hoof walls during lairage. Anecdotal evidence proposing beta-adrenergic agonist (βAA) administration as one possible etiology led to the voluntary suspension of zilpaterol hydrochloride (Zilmax®, Merck Animal Health, Desoto, KS) from U.S. and Canadian markets. However, this condition termed Fatigued Cattle Syndrome (FCS;

Thomson et al. 2015) is likely multifactorial and strategies to prevent this condition are needed to protect the welfare of cattle presented for slaughter.

Fatigued Pig Syndrome (FPS) is a condition in swine where market hogs without obvious disease or injury have difficulty walking or become non-responsive to handling during transport for slaughter (Ritter et al., 2005). Development of metabolic acidosis and elevated stress hormones have been recognized as major contributors for FPS, and factors identified which contribute to FPS include porcine stress syndrome, hot weather, aggressive handling with electric prods, transport space, and administration of βAA at high dosages (Benjamin et al., 2001; Ritter et al., 2007, 2009b).

Frese et al. (2016a) reported fatigued market-weight cattle after aggressive handling with similar clinical signs and blood abnormalities as FPS-affected swine; however, there is little research reported which evaluates the relationship of handling techniques on physiological responses during transport for slaughter in contemporary cattle fed βAA. Therefore, our objective was to determine the effects of handling intensity during transport for slaughter on the physiological response and carcass characteristics of beef cattle fed ractopamine hydrochloride.

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).

Animals and Study Enrollment

Twelve Bos taurus steers were gate-sorted on d 0 at a commercial feedlot from 10 lots of similar age, breed type, frame size and degree of finish. Each lot had been raised in 30.5 × 65.0

m dirt floored pens in accordance with the feedlot's standardized operating procedures consistent with the Ag Guide (Guide for the Care and Use of Agricultural Animals in Research and Teaching, FASS 2010). Before the study, cattle were provided ad libitum access to water and fed a 65.7% DM finishing ration formulated to meet or exceed the requirements for growing cattle (NRC, 2000) with the inclusion of 400 mg·animal-1·d-1 ractopamine hydrochloride (RAC; Optaflexx®; Elanco Animal Health, Greenfield, IN) for 35 to 36 d. On the day of slaughter, 8 steers within each of the 10 lots (n = 80) deemed to be free of disease and lameness by an attending veterinarian were restrained in a hydraulic chute (Silencer®, Moly Manufacturing, Lorraine, KS) and enrolled in the study.

Experimental Design and Treatments

A total of 80 steers (BW = 668 ± 36 kg) were utilized over 2 d in a complete randomized block design study at a commercial feedlot during August of 2014, and subjected to 1 of 2 handling intensity (**HI**) treatments: 1) Low-stress handling (**LSH**) or 2) High-stress handling (**HSH**). The HI treatments were defined as:

LSH: Groups of 4 penmates handled together at a walk around a 1,600 m dirt alley course. One handler was ahead of the cattle on an all-terrain vehicle (**ATV**) serving as a lead rider and 1 handler was behind the cattle on an ATV to prevent cattle from reversing direction. Electric prods were not applied to LSH cattle at any time point during the study.

HSH: Groups of 4 penmates handled together at a minimum of a trot around the same 1,600 m course. Two handlers were behind the cattle on ATVs and no lead rider to prevent the cattle from trotting. An electric prod (DuraProd DX1 motor with 32" flexible fiberglass shaft, Miller Manufacturing Company, Savage, MN) was applied twice (approximately 1 s per impulse) at 2

separate instances: first while in the alley before restraint for post-handling procedures, and again during loading onto semi-trailers at the feedlot before transport to the abattoir.

Cattle were handled and slaughtered over 2 d after they had been fed RAC for a total of 35 to 36 d. The day that specific lots were handled was determined randomly using the RAND function in Microsoft Excel (Microsoft Corporation, Redmond, WA). Cattle from 6 lots were handled on d 1 (block 1; n = 48 steers), while the remaining 4 lots (block 2; n = 32 steers) were handled on d 2. Behavioral observations and physical indicators of stress were recorded a minimum of 1 h before handling (**baseline**), immediately after handling before chute restraint (**POSTHAND**), and while in lairage at the abattoir (**lairage**). Vital parameters were recorded before blood collection during chute restraint at baseline and POSTHAND. Venous blood samples were collected at baseline and POSTHAND, and mixed arterial and venous blood samples were collected during exsanguination at slaughter (**slaughter**). Cattle were walked in a low-stress manner with no electric prod usage during baseline procedures, which were performed simultaneous to study enrollment beginning at 0600 h each day. The first 8 steers eligible for enrollment within a lot were enrolled on the study until there was a total of 80 steers across d 1 (block 1; n = 48) and d 2 (block 2; n = 32). The remaining 4 steers from each lot were not included in the study. Electronic individual identification (**eID**) numbers were recorded and each animal enrolled received corresponding duplicate ear tags (Allflex USA Inc., Dallas, TX) with a unique study identification number. After enrollment, steers were blocked by lot, stratified by weight and allocated to groups of 4 which were randomly assigned to HI treatment. The HI groups within a replicate were handled consecutively to prevent circadian bias, and the order within the first replicate was determined by coin flip and then alternated between every

subsequent replicate. The mean time elapsed after completion of the handling course and before chute restraint of the last animal for POSTHAND measurements was 9.9 min over all replicates.

Handling Course Stop Criteria

This study utilized handling stop criteria to minimize pain and distress in experimental cattle. If an animal was deemed fatigued by the attending veterinarian, it was withdrawn from the HI treatment before completion of the handling course. The handling stop criteria enforced were similar to those used by Frese et al. (2016a), and an animal was deemed fatigued based on the following conditions: 1) resistance or inability to move, 2) non-ambulatory and or inability to stand without assistance, 3) heart rate exceeding 170 beats per min, 4) respiration rate exceeding 120 respirations per min, or 5) rectal temperature exceeding 42.2°C. Fatigued steers (n = 4) were removed from the HI treatment and restrained for POSTHAND procedures before being placed in recovery pens with penmates on the same HI treatment that completed the handling course (**non-fatigued**). Fatigued cattle were evaluated by the attending veterinarian who monitored their recovery and determined all 4 steers were fit for transportation to the abattoir with their penmates.

Handling/Transport/Lairage Conditions

Baseline sampling began at 0600 h on each day cattle were slaughtered. Cattle were handled down a 4.5 m wide dirt alley around a 110 × 90 m rectangular course for a maximum of 4 laps between 0800 and 1700 h on d 1, and between 0800 and 1200 h on d 2. The 8 steers representing both HI groups within a replicate were commingled after POSTHAND sampling, and loaded onto the same compartment of 2 floor aluminum semi-trailers so that LSH and HSH cattle were allotted the same amount of trailer space. During loading, HSH cattle were identified

and an electric prod was applied on the hip twice while ascending the load-out ramp. Cattle were then transported approximately 200 km to the abattoir in accordance with the National Cattlemen's Beef Association Beef Quality Assurance guidelines. Two trailers each containing 3 replicates were transported on d 1 (n = 24 steers per trailer), and 4 replicates were transported on a single trailer on d 2 (n = 32 steers). The mean time replicates spent on the trailer was 3 h. Upon arrival to the abattoir, cattle were unloaded and walked in a low-stress manner to sloping concrete-floored lairage pens for housing until slaughter. While in lairage, all cattle were provided water *ad libitum* and handled without the use of electric prods in accordance with the abattoir's standard operating procedures. All cattle enrolled in the study passed USDA ante-mortem inspection before being removed from lairage pens and walked 125 m to the stunner. The mean amount of time spent in lairage for replicates was 3.9 h.

Weather Data

Ambient temperature and relative humidity data were collected every 15 min using Veriteq data loggers (Vaisala Inc., Boulder, CO). Five loggers were used for data collection: 1 at the feedlot, 1 in the rear compartment of each trailer transporting experimental cattle (n = 3), and 1 in the lairage area at the abattoir. Descriptive weather data is reported for each transport groups' time spent at the feedlot, during transport, and while in lairage (Table 4-1).

Mobility Scores

Qualitative scoring (mobility, temperament, chute-exit), observations for physical signs of stress, and measurement of vital parameters were performed by trained personnel blinded to HI treatments during each applicable phase of the study. Cattle mobility was assessed at 3 time points (baseline, POSTHAND, and lairage) using a 4-point scoring system (NAMI, 2015) where:

1 = normal, walks easily with no apparent lameness or change in gait; 2 = keeps up with normal cattle when the group is walking, exhibits 1 or more of the following: stiffness, shortened stride, or slight limp; 3 = lags behind normal cattle when the group is walking, exhibits 1 or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = extremely reluctant to move, even when encouraged by handlers. The left rear foot was collected at slaughter from all cattle that received a lairage mobility score of 3 or greater (n = 4) for histopathologic evaluation. Grossly, sections were taken through the hoof to sample the stratum medium, stratum internum, laminar corium and the coronary dermis. The sections were routinely processed through different graded concentrations of alcohol, xylene and then into paraffin. After embedding in paraffin and facing it on the microtome, Nair (Church & Dwight Co., Inc., Princeton, NJ) was applied to help soften the hoof for cutting on the microtome. Sections were cut at 4 µm and stained routinely with hematoxylin and eosin for examination.

Behavioral Scoring and Physical Indicators of Stress

Temperament and chute-exit scores were recorded for each animal at baseline and POSTHAND time points. Temperament scores were assigned using a 4 point scoring system where: 1 = calm, no movement; 2 = restless shifting; 3 = continuous squirming and shaking of the chute; 4 = rearing, twisting, continuous violent struggle (Voisinet et al., 1997). Chute exit scores were assigned using a modified 4-point version of the scoring system by Lanier and Grandin (2002) where: 1 = walk; 2 = trot; 3 = run, 4 = jump. The prevalence of cattle exhibiting physical signs of stress were also recorded at baseline, POSTHAND and lairage time points and included open-mouth breathing (defined as the lower jaw being held open exposing the tongue and panting characterized by increased inhalation and exhalation effort with abdominal

involvement), vocalization, and muscle tremors (muscular fasciculation observed on the body trunk or extremities; Johnson et al., 2010).

Physiological Measurements and Blood Assays

Vital parameters (heart rate (**HR**), respiratory rate (**RR)** and rectal temperature (**RT**)) were measured at baseline and POSTHAND by a veterinarian. Heart rate was measured by auscultation of the left thorax and RR was measured by flank visualization. The number of heart beats and respirations were measured for a total of 30 s and multiplied by 2 to convert to beats per min (**bpm**) and respirations per min (**rpm**), respectively. Rectal temperatures were measured using a digital thermometer (GLA Agricultural Products, San Luis Obispo, CA) in degrees Fahrenheit and converted to degrees Celsius for reporting.

Blood samples were collected at baseline, POSTHAND, and during exsanguination at slaughter. Blood samples collected at baseline and POSTHAND were obtained via jugular venipuncture using a 60 mL syringe with a 14 gauge × 1 ½" needle. Mixed arterial and venous blood samples were obtained using 50 ml centrifuge tubes during exsanguination. Blood was not collected at slaughter for block 1 cattle due to the inability to keep up with the chain speed, and resultant failure to maintain the integrity and sequence of collected samples. Therefore, only block 2 cattle are represented by the variables reported and discussed from blood collected at slaughter.

Blood was immediately transferred into 3 types of 10 mL tubes after collection, including: clotting, 158 USP lithium-heparin and 18 mg spray-dried K_2EDTA tubes (Becton, Dickinson and Company, Franklin Lakes, NJ). Before centrifugation, baseline and POSTHAND whole blood from 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 Corporation, Princeton, NJ). Blood-gas assays were not performed on blood samples collected at slaughter because exposure to atmospheric gases could not be prevented during collection. Immediately after completion of iSTAT analyses, blood in lithium-heparin and K_2EDTA tubes were centrifuged at 1,400 × *g* for 15 min at 4ºC. Blood in clotting tubes was allowed to sit for 35 min to allow clot formation before being centrifuged at 1,400 × *g* for 15 min in room temperature. Supernatants were transferred into cryovials immediately after harvest and placed on dry ice for transport to -80 °C storage until analysis.

Plasma harvested from lithium-heparin tubes was assayed for lactate in singlet using a Nova CCX analyzer (Nova CCX analyzer, Nova Biomedical, Waltham, MA). Full serum chemistry panels were performed in singlet to analyze potassium, glucose, bicarbonate (**HCO_3**) 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). Plasma harvested from K_2EDTA tubes was assayed for epinephrine and norepinephrine in duplicate with a commercially available RIA kit (2-CAT RIA, #IB88165, IBL America, Minneapolis, MN). The intraassay CV for cortisol, epinephrine and norepinephrine was 3.9%, 8.1%, and 13.2%, respectively.

Semimembranosus Glycolytic Potential

Tissue samples were collected from the semimembranosus muscle (**SM**) on 73 of 80 steers post-rigor development (80 h after slaughter) and placed on dry ice for transport to -80° C

storage until analysis. The equation of Monin and Sellier (1985) was used for determination of glycolytic potential (**GP**) where: GP = 2 (glycogen + glucose + glucose-6-phosphate) + lactate. The assay was performed using the procedures described by Souza et al. (2011) where duplicate samples of SM were homogenized in 0.6 N perchloric acid and glycogen was converted to glucose by the addition of amyloglucosidase and 20 µL of 5.4 N KOH. Glucose assays were performed using a coupled enzyme assay (hexokinase and glucose-6-phosphate dehydrogenase, Sigma Aldrich, St. Louis, MO) which measured the conversion of nicotinamide adenine dinucleotide phosphate (NAD+) to the reduced form nicotinamide adenine dinucleotide phosphate (NADH) at 340 nm (Keppler and Decker, 1974). Fourteen cattle (LSH = 9; HSH = 5) had glucose equivalents (glycogen + glucose + glucose-6-phosphate) below the detectable limit of the assay (0.5 µmol/g). As a result, glucose equivalent values were designated as equal to 0 for these cattle in the statistical analysis. An enzyme assay was used to determine lactate concentrations which measured the conversion of NAD+ to NADH at 340 nm using lactate dehydrogenase. Concentrations of SM glucose equivalents, lactate, and total GP are expressed in µmol/g of muscle tissue.

Carcass Data

Experimental cattle were slaughtered in accordance with the abattoir's standard operating procedures and carcass data were provided for analysis. Carcasses were identified by recording the kill sequence by study ID and cross-matching study IDs with unique carcass numbers that also corresponded with an animal's eID. Carcasses were ribbed between the 12^{th} and 13^{th} rib and quality grades were determined in accordance with the official USDA beef grading standards using overall maturity combined with marbling score. The following carcass characteristics were obtained from the abattoir: HCW, LM area, 12^{th} rib fat thickness, marbling score and quality

grade. Dressing percentage was calculated by adjusting the final BW for a 4% shrink (BW × 0.96) and dividing the HCW by the adjusted BW.

Statistical Analysis

Data were analyzed using SAS version 9.3 (SAS Inst. Inc., Cary, NC). Cattle exhibiting physical signs of stress (vocalization, open-mouth breathing and muscle tremors) or having a temperament, chute-exit, or mobility score greater than 1 were considered an abnormal event, and χ^2 tests were performed for these variables using the FREQ procedure. Due to the high event rates of 0 and expected cell counts less than 5, the χ^2 test was not valid for analysis of baseline mobility scores and POSTHAND muscle tremors and mobility scores. Consequently, Fisher's exact tests were performed within the FREQ procedure to analyze the frequency distributions for these variables.

Continuous variables were analyzed using the GLIMMIX procedure. For all continuous variables, HI groups of 4 penmates were considered the experimental unit and the group mean was used for analysis. The 2 groups of 4 steers representing both HI treatments within a lot served as the replicate and day served as the blocking factor. The statistical model included the fixed effects of HI and random effects of replicate and block. The Kenward-Roger approximation was included in the model to calculate degrees of freedom. To normalize the data, CK concentrations were logarithmically transformed for all analyses. Logarithmic transformations were also used for the analysis of the difference between baseline and POSTHAND for pO_2, and for epinephrine and norepinephrine concentrations at each time point. However, the difference between time points for epinephrine and norepinephrine was performed on original values. All variables requiring logarithmic transformations were back transformed for

reporting and are denoted by superscripts within the data tables. Carcass quality grade was analyzed by calculating the prevalence of cattle grading choice or greater within HI groups of 4.

Comparisons between fatigued and non-fatigued HSH were also analyzed with individual animal serving as the experimental unit. The statistical model was similar to the model used to evaluate the effect of HI on continuous variables, except fatigue status replaced HI as a fixed effect. Due to all fatigued cattle coming from the HSH treatment, HI was removed from the model and data for LSH cattle were omitted from the analyses. Only POSTHAND comparisons are reported because all fatigued cattle occurred on d 1 and blood samples were not properly collected for these 4 steers at the time of slaughter.

In addition to HI and fatigue status, a secondary analysis was performed to evaluate the effect of time on the subset of variables measured in blood samples collected at slaughter in addition to baseline and POSTHAND (excludes iSTAT parameters). Time point was included as a fixed effect and HI was removed from the statistical model. Analysis of CK, epinephrine and norepinephrine were performed on logarithmically transformed data, as in the analysis of HI.

Comparisons between the least squares means of factor levels were performed using two-sided Student's t-tests with the PDIFF option of the LSMEANS statement. Statistically significant differences between estimates were determined by $P \leq 0.05$ and tendencies were declared when $0.06 \leq P \leq 0.10$.

RESULTS

Qualitative Scoring and Indicators of Stress

Mobility Scores. No differences in mobility scores greater than 1 were observed between HI treatments ($P \geq 0.24$; Table 4-2), and no steers from either HI received a mobility score of 4 at any time point.

Histological evaluation of the left rear foot from the 4 steers (LSH = 1; HSH = 3) receiving a mobility score of 3 at lairage revealed the LSH and 1 HSH animal had no appreciable lesions. One HSH animal had mild tubular and intratubular necrosis characterized by pyknotic cellular debris within the stratum medium. The final HSH animal had mild ballooning, degeneration and vacuolation of the epithelial cells in the stratum internum and occasional apoptotic keratinocytes within the stratum internum. Similar to the other HSH animal with histopathological lesions, this animal also had pyknotic debris and necrosis in the tubular and intratubular horns of the stratum medium. A few vessels with fibrinoid necrosis of the vascular wall and 1 with thickening of the vascular wall were present in the dermis near the level of the coronary band. Because only 1 foot could be collected from each animal with a mobility score of 3, and no feet were collected from cattle with mobility scores of 1 or 2, the absolute significance of these lesions in relation to mobility status cannot be determined.

Behavioral scoring and physical signs of stress. The prevalence of cattle exhibiting muscle tremors tended to be greater in HSH cattle POSTHAND ($P = 0.10$; Table 4-3). There was no effect of HI on temperament or chute-exit score, vocalization or open-mouth breathing at any time point ($P \geq 0.11$; Tables 4-2 and 4-3). No cattle were vocalizing, open-mouth breathing or exhibiting muscle tremors when observed during lairage.

Physiological response to the handling model

Baseline. No differences were detected between HI treatments at baseline for any blood variable or vital parameter ($P > 0.10$; Table 4-4). This was expected as cattle were commingled and handled in a low-stress manner before measurements.

POSTHAND. Mean time to complete the course was 25.6 min for LSH cattle and 10.1 min for HSH cattle (Fig. 4-1). High-stress handled cattle tended to have greater HR ($P = 0.08$; Table 4-4) and a greater increase in HR from baseline to POSTHAND than LSH cattle ($P = 0.10$; Table 4-5). However, there was no effect of HI on the POSTHAND value or change from baseline to POSTHAND for RR or RT ($P \geq 0.29$). The HSH cattle had greater POSTHAND values and greater increases from baseline to POSTHAND for lactate, epinephrine, norepinephrine, cortisol, glucose and pO_2 ($P \leq 0.02$), and lower POSTHAND values and greater decreases from baseline to POSTHAND for pH, HCO_3, pCO_2, TCO_2, and base excess ($P < 0.01$). Percent sO_2 and potassium concentrations tended to be greater POSTHAND ($P = 0.08$) and the increase in sO_2 from baseline to POSTHAND was greater in HSH cattle ($P = 0.02$). Creatine kinase concentration at POSTHAND and the change from baseline to POSTHAND for CK and potassium concentrations did not differ between HI ($P \geq 0.12$; Tables 4-4 and 4-5).

Four HSH steers became reluctant to move and non-responsive to handling pressure. As a result, these cattle were deemed fatigued and withdrawn from HI treatment before completion of the 1,600 m course. Their data were included for HSH treatment estimates; however, their POSTHAND values and the change from baseline to POSTHAND are also reported separately (Table 4-6). Compared to non-fatigued HSH cattle that completed the course, fatigued cattle had greater POSTHAND lactate and pO_2 ($P < 0.01$), tended to have greater norepinephrine ($P = 0.10$)

and glucose ($P = 0.08$), and had lower pH, HCO_3, TCO_2, and base excess ($P \leq 0.03$). Furthermore, fatigued cattle had greater increases from baseline to POSTHAND in HR, lactate, norepinephrine, glucose, pO_2 and potassium ($P \leq 0.04$), and tended to have greater increases in epinephrine ($P = 0.06$) and cortisol ($P = 0.09$). Finally, fatigued cattle had greater decreases from baseline to POSTHAND in pH, HCO_3, TCO_2, and base excess ($P < 0.01$).

Slaughter. Low-stress handled cattle had lower HCO_3 concentrations than HSH cattle ($P = 0.05$; Table 4-4), otherwise HI had no effect on any other blood variable measured at slaughter or on changes in blood variables from baseline to slaughter ($P \geq 0.11$; Tables 4-4 and 4-5). From POSTHAND to slaughter, LSH cattle had greater increases in lactate and glucose, and greater decreases in HCO_3 ($P \leq 0.02$). Handling intensity did not affect the change from POSTHAND to slaughter in epinephrine, norepinephrine, cortisol and CK ($P \geq 0.20$).

In the secondary analysis of blood parameters measured at each of the 3 time points, there was a temporal effect for lactate, CK, cortisol, epinephrine, and norepinephrine, where concentrations of each variable were greatest at slaughter compared to baseline and POSTHAND ($P < 0.05$, data not shown). There was also an effect of time on HCO_3 and glucose ($P < 0.01$), although these variables differed as concentrations were greatest at baseline and POSTHAND, respectively, compared to the other time points (data not shown).

Carcass characteristics

SM Glycolytic Potential and Carcass Quality. Lactate concentrations tended to be greater ($P = 0.08$) and glucose equivalents tended be lower ($P = 0.10$) in HSH SM tissue samples (Fig. 4-2), yet there was no effect of HI on overall SM glycolytic potential ($P = 0.58$). There were no effects of HI on any other carcass characteristic ($P \geq 0.17$; Table 4-7).

DISCUSSION

The welfare of animals presented to abattoirs is a growing welfare concern for all livestock species. Recent anecdotal reports from beef packers suggest an increased prevalence of cattle having difficulty walking and becoming non-responsive to handling while in lairage. In severe cases where cattle have sloughed hoof walls and required euthanasia, consistent blood abnormalities include elevated blood lactate and CK concentrations (Thomson et al., 2015). Although initial dialogues directed attention to administration of the βAA zilpaterol hydrochloride (ZIL), Boyd et al. (2015) reported that ZIL had minimal effects on the mobility of feedlot cattle and that the proportion of cattle with abnormal mobility scores was greatest after overnight lairage at the abattoir. These findings indicate other factors, such as long periods standing on concrete, can negatively impact mobility (Boyd et al., 2015). Such factors that may negatively affect the mobility of cattle presented for slaughter need to be considered to aid the development of strategies to mitigate ongoing welfare concerns, and includes the factors shown to predispose swine to FPS such as genetics, structural correctness, handling methods, and transportation to abattoir (Benjamin et al., 2001; Anderson et al., 2002; Ritter et al., 2009b).

González et al. (2012a) reported non-ambulatory cattle were more likely during episodes of hot weather and after long durations of transport. However, it is interesting to note the effects of handling methods on the physiological responses and mobility of contemporary cattle being presented to abattoirs have not been extensively studied, and to these authors' knowledge no studies have been reported which evaluate the effects of handling before transport for slaughter in cattle fed βAA. Furthermore, only a few studies have evaluated behavior during routine handling in cattle fed RAC which conclude no apprehension to handling as a result of administering the drug (Baszczak et al., 2006; Hagenmaier et al., 2017b).

The results of the current study suggest aggressive handling profoundly effects regulation of blood acid-base status and concentrations of stress hormones in finished beef cattle fed RAC. Although it was not measured in this study, it is likely beef cattle are less capable than other species at increasing oxygen consumption to levels adequate to meet metabolic demands during periods of high workloads (Kuhlmann et al., 1985). During periods of intense exercise, oxygen becomes depleted and chemoreceptors throughout the vasculature trigger the body to increase respiratory rate to decrease blood CO_2 and increase oxygen availability for metabolism in the muscle. If oxygen availability is insufficient, energy production shifts from mitochondrial oxidative metabolism to cytoplasmic anaerobic glycolysis, which is much less efficient at generating ATP and also produces lactate, hydrogen and heat (Kreisberg, 1984). Increased concentrations of blood lactate and decreased pH, HCO_3 and pCO_2 concentrations in our study confirm cattle experienced metabolic acidosis, as opposed to respiratory acidosis which is characterized by increased blood pCO_2 due to inadequate ventilation (Nagy et al., 2006).

Our findings are supported by earlier studies where exercised cattle developed metabolic acidosis (Holmes et al. 1973; Gustin et al., 1988; Frese et al., 2016a). Gustin et al. (1988) reported Fresian calves were able to adequately increase oxygen consumption during exercise and prevent development of acidosis, whereas double-muscled calves developed metabolic acidosis attributable to muscle type (greater proportion of type II, glycolytic white muscle fibers) and the inability to sufficiently increase oxygen consumption to meet metabolic requirements. Kuhlmann et al. (1985) and Gustin et al. (1988) reported decreased pO_2 and increased pCO_2 in light-weight Hereford and Fresian calves after exercise. Escalera-Valente et al. (2013) reported much more severe acidosis after intense exercise in 4 to 5 yr old Libia bulls as lactate concentrations were more than triple and pH dropped substantially greater compared to the

current study. On the other hand, Frese et al. (2016a) and our study report the opposite as pCO_2 decreased and pO_2 increased in HSH cattle. Collectively, these findings suggest genetics, BW, workload, degree of finish, and muscle character may play an important role in the physiological response to exercise. Of equal importance is the fact no appreciable deviations to blood acid-base status were noted when cattle were handled 1,600 m in a low-stress manner with the use of a lead rider in the current study.

It has been documented cattle administered βAA have greater resting heart rates (Bruckmaier and Blum, 1992; Frese et al., 2016b), most likely attributable to interactions with myocardial β_1 receptors, although arterial vasodilation, hypotension and reflex tachycardia must also be considered (Mersmann et al., 1989). Furthermore, it is well-established stimulation of the sympathetic nervous system in response to exercise increases HR (Hays et al., 1978; Kuhlmann et al., 1985; Bruckmaier and Blum, 1992). Gruber et al. (2010) reported that cattle exhibiting adverse behavioral reactions during chute restraint had correlated increases in HR, yet chute behavioral score was a poor predictor of RR. In a study conducted by Kuhlman et al. (1985) in Hereford calves, respiratory rate increased with exercise, but plateaued near 60 rpm. Multiple studies reported cattle increase tidal volume during exercise (Kuhlmann et al., 1985; Piguet et al. 1994), which may explain why HI had no effect on RR. The absence of differences in RT between HI in the current study is supported by other reports where exercise did not affect rectal temperature (Kuhlmann et al., 1985; Bruckmaier and Blum, 1992). Conversely, Frese et al. (2016a) utilized a similar handling model as our study and reported greater RT in aggressively handled cattle after 1,540 m. The authors of the current study speculate this could be explained by differences in environment ambient temperatures, however weather data were not reported in the other study.

The greater elevation in stress hormone concentrations in HSH cattle was not surprising, as this has been reported in previous studies (Kuhlmann et al., 1985; Blum and Eichinger, 1988; Frese et al., 2016a). This increase is well-documented to occur through upregulation of the sympathetic nervous system and hypothalamic-pituitary-adrenal (**HPA**) axis activation during periods of excitement or fear. As a review, stressful events cause secretion of corticotropin releasing hormone (CRH) from the hypothalamus, secondary release of ACTH from the anterior pituitary, and subsequent release of cortisol and catecholamines from the adrenal cortex and medulla (Axelrod and Reisine, 1984; Gruber et al., 2010; Carroll and Sanchez, 2014). Holmes et al. (1973) reported double-muscled cattle injected with epinephrine had elevated lactate concentrations and became difficult to handle, suggesting elevated catecholamines associated with excitability or stress play an important role in lactate production, the development of metabolic acidosis, and, consequently, apprehension to handling. Gruber et al. (2010) reported similar findings where increased epinephrine concentrations were associated with greater lactate concentrations in cattle exposed to handling and transportation stress. Warner et al. (2007) reported increased plasma lactate concentrations in cattle exposed to electric prod usage 15 min before slaughter. Bruckmaier and Blum (1992) speculated skeletal muscle glycogenolysis is mediated by β receptors, thereby increasing lactate production during periods of high catecholamine concentrations. Blum and Eichinger (1988) speculated a possible causal relationship between elevated epinephrine and norepinephrine concentrations leading to increased oxygen consumption to compensate for greater metabolic rates during stress, which is consistent with our findings as HSH and fatigued cattle had greater pO_2 compared to LSH and non-fatigued HSH cattle, respectively.

In a study reported by Mitchell et al. (1988) where cattle were subjected to handling, restraint, transport, and slaughter stressors, cattle had greatest cortisol concentrations after handling, while catecholamines remained unchanged from basal levels. In the same study, cortisol concentrations were lowest after captive bolt euthanasia, and epinephrine and norepinephrine individually increased 6-fold after transport and captive bolt euthanasia, respectively. Because of this, the authors speculate the stress response displays 2 separate patterns: 1) an HPA phase linked to environmental stress (handling) where cortisol is predominately secreted, and 2) a sympathomedullary phase linked to neurogenic stress (transport, captive bolt penetration) where catecholamines are primarily secreted. The results of the current study differed as a biphasic response was not observed. Instead, although cattle from each HI had increased epinephrine, norepinephrine, and cortisol concentrations after handling, concentrations of all 3 hormones were greatest at slaughter regardless of HI. Given the large magnitude of increase in cortisol and catecholamine concentrations at slaughter relative to baseline and POSTHAND, this is most likely attributable to captive bolt destruction of neural tissue and a corresponding surge in sympathetic tone (Althen et al., 1977).

The greater increases in glucose concentrations in response to handling observed in HSH cattle is likely attributed to hypercortisolemia and increased catecholamines leading to mobilization of glucose through hepatic glycogenolysis and gluconeogenesis (Mitchell et al., 1988; Gruber et al. 2010). Additionally, the authors of the current study speculate the magnitude of increase in glucose levels measured in blood collected at slaughter was not as high relative to the large increase in catecholamines due to minimal time for glucose mobilization to occur after euthanasia and before exsanguination.

Creatine kinase is a muscle isoenzyme that enters systemic circulation through the interstitial space secondary to rhabdomyolysis from physical exertion or trauma (Gruber et al., 2010), and reaches maximum concentrations in the blood approximately 6 h after muscle damage occurs. Although concentrations at each time point and the changes between time points were not affected by HI for CK, it should be pointed out that concentrations were greater than published reference ranges for cattle (159 – 332 U/L, Thomson et al., 2015) in both LSH and HSH cattle at POSTHAND (1,851 vs. 1,278 U/L) and slaughter (7,810 vs. 8,502 U/L). Thomson et al. (2015) reported similar CK concentrations at the abattoir in non-ambulatory cattle that had sloughed hoof walls; however, no cattle sloughed hooves in the present study. Frese et al. (2016a) reported HI did not impact CK concentrations when measured immediately subsequent to and after 2 h rest post handling over a 1,540 m course. Warriss et al. (1995) and Lambert et al. (2000) reported elevated CK concentrations in market-weight beef cattle after transportation and lairage. It is probable some degree of muscle trauma or fatigue or both was experienced during chute-restraint and transport in the current study. Given the temporal component of CK, the authors speculate this may have contributed to the large CK concentrations observed at POSTHAND and slaughter and obscured the effects of HI. Regardless, the consequences of failing to prevent avoidable sources of muscle damage and fatigue such as crushing injuries during weigh-out and long-haul transportation on cattle mobility warrants further investigation.

Although the primary focus for the current study was on the effect of HI on changes in blood parameters across time points, it should be noted that baseline vital parameters and blood variables were comparable to values reported in a recent study where blood was collected via jugular venipuncture from late day feedlot cattle that had not been fed βAA (Frese et al., 2016a). Furthermore, Frese et al. (2016a) also reported fatigued cattle with comparable hematological

abnormalities as the fatigued cattle in the current study. Fatigued cattle developed more severe acidosis evident by greater concentrations of blood lactate, lower blood pH, and lower pCO_2 compared to non-fatigued HSH cattle. Additionally, our findings where fatigued cattle had greater increases in potassium agree with Ivers et al. (2002) who reported increased extracellular potassium in downer pigs at abattoirs. Elevations in blood potassium concentration during exercise can be explained by multiple mechanisms, including release from skeletal myocytes during muscle contraction, and acidosis causing an intracellular infiltration of hydrogen and consequential extracellular shift of potassium to maintain normal resting membrane potentials. Kuhlmann et al. (1985) reported calves can increase serum potassium concentrations by 40% in response to exercise, which is noteworthy as severe cases of hyperkalemia have the potential to elicit profound effects on cardiovascular function by limiting myocardial contractility and thereby limiting the ability to increase stroke volume, blood perfusion, and oxygen delivery to the skeletal muscles involved in locomotion. Lastly, it is important to note that fatigued cattle only occurred on d 1 in our study, which had considerably greater ambient temperatures during handling periods than d 2 and aligns with industry reports suggesting heat stress plays a key role in the development of FCS.

Availability of muscle glycogen for post-mortem glycolysis is the major determinant for ultimate carcass pH (Lambert et al., 2000; Apple et al., 2006; Coombes et al., 2014). Pre-slaughter stressors which can cause muscle fatigue such as improper handling and transportation have potential to deplete muscle glycogen stores so much that the glycogen available for lactate production through glycolysis is inadequate to effectively decrease carcass pH post-mortem and prevent dark cutters (Scanga et al., 1997; Lambert et al., 2000; Coombes et al., 2014). However, Apple et al. (2006) reported treadmill exercise did not affect LM glycogen and lactate

concentrations in light-weight Holstein calves. In separate studies where cattle were characterized as stressed based on flight speeds (Coombes et al., 2014) and chute scores (Gruber et al., 2010), similar findings were reported as carcass pH and dark cutting incidence did not differ. Likewise, Lambert et al. (2000) reported that 4 h transport and 24 h lairage had no effect on longissimus dorsi muscle glycogen in Hereford-cross steers. Research conducted in swine has also reported aggressive handling had no effect on total muscle glycolytic potential (Bertol et al., 2005; Ritter et al., 2009b), although other studies have described decreased glycolytic potential associated with feed withdrawal, stressful handling, and electric prod use (D'Souza et al., 1998; Hambrecht et al., 2004; Bertol et al., 2011).

In conclusion, the findings of the current study demonstrate finished beef cattle fed ractopamine hydrochloride develop metabolic acidosis when trotted 1,600 m, similar to the cattle not fed βAA in a recent study reported by Frese et al. (2016a). From our field experience, the trot at which HSH cattle were handled in this study is not uncommon to occur in feedlots and illustrates the need for proper training in low-stress cattle handling. Furthermore, the resemblances in clinical presentation and blood abnormalities of cattle affected with FCS compared to FPS-affected pigs suggest this condition is also multi-factorial. Practical approaches at the feedlot to reduce the risk for FCS include staging pens at re-implanting to position heavy cattle near load-out facilities, moving cattle with a lead rider, identifying cattle unfit for transport, and minimizing durations cattle are required to stand on hard surfaces such as concrete-floored holding pens. Further investigation is warranted to define the prevalence of cattle presented to abattoirs with compromised mobility and identify contributing factors to develop additional strategies for mitigating this problem.

Table 4-1. Maximum, minimum, and mean ambient temperature, relative humidity and temperature humidity index (THI) at the feedlot, abattoir, and on the trucks for transport groups[1].

		Ambient temperature, ºC			Relative humidity			THI[2]		
Site	No. of Reps[3]	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
Feedlot[4]										
Group 1	3	40.5	17.9	28.5	73.7	24.1	47.2	85.1	63.2	74.7
Group 2	3	43.4	17.9	32.7	73.7	17.6	39.0	86.5	63.2	78.2
Group 3	4	32.1	20.0	25.0	81.0	52.2	69.6	81.5	66.8	73.3
Truck[5]										
Group 1	3	36.7	32.2	34.2	40.3	28.8	36.2	83.4	78.6	80.9
Group 2	3	36.7	35.0	35.9	38.5	28.1	32.5	83.0	80.6	82.1
Group 3	4	38.4	29.2	33.3	62.8	34.1	47.8	85.5	78.2	81.7
Lairage[6]										
Group 1	3	39.4	29.5	35.0	51.0	29.5	36.4	85.5	75.8	81.8
Group 2	3	36.2	29.5	32.3	47.1	34.3	41.2	82.9	77.1	79.5
Group 3	4	39.5	31.5	26.0	80.3	57.0	27.2	86.4	75.7	80.7

[1]Data was collected using Veriteq data loggers (Vaisala Inc., Boulder, CO). Groups 1 and 2 were transported on d 1 and group 3 was transported on d 2.
[2]THI was calculated using the same equation as Mader et al. (2006) where THI = (0.8 × TA) + [(RH × 0.01) × (TA – 14.4)] + 46.4; TA = Ambient Temperature; RH = Relative Humidity.
[3]Rep = 8 penmates comprised of pairs of handling intensity treatment groups of 4 representing both handling intensity treatments (n = 10).
[4]Data was collected at the feedlot throughout the time animals were housed at the feedlot on d 1 and 2.
[5]Data was collected on the trucks from the time cattle were loaded at the feedlot until unloaded at the abattoir.
[6]Data was collected in the holding pen area throughout the time cattle spent in lairage at the abattoir.

Table 4-2. Least squared means for the effects of different handling intensities (HI) on temperament, chute-exit, and mobility scores in beef cattle at baseline, POSTHAND and lairage time points.

	Baseline[1]						POSTHAND[2]						Lairage[3]					
	Temperament score[6]																	
HI treatment[4]	1	2	3	4	% > 1	*P*-value[5]	1	2	3	4	% > 1	*P*-value[5]						
LSH	26	14	0	0	35.0	0.81	27	13	0	0	32.5	0.11						
HSH	27	13	0	0	32.5		20	19	1	0	50.0		N/A					
Total	53	27	0	0	33.8	(n = 80)	47	32	1	0	41.3	(n = 80)						
	Chute-exit score[7]																	
HI treatment[4]	1	2	3	4	% > 1	*P*-value[5]	1	2	3	4	% > 1	*P*-value[5]						
LSH	22	17	1	0	45.0	0.82	25	13	1	1	37.5	0.64						
HSH	23	15	2	0	42.5		26	9	3	1	33.3		N/A					
Total	45	32	3	0	43.8	(n = 80)	51	22	4	2	35.4	(n = 79)						
	Mobility score[8]																	
HI treatment[4]	1	2	3	4	% > 1	*P*-value[5]	1	2	3	4	% > 1	*P*-value[5]	1	2	3	4	% > 1	*P*-value[5]
LSH	37	3	0	0	7.5	0.24	34	2	0	0	5.5	1.0	30	9	1	0	25.0	0.59
HSH	40	0	0	0	0.0		29	3	0	0	9.4		32	5	3	0	20.0	
Total	77	3	0	0	3.8	(n = 80)	63	5	0	0	7.4	(n = 68)	62	14	4	0	22.5	(n = 80)

[1]Baseline observations were made a minimum of 1 h prior to HI treatment.

[2]POSTHAND observations were made immediately following the application of HI treatments over a 1,600 m handling course.

[3]Lairage observations were made approximately 1 h prior to slaughter while cattle were standing in the lairage area at the abattoir.

[4]On the day of slaughter, cattle were handled in groups of 4 randomly assigned to 1 of 2 HI treatments: 1) low-stress handling (LSH) or 2) high-stress handling (HSH). Cattle were handled around a 400 m dirt alley course for a maximum of 4 laps and either kept at a walk with no electric prod use (LSH) or kept at a minimum of a trot with electric prods applied after handling and during loading at the feedlot (HSH).

[5]Scores greater than 1 was considered an abnormal event and statistical analyses were performed using the χ^2 and Fisher's exact test in PROC FREQ of SAS. Statistical significance was declared when $P \leq 0.05$; tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.

[6]Temperament scores were assigned using a 4 point scoring system where: 1 = Calm, no movement; 2 = Restless shifting; 3 = Continuous squirming and shaking of the chute; 4 = Rearing, twisting, continuous violent struggle (Voisinet et al., 1997).

[7]Chute-exit scores were assigned using a 4 point scoring system where: 1 = Walk; 2 = Trot; 3 = Run, 4 = Jump (Lanier and Grandin, 2002). One HSH steer did not have a POSTHAND chute-exit score recorded and was treated as a missing data point for analysis.

[8]Mobility scores were assigned using a 4 point scoring system where: 1 = Normal, walks easily with no apparent lameness or change in gait; 2 = Keeps up with normal cattle when the group is walking, exhibits 1 or more of the following: stiffness, shortened stride, or slight limp; 3 = Lags behind normal cattle when the group is walking, exhibits 1 or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = Extremely reluctant to move, even when encouraged by handlers (NAMI, 2015). Four LSH animals and 8 HSH animals did not have mobility scores reported and were treated as missing data points for analysis.

Table 4-3. Least squared means for the effects of different handling intensities (HI) on the frequency of cattle exhibiting acute signs of stress at baseline, POSTHAND and lairage time points.

	Baseline[1]			POSTHAND[2]			Lairage[3]		
	Open-mouth breathing[8]								
	HI treatment[4]			HI treatment[4]			HI treatment[4]		
	LSH	HSH	*P*-value[5]	LSH	HSH	*P*-value[5]	LSH	HSH	*P*-value[5]
No. of observations[6]	40	40	1.00	40	40	1.00	40	40	1.00
No. abnormal[7]	0	0		3	3		0	0	
Prevalence, %	0	0	(n = 80)	7.5	7.5	(n = 79)	0	0	(n = 80)
	Muscle tremors[9]								
	HI treatment[4]			HI treatment[4]			HI treatment[4]		
	LSH	HSH	*P*-value[5]	LSH	HSH	*P*-value[5]	LSH	HSH	*P*-value[5]
No. of observations[6]	40	40	1.00	40	40	0.10	40	40	1.00
No. of abnormal[7]	0	0		1	6		0	0	
Prevalence, %	0	0	(n = 80)	2.5	15.0	(n = 79)	0	0	(n = 80)
	Vocalization								
	HI treatment[4]			HI treatment[4]			HI treatment[4]		
	LSH	HSH	*P*-value[5]	LSH	HSH	*P*-value[5]	LSH	HSH	*P*-value[5]
No. of observations[6]	40	40	0.22	40	40	0.79	40	40	1.00
No. of abnormal[7]	14	9		10	9		0	0	
Prevalence, %	35.0	22.5	(n = 80)	25.0	22.5	(n = 79)	0	0	(n = 80)

[1]Baseline observations were made a minimum of 1 h prior to HI treatment.
[2]POSTHAND observations were made immediately following the application of HI treatments over a 1,600 m handling course.
[3]Lairage observations were made approximately 1 h prior to slaughter while cattle were standing in the lairage area at the abattoir.
[4]On the day of slaughter, cattle were handled in groups of 4 randomly assigned to 1 of 2 HI treatments: 1) low-stress handling (LSH) or 2) high-stress handling (HSH). Cattle were handled around a 400 m dirt alley course for a maximum of 4 laps and either kept at a walk with no electric prod use (LSH) or kept at a minimum of a trot with electric prods applied after handling and during loading at the feedlot (HSH).
[5]Statistical analyses were performed using the χ^2 and Fisher's exact test in PROC FREQ of SAS. Statistical significance was declared when $P \leq 0.05$; tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.
[6]No. of observations = no. of animals observed.
[7]No. abnormal = no. of animals exhibiting trait.
[8]Open mouth breathing observations were performed by a trained observer and are defined as the upper and lower jaw being held open exposing the tongue and panting characterized by increased inhalation and exhalation effort with abdominal involvement.
[9]Muscle tremor observations were performed by a trained observer and are defined as muscular fasciculations observed on the body trunk or extremities.

Table 4-4. Least squares means for the effect of HI on baseline, POSTHAND and slaughter physiological measurements and blood variables in beef cattle.

	Baseline[1]				POSTHAND[2]				Slaughter[3]			
	HI treatment[4]				HI treatment[4]				HI treatment[4]			
Variable	LSH	HSH	SEM[5]	*P*-value[6]	LSH	HSH	SEM[5]	*P*-value[6]	LSH	HSH	SEM[5]	*P*-value[6]
BW, kg	670	667	4.7	0.51	-	-	-	-	-	-	-	-
Vital parameters												
Respiratory rate, rpm[7]	50.9	53.0	1.94	0.44	75.7	76.7	2.84	0.82	-	-	-	-
Heart rate, bpm[7]	76.6	79.0	2.87	0.57	86.2	99.8	5.27	0.08	-	-	-	-
Rectal temperature, ºC	39.6	39.6	0.18	0.89	40.3	40.5	0.12	0.34	-	-	-	-
Blood variables												
Lactate, mmol/L	5.8	5.2	0.74	0.49	4.5	14.3	0.98	< 0.0001	12.2	11.0	0.45	0.11
HCO_3, mmol/L[8]	25.4	25.4	1.28	0.97	25.9	17.1	0.77	< 0.0001	23.8	24.4	0.27	0.05
pH	7.38	7.38	0.009	0.94	7.44	7.29	0.015	< 0.0001	-	-	-	-
pCO_2, mmHg[8]	47.1	46.9	2.09	0.86	40.6	34.5	1.12	< 0.0001	-	-	-	-
TCO_2, mmol/L[8]	29.0	28.7	1.30	0.74	28.5	18.2	0.80	< 0.0001	-	-	-	-
pO_2, mmHg[8]	29.5	29.3	0.67	0.84	31.8	39.0	3.28	< 0.01	-	-	-	-
sO_2, %[8]	53.3	53.1	1.55	0.90	61.6	65.2	5.34	0.08	-	-	-	-
Base excess, mmol/L	2.3	2.1	1.35	0.82	3.1	- 9.6	1.01	< 0.0001	-	-	-	-
Epinephrine, pg/mL[9]	1,176	1,045	366.8	0.61	1,383	2,348	257.0	0.02	5,657	5,987	524.8	0.60
Norepinephrine, pg/mL[9]	1,239	1,178	387.4	0.82	1,071	2,373	414.6	0.001	7,346	8,327	1,157.8	0.51
Cortisol, ng/mL	39.1	37.5	4.63	0.73	41.1	49.0	2.20	0.02	68.4	63.0	6.01	0.56
Creatine kinase, U/L[9]	266	250	15.1	0.58	1,851	1,278	263.3	0.12	7,810	8,502	4,744.1	0.90
Glucose, mg/dL	101	95	4.3	0.29	102	260	12.4	< 0.0001	148	131	9.8	0.18
Potassium, mmol/L	5.06	5.17	0.072	0.30	5.13	5.30	0.070	0.08	7.36	7.34	0.184	0.88

[1]Baseline measurements occurred a minimum of 1 h before HI treatment.
[2]POSTHAND measurements occurred immediately after the application of HI treatments over a 1,600 m handling course.
[3]Cattle were slaughtered after a 200 km transport to the abattoir and mean 3.9 h lairage. Blood gas analysis was not performed on samples collected during exsanguination because blood was exposed to atmospheric gases during collection. Blood samples were not collected at slaughter for d 1 cattle (n = 6 replications).
[4]HI = handling intensity. Cattle were handled over 1,600 m in groups of 4 randomly assigned to 1 of 2 HI treatments: 1) low-stress handling (LSH) or 2) high-stress handling (HSH).
[5]SEM = largest SE in the analysis.
[6]Represents the main effects of HI. Statistical significance was declared when $P \leq 0.05$; tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.
[7]rpm = respirations per min; bpm = beats per min.
[8]HCO_3 = bicarbonate; pCO_2 = partial pressure carbon dioxide; TCO_2 = total carbon dioxide; pO_2 = partial pressure oxygen; sO_2 = saturated oxygen.
[9]Statistical analysis was conducted on log transformed values and treatment estimates were back-transformed for reporting purposes.

Table 4-5. Least squares means for the effect of HI on the change in beef cattle physiological measurements and blood variables among baseline, POSTHAND and slaughter time points on the day of slaughter.[1]

	POSTHAND – Baseline[2]				Slaughter – Baseline[3]				Slaughter – POSTHAND[4]			
	HI treatment[5]				HI treatment[5]				HI treatment[5]			
Variable	LSH	HSH	SEM[6]	*P*-value[7]	LSH	HSH	SEM[6]	*P*-value[7]	LSH	HSH	SEM[6]	*P*-value[7]
Vital parameters												
Respiratory rate, rpm[8]	24.8	23.7	2.80	0.78	-	-	-	-	-	-	-	-
Heart rate, bpm[8]	9.6	20.9	4.64	0.10	-	-	-	-	-	-	-	-
Rectal temperature, ºC	0.7	0.9	0.25	0.17	-	-	-	-	-	-	-	-
Blood variables												
Lactate, mmol/L	- 1.4	9.0	1.37	< 0.0001	5.5	5.4	1.46	0.95	7.2	- 1.4	1.69	0.02
HCO_3, mmol/L[9]	0.5	- 8.3	1.90	< 0.0001	- 0.3	0.1	1.24	0.82	- 1.8	5.6	0.76	< 0.001
pH	0.06	- 0.09	0.018	< 0.0001	-	-	-	-	-	-	-	-
pCO_2, mmHg[9]	- 6.2	- 12.4	2.97	< 0.01	-	-	-	-	-	-	-	-
TCO_2, mmol/L[9]	- 0.4	- 10.6	1.74	< 0.0001	-	-	-	-	-	-	-	-
pO_2, mmHg[9,10]	2.8	8.1	1.42	0.01	-	-	-	-	-	-	-	-
sO_2, %[9]	8.2	12.6	4.33	0.02	-	-	-	-	-	-	-	-
Base excess, mmol/L	0.9	- 11.7	1.87	< 0.0001	-	-	-	-	-	-	-	-
Epinephrine, pg/mL	152	1,131	535.3	0.02	3,431	4,371	882.1	0.48	3,544	4,031	824.8	0.70
Norepinephrine, pg/mL	- 445	1,013	1,174.3	0.02	5,015	5,879	1,138.0	0.61	6,713	5,969	1,134.0	0.64
Cortisol, ng/mL	1.6	11.0	5.59	< 0.01	27.5	18.6	5.37	0.20	27.9	16.7	5.69	0.20
Creatine kinase, U/L[10]	1,537	973	256.0	0.13	7,309	8,253	4,738.4	0.86	5,626	6,511	4,504.9	0.85
Glucose, mg/dL	0.9	166	11.6	< 0.0001	40	40	5.1	0.99	30	- 117	17.4	< 0.01
Potassium, mmol/L	0.08	0.13	0.069	0.46	2.28	2.12	0.210	0.38	2.19	2.02	0.145	0.39

[1]Baseline measurements occurred a minimum of 1 h before HI treatment, POSTHAND measurements occurred immediately after HI treatment, and slaughter blood samples were collected during exsanguination inside the abattoir. Blood gas analysis was not performed on samples collected during exsanguination because blood was exposed to atmospheric gases during collection. Blood samples were not collected at slaughter for d 1 cattle (n = 6 replications).
[2]Change = POSTHAND value – baseline value.
[3]Change = Slaughter value – baseline value.
[4]Change = Slaughter value – POSTHAND value.
[5]HI = handling intensity. Cattle were handled over 1,600 m in groups of 4 randomly assigned to 1 of 2 HI treatments: 1) low-stress handling (LSH) or 2) high-stress handling (HSH).
[6]SEM = largest SE in the analysis.
[7]Statistical significance was declared when $P \leq 0.05$; tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.
[8]rpm = respirations per min; bpm = beats per min.
[9]HCO_3 = bicarbonate; pCO_2 = partial pressure carbon dioxide; TCO_2 = total carbon dioxide; pO_2 = partial pressure oxygen; sO_2 = saturated oxygen.
[10]Statistical analysis was conducted on log transformed values and treatment estimates were back-transformed for reporting purposes.

Table 4-6. Least squares means for the effects of fatigued status (fatigued vs. non-fatigued) on beef cattle physiological measurements and blood variables at POSTHAND and on the change from baseline to POSTHAND time points on the day of slaughter.

	POSTHAND				POSTHAND – Baseline[1]			
	STATUS[2]				STATUS[2]			
Variable	Fatigued	Non-fatigued	SEM[3]	*P*-value[4]	Fatigued	Non-fatigued	SEM[3]	*P*-value[4]
Vital parameters								
Respiratory rate, rpm[5]	72.8	77.1	9.1	0.66	23.1	23.7	8.8	0.94
Heart rate, bpm[5]	114.1	98.2	13.0	0.24	52.0	17.4	14.7	0.03
Rectal temperature, ºC	40.5	40.5	0.3	0.92	1.3	0.9	0.3	0.15
Blood variables								
Lactate, mmol/L	22.1	13.4	2.9	< 0.01	18.8	8.1	2.9	< 0.001
HCO_3, mmol/L[6]	11.1	17.7	3.0	0.03	- 15.2	- 7.5	3.2	< 0.01
pH	7.15	7.30	0.05	< 0.01	- 0.25	- 0.07	0.06	< 0.01
pCO_2, mmHg[6]	30.4	34.8	3.5	0.21	- 16.3	- 12.0	4.6	0.27
TCO_2, mmol/L[6]	11.8	18.9	2.8	0.02	- 19.1	- 9.7	3.4	< 0.01
pO_2, mmHg[6]	51.4	37.7	5.0	< 0.01	23.3	8.3	5.4	< 0.01
sO_2, %[6]	74.5	64.5	7.8	0.16	23.0	11.1	8.8	0.13
Base excess, mmol/L	- 18.2	- 8.6	3.4	0.01	-23.4	- 10.6	4.1	< 0.01
Epinephrine, pg/mL	3,317	2,307	748	0.21	3,000	1,011	989	0.06
Norepinephrine, pg/mL	4,533	2,494	1,153	0.10	3,390	871	1,435	0.04
Cortisol, ng/mL	51.4	48.4	3.8	0.39	22.1	9.3	10.0	0.09
Creatine kinase, U/L[7]	687	907	291	0.63	399	568	286	0.61
Glucose, mg/dL	334	252	43	0.08	251	156	41.8	0.03
Potassium, mmol/L	5.7	5.3	0.3	0.17	0.8	0.1	0.2	< 0.01

[1]Change = POSTHAND value – baseline value. POSTHAND measurements occurred immediately after HI treatment. Baseline measurements occurred a minimum of 1 h before HI treatment.
[2]Cattle were handled over 1,600 m in groups of 4 randomly assigned to 1 of 2 HI treatments: 1) low-stress handling (LSH) or 2) high-stress handling (HSH). Fatigued cattle were HSH cattle withdrawn before completion to completion of the 1,600 m course, whereas normal cattle are HSH cattle were not withdrawn from the course. No LSH cattle became fatigued during the study and therefore their data were not included in the analysis
[3]SEM = largest SE in the analysis.
[4]Statistical significance was declared when $P \leq 0.05$; tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.
[5]rpm = respirations per min; bpm = beats per min.
[6]HCO_3 = bicarbonate; pCO_2 = partial pressure carbon dioxide; TCO_2 = total carbon dioxide; pO_2 = partial pressure oxygen; sO_2 = saturated oxygen.
[7]Statistical analysis was conducted on log transformed values and treatment estimates were back-transformed for reporting purposes.

Table 4-7. Least squares means for the effect of HI on carcass characteristics.

	HI Treatment[1]			
Variable	LSH	HSH	SEM[2]	*P*–value[3]
HCW, kg	414	412	4.4	0.64
Dressing percentage[4]	64.3	64.3	0.4	0.94
LM area, cm^2	95.5	93.5	2.6	0.44
12^{th} rib fat thickness, cm	1.40	1.37	1.5	0.72
Marbling Score[5]	472	469	16.6	0.88
Choice or greater, %[6]	65	80	7.5	0.17

[1]HI = handling intensity. Cattle were handled over 1,600 m in groups of 4 randomly assigned to 1 of 2 HI treatments: 1) low-stress handling (LSH) or 2) high-stress handling (HSH).
[2]SEM = largest SE in the analysis.
[3]Statistical significance was declared for $P \leq 0.05$; tendencies for main effects were declared when $0.06 \leq P \leq 0.10$.
[4]Calculated based on 4% shrinkage in final BW.
[5]Evaluated in the longissimus dorsi m. between the 12^{th} and 13^{th} ribs; Slight = 300, Small = 400, Modest = 500 (USDA, 1997).
[6]Prevalence of cattle grading choice or greater within handling groups of 4 was used for statistical analysis.

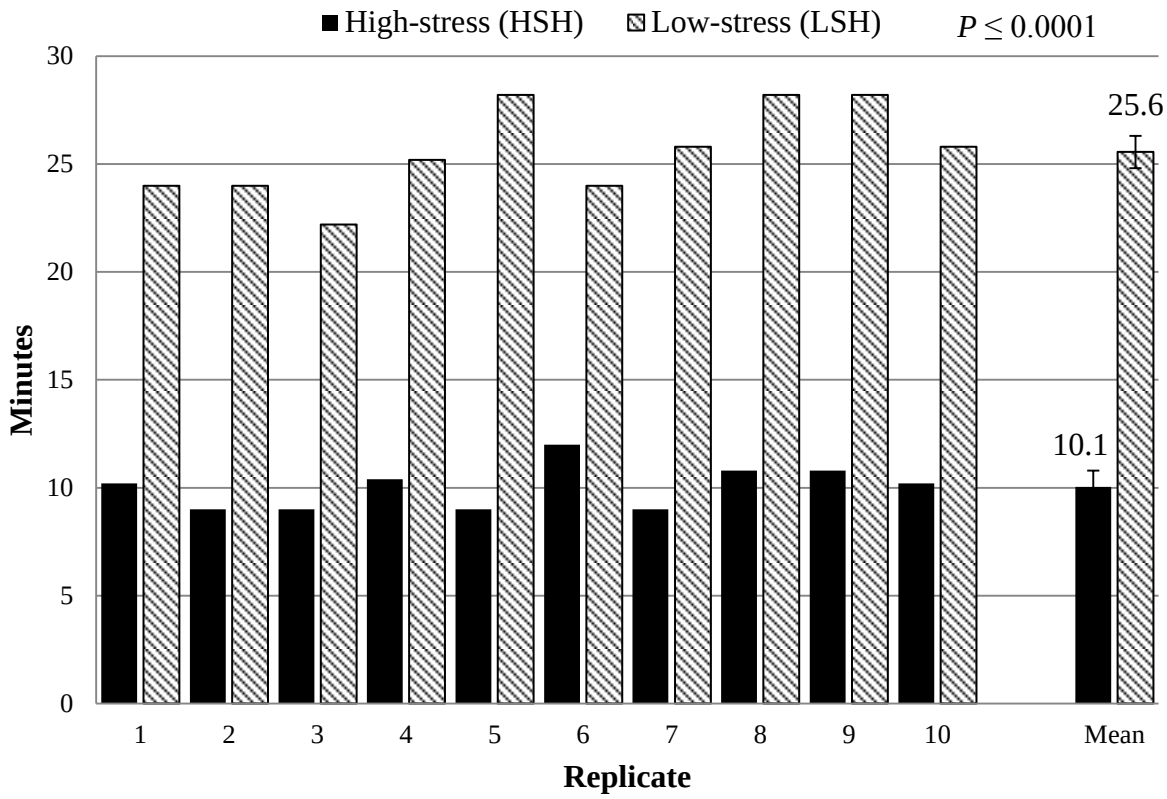

Figure 4-1. Time to complete the 1,600 m handling course. On the day of slaughter, cattle were handled in groups of 4 penmates randomly assigned to 1 of 2 handling intensities: 1) low-stress handling (LSH) or 2) high-stress handling (HSH). Cattle were handled around a 1,600 m dirt alley course and either kept at a walking pace with no electric prod use (LSH) or kept at a minimum of a trot with electric prods applied after handling and during loading (HSH). Pairs of LSH and HSH handling groups of 4 within a lot served as the replicate.

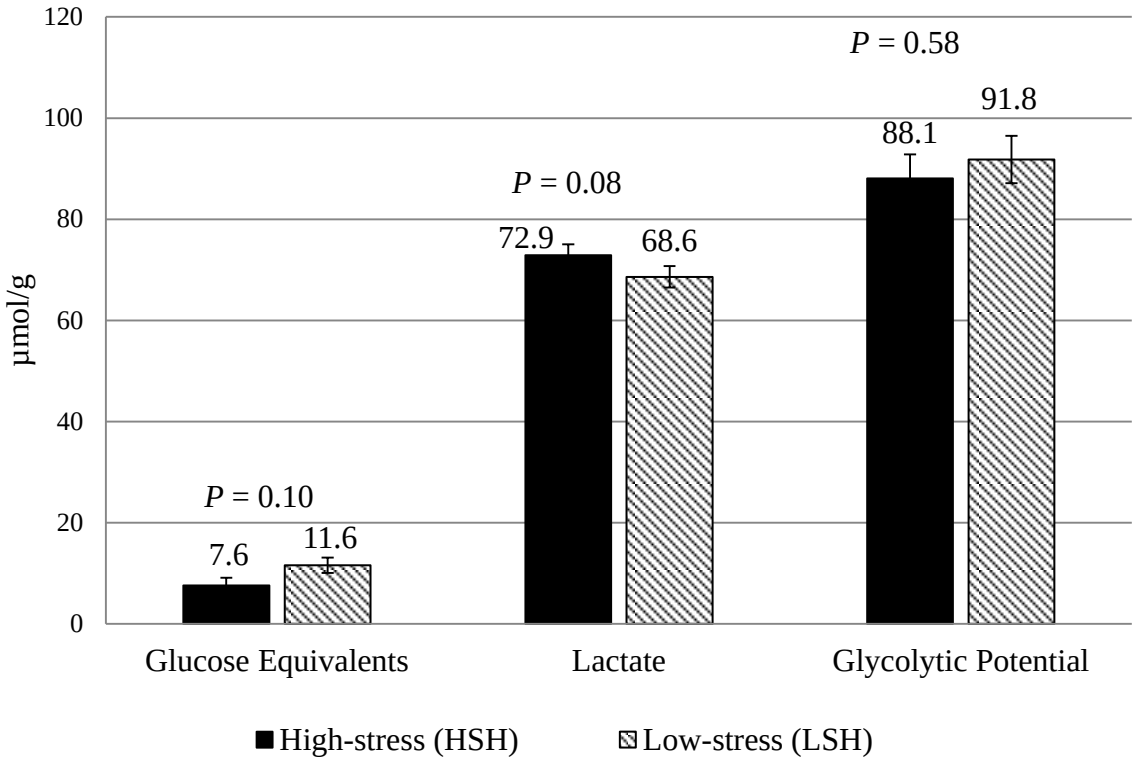

Figure 4-2. Effect of handling intensity on glycolytic potential of semimembranosus muscle (SM) taken approximately 80 h after exsanguination. On the day of slaughter, cattle were handled in groups of 4 penmates randomly assigned to 1 of 2 HI treatments: 1) low-stress handling (LSH) or 2) high-stress handling (HSH). Cattle were handled around a 1600 m dirt alley course and either kept at a walking pace with no electric prod use (LSH) or kept at a minimum of a trot with electric prods applied after handling and during loading (HSH). The equation of Monin and Sellier (1985) was used for determination of GP where: GP = 2 (glycogen + glucose + glucose-6-phosphate) + lactate.

Chapter 5 - Effects of ractopamine hydrochloride on growth performance, carcass characteristics and physiological response to different handling techniques

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ABSTRACT

Feedlot cattle (n = 128; BW = 549 ± 60 kg) were used to evaluate the effects of ractopamine hydrochloride (**RAC**) on growth performance and physiological responses to handling 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 post-handling restraint and during loading for shipment to the abattoir. Sixteen identical pens (8 cattle per pen) were used to evaluate the effect of RAC on growth performance over a 28 d feeding period. Cattle fed RAC tended to have greater ADG and G:F ($P = 0.06$), and had greater HCW and LM area ($P < 0.05$). On the day after the 28 d growth performance period, HI treatments were applied to groups of 8 cattle comprised of 4 representing each diet. Venous blood samples were collected a minimum of 1 h before handling (**baseline**), after handling (**POSTHAND**), and after transport to the abattoir (**POSTTRANS**). An additional mixed arterial and venous blood sample was collected at slaughter during exsanguination (**slaughter**). A diet ×

HI interaction ($P = 0.01$) was detected for the change in cortisol from baseline to POSTTRANS, and there tended ($P < 0.10$) 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 had greater changes from baseline to POSTHAND in blood pH, HCO_3, base excess, pCO_2, lactate, norepinephrine, cortisol and glucose ($P \leq 0.01$). Feeding RAC and HSH increased the overall change in CK concentrations from baseline to slaughter ($P < 0.01$). This study confirms RAC improves growth performance and suggests metabolic acidosis, a precursor to fatigued cattle syndrome, develops in cattle regardless of RAC administration when cattle are allowed to trot without the use of a lead rider. Ractopamine hydrochloride altered the hormonal response to aggressive handling and transport, warranting further investigation to understand the physiological responses of cattle to stressors that occur during shipment for slaughter.

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

INTRODUCTION

Ractopamine hydrochloride (**RAC**; Optaflexx®; Elanco Animal Health, Greenfield, IN) and zilpaterol hydrochloride (**ZIL**; Zilmax®, Merck Animal Health, Desoto, KS) are FDA approved β-adrenergic agonists (**βAA**) fed to beef cattle at the end of the feeding period to repartition nutrients and promote 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 non-responsive to handling cues, sloughed one 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; ref. range: < 4 - 5) and creatine kinase (**CK**; 6,890, ref. range: 159 - 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 which 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., 2017a) have reported improper handling techniques induce clinical signs and blood abnormalities in feedlot cattle 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) **CON** – no βAA vs. 2) **RAC** – 400 mg·animal^{-1}·d^{-1} ractopamine hydrochloride. Phase II occurred on 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 Company, 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 post-handling sampling and once during loading onto semi-trailers at the feedlot before transport to the abattoir. The handling model of the present study utilized the same stop criteria as Hagenmaier et al. (2017a) to minimize pain and distress in experimental cattle. No cattle met the established stop criterion, and 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 (d -1), approximately 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 sex were selected for enrollment. Extra cattle were no longer used in this study. The 128 experimental cattle were then segregated by sex, stratified by BW, assigned uniquely numbered study ear tags (Allflex USA Inc., Dallas, TX) and allocated to 16 identical feeding 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 Corporation, 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. 5-1). Start date of RAC administration was staggered over 2 d based on future slaughter dates and used as a blocking factor. Each block consisted of 4 single gender replicates (2 per sex) from 8 pens for a total of 64 cattle. Ractopamine hydrochloride was fed continuously within each block for 28 d before slaughter, as this is the most common RAC feeding period duration used 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 creating 2 handling groups (LSH and HSH) of 8 cattle (4 CON and 4 RAC) based on the initial allotment to diet and HI treatments (Fig. 5-1). As a result, each handling group of 8 cattle was single-sexed and comprised of 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 commercial feedlot's standardized operating procedures consistent with the Ag Guide (*Guide for the Care and Use of Agricultural Animals in Research and Teaching*, FASS, 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 bunk at the front of the pen which extended over the entire width of the pen. A 3.8 m gate at the bunk 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 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 until the day of slaughter (Table 5-1; NRC, 2000). Feed bunks were assessed daily beginning at 0600 h by a trained individual who would estimate orts and daily consumption to determine the amount of feed needed to be delivered to provide *ad libitum* access to feed.

Health monitoring was performed daily by a veterinarian and was consistent with the feedlot's standard operating procedures. Records were maintained to document abnormal health observations and all concomitant therapy was under the direction of the veterinarian. No mortalities occurred for either diet throughout the duration of the study. Six cattle (5 CON, 1

RAC) were treated with 0.22 – 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 5-2) for the growth performance period was obtained from a weather station located approximately 25 km from the feedlot using the National Oceanic and Atmospheric Administration database (NOAA). On the days cattle were handled and slaughtered, ambient temperature (TA) and relative humidity (RH) were collected every 10 min using Veriteq data loggers (Vaisala Inc., Boulder, CO). The loggers were used for weather data collection 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 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 Conditions and Procedures (Phase II)

Cattle were handled and slaughtered on the days after completion of the 28 d growth performance period for each block (study d 28 and 29) using a similar model as Hagenmaier et

al. (2017a) where 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 pre-determined HI groups, and handled using a bud box system before being restrained for recording of final BW and baseline measurements using the same scale and hydraulic scale as enrollment. Baseline procedures were performed following the procedures described by Hagenmaier et al. (2017a) and included measurement of vital parameters (rectal temperature (**RT**), heart rate (**HR**) and respiratory rate (**RR**)), venous blood collection, 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.

Post-handling. 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 procedures. 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 semi-trailers. During loading, HSH cattle received another impulse from an electric prod on the hip while ascending the load-out ramp. The HI treatment groups within replicates were commingled inside the trailer and shipped together in the deck or belly compartment to prevent confounding HI treatment by trailer compartments. Dimensions for 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. 2012b). Cattle were then transported approximately 100 km to the abattoir in accordance with the National Cattlemen's Beef Association Beef Quality Assurance Guidelines. 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 provided water *ad libitum* throughout lairage according to the abattoir's standard operating procedures. Mobility scores were recorded for all cattle while 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, and subsequently turned to lairage pens to be housed until slaughter. All cattle enrolled in the study passed USDA ante-mortem inspection before being removed from lairage pens for slaughter. The mean time cattle spent in lairage was approximately 6 h.

Slaughter. Cattle were humanely euthanized using a captive bolt gun and then slaughtered in accordance with the abattoir's standard operating procedures. At the time of euthanasia, slaughter sequence was recorded using the study ear tags to identify blood samples collected during exsanguination (**slaughter**) and cross-match with numbers assigned by the plant for evaluation of carcass characteristics. Briefly, carcasses were ribbed between the 12th and 13th rib 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.

Mobility Scores and Behavioral Observations.

Mobility scoring and behavioral observations were performed at baseline, POSTHAND and POSTTRANS time points by trained observers blinded to diet and HI treatments using the same scoring systems and definitions as Hagenmaier et al. (2017a). In addition to the previously mentioned time points, a final mobility score was recorded approximately 1 h before slaughter within the lairage pen (lairage). To maintain blinding to HI, observers at the feedlot remained in the restraining facility where 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, exhibits 1 or more of the following: stiffness, shortened stride, or slight limp; 3 = lags behind normal cattle when the group is walking, exhibits 1 or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = extremely reluctant to move, even when encouraged by handlers (NAMI, 2015). Temperament scores were determined where: 1 = calm, no movement; 2 = restless shifting; 3 = continuous squirming and shaking of the chute; 4 = rearing, twisting, continuous violent struggle. Finally, chute-exit scores

were assigned as: 1 = walk; 2 = trot; 3 = run, 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 at baseline, POSTHAND and POSTTRANS using the same procedures as Hagenmaier et al. (2017a) and transferred into 10 mL serum separator, 158 USP lithium-heparin, and K_2EDTA tubes (Becton, Dickinson and Company, 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 Corporation, 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 due to exposure to atmospheric gases during collection. Whole blood in K_2EDTA and lithium-heparin tubes were centrifuged in a 4ºC refrigerated centrifuge at 1,400 × *g* for 15 min as soon as feasible following each respective collection. Blood in serum-separator tubes were 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 in -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. Plasma was assayed in duplicate for epinephrine and norepinephrine 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 was 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 for 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 non-adjusted values, whereas dressing percentage was calculated using an adjusted final BW where 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 where HI was the main plot and diet was the sub-plot. Handling groups of 8 cattle were considered the experimental unit for the whole plot effect of HI (n = 8 groups per HI), and the

sub-groups of 4 cattle representing each diet were considered the experimental unit for the sub-plot (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 non-parametric analyses were performed using the GENMOD procedure in SAS. Frequency distributions of mobility score events were also analyzed across diet × HI combinations at each time point using the Fisher's Exact Test within the FREQ procedure. The diet × HI interaction could not be tested for multiple variables analyzed with non-parametric models due to "0" observed events within the diet × HI treatment subclass categories, and these variables are denoted by superscripts within the data table. Continuous variables were summarized by calculating the mean of the appropriate experimental unit (i.e. group of 8 for HI or sub-group 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. Variables with significant ($P \leq 0.05$) diet × HI interactions are denoted by superscripts within the data tables. 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 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 $0.06 \leq P \leq 0.10$.

RESULTS

Only the changes in blood variables and vital parameters between time points are reported and discussed in this chapter. Blood and vital parameter measurements for diet and HI treatments at POSTHAND, POSTTRANS and EXSANG time points are included in the appendix of this dissertation.

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 5-3); however, there was no effect of diet on DMI or final BW ($P = 0.11$). Furthermore, RAC cattle had greater HCW and LM area ($P = 0.04$), while no effect of RAC was observed for dressing percentage, 12th rib fat thickness or marbling score ($P \geq 0.34$).

Qualitative Scoring and Physical Indicators of Stress

Mobility Scores. Distribution of mobility score events differed across diet × HI combinations at POSTTRANS ($P = 0.03$; Table 5-4) with prevalence being numerically greatest in RAC/LSH followed by CON/LSH cattle. There was no difference in mobility scores > 1 across treatment combinations at any other time point ($P \geq 0.17$). The proportion of cattle with POSTTRANS mobility scores > 1 was greater in LSH cattle ($P = 0.01$; Table 5-5) and tended ($P = 0.09$) to be greater in RAC cattle compared 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 5-5), 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 measured ($P \geq 0.19$; Table 5-6). However, effects of diet were observed as CON cattle had greater RR and HR, greater concentrations of lactate, pO_2, sO_2, and epinephrine ($P < 0.05$), and tended to have greater RT ($P = 0.07$) 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 ($P = 0.10$).

Post-handling. 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 sub-plots 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. 5-2) as RAC cattle had a greater increase than CON cattle in 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 5-7). However, HI did not affect the change from baseline to POSTHAND for RR, RT, pO_2, sO_2, CK or potassium ($P \geq 0.21$). Cattle fed RAC had greater increases in RR and norepinephrine, and greater decreases in pH from baseline to POSTHAND ($P \leq 0.05$). Otherwise, there was no effect of RAC on the changes from baseline to POSTHAND for the remaining blood variables and vital parameters ($P \geq 0.12$).

Post-transport. The diet × HI interaction was significant ($P = 0.01$) for changes in cortisol from baseline to POSTTRANS (Fig. 5-3), as HSH increased the change in cortisol in RAC but not CON cattle. There tended ($P = 0.07$) to be a similar diet × HI interaction for changes in CK where HSH increased the change in CK in RAC cattle only (Fig. 5-4). Changes in HCO_3 ($P = 0.06$) and norepinephrine ($P = 0.07$) concentrations tended to be greater for CON cattle (Table 5-8), although 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 increased the overall change in CK from baseline to slaughter ($P < 0.01$; Table 5-9). Otherwise, there was no effect of diet or HI on any other blood variable measured in samples collected during exsanguination.

DISCUSSION

β-adrenergic agonists such as RAC and ZIL function through a mode of action whereby nutrients are diverted away from adipose tissue 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) comprising 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 in 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 %, feed efficiency by 20 %, increased HCW by 7 kg and increased LM area by 4 cm^2. These findings are in alignment with the conclusions of the βAA meta-analysis performed by Lean et al. (2014) which included over 50 comparisons involving RAC versus 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 2 events in 2013 where distressed cattle had difficulties walking, sloughed hoof walls and were euthanized while in lairage at the abattoir. A major U.S. packer announced the decision to stop accepting cattle fed ZIL soon after these events, which led to Merck Animal Health (Desoto, KS) announcing a self-imposed suspension of ZIL sales in the United States and Canadian markets (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 (Thomson et al., 2015), is believed to be multi-factorial and other factors proposed to play a role include heat stress, high-stress handling, heavy muscling, increasing slaughter weights, subclinical laminitis, long-haul transport and lairage conditions (Thomson et al., 2015; Boyd et al., 2016). Although anecdotal reports implicate the use of βAA, there is no data to either confirm or refute this supposition.

Shortly after the approval of RAC in swine, reports surfaced proposing increased rates of non-ambulatory pigs and a caution statement was added to the U.S. Paylean label (FDA, 2002). Non-ambulatory pigs are defined as pigs unable to walk or keep up with contemporaries during movement at the packing plant (Anderson et al., 2002). Research conducted in swine demonstrated that metabolic acidosis and elevated stress hormones are major determinants for the vast majority of non-ambulatory 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 and HCO_3 (Benjamin et al., 2001; Anderson et al., 2002; Ivers et al., 2002). Dead and non-ambulatory pigs due to FPS are a multi-factorial issue involving, but not limited to, improper handling with use of an electric prod, inappropriate trailer stocking densities, and poorly designed facilities where pigs are handled far distances and required to 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). Due to 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, the North American Meat Institute (NAMI) has recently adopted a new mobility scoring system designed to evaluate fatigued cattle (NAMI, 2015), rather than traditional scoring systems such as the Zinpro Step-Up Locomotion Scoring System primarily used for determining the gradea of lameness (Zinpro, 2016). 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 (for full definitions, please refer to the materials and methods section). A June 2016 industry report by Elanco Animal Health assessing mobility on over 200,000 U.S. market cattle using the same scoring system as our study revealed that 8.5% of cattle received a score of 2 or greater upon presentation to abattoirs (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, and increased to 20.3% after lairage.

The findings of the current study demonstrate feeding RAC did not adversely affect mobility at the feedlot or at the slaughter plant. 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 (Burson, 2014, Bernhard et al., 2014; Boyd et al., 2015). Still, it is important to note that mobility scores worsened after transportation and lairage regardless of treatment, and is in agreement with multiple recent studies which reported proportions of cattle with compromised mobility increased late in the marketing channel compared to at the feedlot (Boyd et al., 2015; Hagenmaier et al., 2017a).

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 endured during transport to the abattoir. The former seems counterintuitive as one can assume fatigue would have been greater in cattle handled aggressively. Not only does this stimulate one to consider other sources of pre-slaughter stress at the feedlot predisposing cattle to fatigue, but it also leads one to speculate that additional factors exist during transport and at the abattoir which may contribute to an animal's willingness to respond to handling. In addition to

long-haul transport and adverse weather, González et al. (2012a) reported both inappropriately low and high trailer stocking densities in the belly compartment of trailers increased the prevalence of non-ambulatory 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 due to unfamiliarity of slated concrete floors in the lairage area when indeed they had no mobility issue, or that the fatigue was only temporary and able to be overcome when cattle were allowed to rest following transport. In addition to HI and βAA, an array of other factors exist which warrant investigation due to their potential to impact mobility, including body weight, musculoskeletal confirmation (genetics), subclinical disease (foot rot, laminitis, etc.), injury, transport conditions, lairage duration, and facility designs (flooring, sloping, etc.).

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 as these measurements were within normal reference ranges reported in cattle and comparable to 2 other recent studies where blood was collected via jugular venipuncture from feedlot cattle that were either fed RAC (Hagenmaier et al., 2017a) 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 similar findings to our study where cattle fed ZIL had decreased blood lactate and glucose concentrations, and speculate 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 due to 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 the of ZIL administration in beef cattle is most prominent during periods of environmental stress in the summer, and speculate it 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, KS, personal communication). The HSH cattle in the present study developed metabolic acidosis characterized by elevations in blood lactate and decreases pH, HCO_3, and pCO_2, and is consistent with other studies in cattle where exercise has been shown to cause development of metabolic acidosis (Holmes et al., 1972; Frese et al., 2016; Hagenmaier et al., 2017a). Compared to recent studies conducted by Hagenmaier et al. (2017a) and Frese et al. (2016) where HSH cattle were handled at comparable speeds, the degree of acidosis experienced in the current study was less severe based on lower lactate concentrations and the pH being closer to physiological normal. This could be attributed to several factors including 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 in response to HI, which supports previous research where stress or excitement or both due to increased workloads from exercise elevated both epinephrine and norepinephrine concentrations in cattle (Blum and Echinger, 1988; Blum and Flueckiger, 1988; Hagenmaier et al,. 2017a). 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., 2017a).

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 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 stress hormones has not been reported in cattle to these authors' knowledge and warrants further investigation. Swine studies have also reported increased epinephrine concentrations after being administered RAC, and suggest the effects of RAC on stress responses are dependent upon 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 mg/kg but not 5.0 mg/kg RAC, indicating the effect of RAC on response to stress may be dose dependent. Both down-regulation and desensitization of β-receptors have been proposed to ensue after βAA usage, which may

consequently lead to up-regulation 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 suggest long-term exposure of 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, either attributable to increased 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 which has been identified as a potential indicator of metabolic stress associated with βAA administration and in non-ambulatory cattle at abattoirs (Loneragan et al., 2014; Thomson et al., 2015). Frese et al. (2016) and Hagenmaier et al. (2017a) also reported HI did not affect CK immediately after handling. The larger increases in CK from baseline to slaughter in cattle fed RAC was not surprising, as similar results have been reported in cattle fed ZIL (Zilmax® Freedom of Information summary, FDA, 2006; Fuller et al., 2014). The diet × HI interaction for the change from baseline to POSTTRANS is of particular interest and suggest that aggressive handling combined with transport may be more harmful and cause greater degrees of muscle breakdown in cattle fed RAC than 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 speculated that RAC increased CK concentrations due to microlesions causing

enlarged muscle fibril diameters and stimulation of CK release. 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, leading to an increased amount of CK released from the muscle. Other studies in beef cattle have reported similar findings where CK concentrations 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., 2017a). 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 incurred 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., 2017a). Although inclement weather and excitable temperaments may hinder the ability to completely avoid a small degree of muscle fatigue and trauma in subsets of cattle during loading and shipment to slaughter, the relationship between decreased mobility and preventable muscle fatigue due to aggressive handling and crushing injuries during weigh-out at the feedlot warrants further investigation.

In conclusion, the results of this study confirm the advantages of feeding RAC on growth performance and negative implications of aggressive handling without the use of a lead rider on physiological and metabolic responses in beef cattle, regardless of whether or not they had been fed the βAA. Cattle appear to have the highest likelihood of developing mobility issues during transport and while in lairage at the abattoir, which warrants further investigation to protect the welfare of beef cattle presented for slaughter

Table 5-1. Ingredient composition and analyzed nutrient content of the finishing ration fed during phase I.

Ingredient, %DM basis	
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.

Table 5-2. Maximum, minimum and mean ambient temperature (TA), relative humidity (RH) and temperature humidity index (THI) summarized for Phases I and II.[1]

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

[2]THI was calculated using the same equation as Mader et al. (2006) where THI = (0.8 × TA) + [(RH × 0.01) × (TA – 14.4)] + 46.4; TA = Ambient Temperature; RH = Relative Humidity.

[3]RH was not reported on 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, TA = Ambient temperature.

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

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

Table 5-3. 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	2.3	0.04
12^{th} rib fat thickness, cm	1.18	1.11	0.07	0.34
Marbling score[5]	451	455	12.7	0.78

[1]CON – No β-agonist; RAC – 400 mg·animal-1·d-1 ractopamine hydrochloride for 28 d.
[2]SEM = largest SE in the analysis
[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 longissimus dorsi m. between the 12^{th} and 13^{th} ribs; Slight = 300, Small = 400, Modest = 500 (USDA, 1997).

Table 5-4. Distribution of mobility scores by each time point for each diet × HI treatment combinations.[1]

Treatment[6] (Diet/HI)	Baseline[2]					POSTHAND[3]					POSTTRANS[4]					Lairage[5]				
	1	2	3	4	% > 1	1	2	3	4	% > 1	1	2	3	4	% > 1	1	2	3	4	% > 1
CON/LSH (32)	31	1	0	0	3.1	31	1	0	0	3.1	29	2	0	0	6.4	26	4	2	0	18.8
RAC/LSH (32)	32	0	0	0	0.0	29	3	0	0	9.4	26	3	3	0	18.8	21	6	5	0	34.4
CON/HSH (32)	32	0	0	0	0.0	32	0	0	0	0.0	32	0	0	0	0.0	27	5	0	0	15.6
RAC/HSH (32)	32	0	0	0	0.0	32	0	0	0	0.0	30	1	0	0	3.2	28	3	1	0	12.5
Total	127	1	0	0	0.8	124	4	0	0	3.1	117	6	3	0	7.1	102	18	8	0	20.3
***P*-value[7]**	(n = 128)			*P* = 1.00		(n = 128)			*P* = 0.19		(n = 126)			*P* = 0.03		(n = 128)			*P* = 0.17	

[1]Mobility scores were assigned by observers blinded to diet and HI using a 4 point scoring system (see Materials and Methods).
[2]Baseline observations were recorded a minimum of 1 h before HI treatment.
[3]POSTHAND observations occurred immediately after the application of HI treatments over a 1,500 m handling course.
[4]POSTTRANS observations occurred immediately after unloading from semi-trailers at the abattoir. Mobility scores were not obtained at POSTRANS for 1 CON/LSH and 1 RAC/HSH animal.
[5]LAIRAGE observations were recorded approximately 1 h before slaughter while cattle were standing in the lairage pen at the abattoir.
[6]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 $mg \cdot animal^{-1} \cdot d^{-1}$ ractopamine hydrochloride for 28 d, and 2) Handling intensity (HI) over a 1,500 m course the 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.
[7]*P*-value represents probability that differences in frequency distribution of mobility scores across different across treatment combinations at that time point. Statistical significance was declared between treatment combinations when $P \leq 0.05$

Table 5-5. 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 %	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: 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 the 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]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 semi-trailers 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 126 experimental cattle (mobility scores were not obtained at POSTRANS for 1 CONLSH and 1 RACHSH animal); all other POSTTRANS variables represent block 1 cattle only (n = 32 per treatment).

Table 5-6. 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, rpm[5]	42.1	40.0	1.33	0.04	41.1	41.0	1.33	0.97
Heart Rate, bpm[5]	115.3	105.3	7.83	0.02	109.9	111.8	7.88	0.84
Rectal Temperature, ºC	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, mmol/L[6]	23.7	24.6	0.28	< 0.01	24.2	24.2	0.28	0.92
pCO_2, mmHg[6]	43.2	44.2	0.68	0.10	43.6	43.8	0.68	0.71
TCO_2, mmol/L[6, 7]	29.3	30.4	0.36	0.02	29.9	29.8	0.36	0.81
pO_2, mmHg[6]	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, mmol/L[7]	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, U/L[8]	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: 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 the 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.
[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 min; bpm = beats per min.
[6]HCO_3 = bicarbonate; pCO_2 = partial pressure carbon dioxide; TCO_2 = total carbon dioxide; pO_2 = partial pressure 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.

Table 5-7. 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 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, rpm[5]	9.8	12.7	1.85	0.02	10.0	12.5	1.99	0.21
Heart Rate, bpm[5]	17.3	17.8	7.49	0.89	6.5	28.6	8.01	0.01
Rectal Temperature, ºC	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, mmol/L[6]	- 2.1	- 2.9	0.76	0.19	1.4	- 6.4	0.81	< 0.0001
pCO_2, mmHg[6]	- 6.0	- 6.1	0.81	0.83	- 4.4	- 7.7	0.85	< 0.01
TCO_2, mmol/L[6]	- 3.0	- 4.0	0.82	0.14	1.2	- 8.2	0.92	< 0.0001
pO_2, mmHg[6]	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, pg/mL[7]	699	858	103.9	0.23	406	1,151	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, U/L[8]	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: 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 the 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.

[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 min; bpm = beats per min.

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

[7]The diet × HI interaction tended to be significant for epinephrine ($P = 0.06$; Fig. 5-2) 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.

Table 5-8. 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 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, ºC	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, mmol/L[5]	2.3	1.2	0.51	0.06	2.2	1.2	0.64	0.34
pCO_2, mmHg[5]	- 1.2	- 2.4	1.29	0.50	- 1.4	- 2.2	1.29	0.62
TCO_2, mmol/L[5]	0.7	0.0	0.53	0.20	0.9	- 0.2	0.62	0.26
pO_2, mmHg[6]	- 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, ng/mL[6]	- 1.8	3.7	3.42	0.14	- 4.9	6.7	3.42	0.07
Creatine kinase, U/L[6,7]	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: 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 the 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.

[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 carbon dioxide; TCO_2 = total carbon dioxide; pO_2 = partial pressure oxygen; sO_2 = saturated oxygen.

[6]The diet × HI interaction was significant for cortisol ($P = 0.01$; Fig. 5-3) and tended to be significant for creatine kinase ($P = 0.07$; Fig. 5-4), 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.

Table 5-9. 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]
Blood variables								
Lactate, mmol/L	6.3	7.0	0.52	0.12	6.3	6.9	0.52	0.16
HCO_3, mmol/L[5]	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, U/L[6]	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	74.7	61.3	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: 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 the 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.

[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.

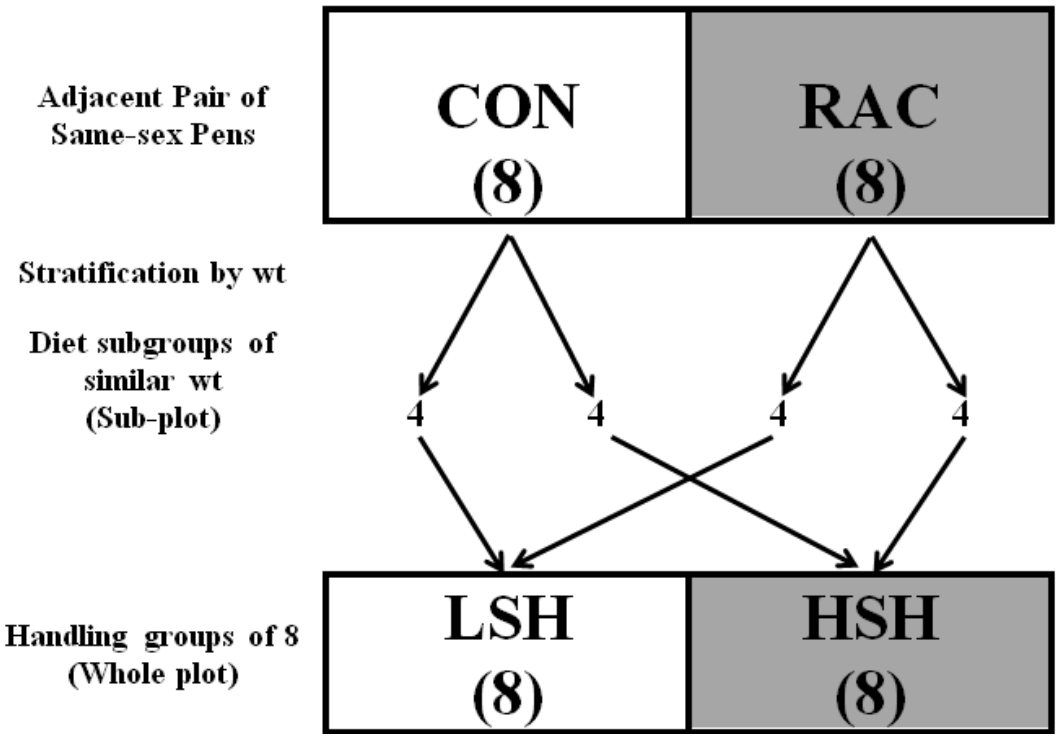

Figure 5-1. Schematic overview for how pairs of single-sex pens of 8 cattle were stratified by weight and allocated to handling intensity treatments in phase II. Pens of 8 cattle fed 400 $mg \cdot animal^{-1} \cdot d^{-1}$ ractopamine hydrochloride (RAC) or not fed a β-agonist (CON) were stratified by wt into subgroups of 4 which were randomly allocated to 1 of 2 handling intensity (HI) treatments: 1) LSH – cattle moved at a walking pace through a 1,500 m handling course with no electric prod use vs. 2) HSH – cattle moved at a minimum of trot through a 1,500 m handling course with no lead rider and an electric prod applied twice (approximately 1 s per impulse) on the hip: once while in the alley before restraint for post-handling sampling and once during loading onto semi-trailers at the feedlot. Handling groups of 8 served as the whole plot (n = 8) and subgroups of 4 representing each diet served as the sub-plot (n = 16).

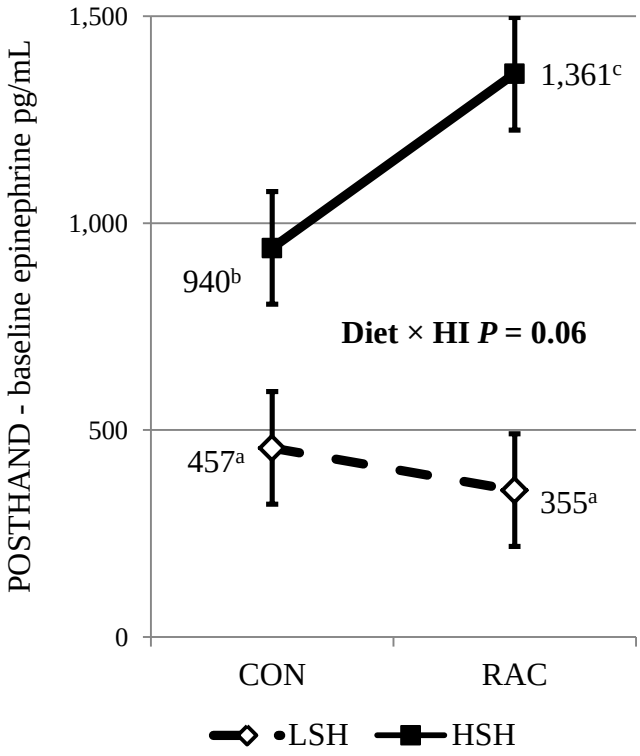

Figure 5-2. Diet × handling intensity (HI) interaction for the change in epinephrine concentrations from baseline to POSTHAND. Treatments were assigned in a 2 × 2 factorial arrangement in a split-plot design with the following treatments: 1) Diet: CON – no β-agonist vs. RAC – 400 mg·animal-1·d-1 ractopamine hydrochloride for 28 d and 2) HI on the day of slaughter: LSH – cattle moved at a walking pace through a 1,500 m handling course with a lead rider and no electric prod use vs. HSH – cattle moved at a minimum of trot through a 1,500 m handling course with no lead rider and an electric prod applied twice (approximately 1 s per impulse) on the hip: once while in the alley before restraint for post-handling sampling and once during loading onto semi-trailers at the feedlot. The HI was the whole plot 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 ($P < 0.05$).

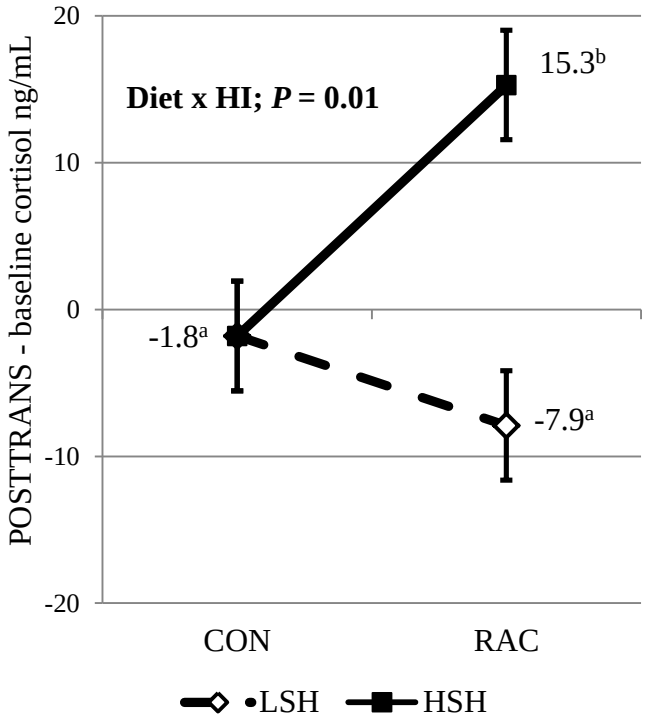

Figure 5-3. Diet × handling intensity (HI) interaction for the change in cortisol concentrations from baseline to POSTTRANS. Treatments were assigned in a 2 × 2 factorial arrangement in a split-plot design with the following treatments: 1) Diet: CON – no β-agonist vs. RAC – 400 mg·animal^{-1}·d^{-1} ractopamine hydrochloride for 28 d and 2) HI on the day of slaughter: LSH – cattle moved at a walking pace through a 1,500 m handling course with a lead rider and no electric prod use vs. HSH – cattle moved at a minimum of trot through a 1,500 m handling course with no lead rider and an electric prod applied twice (approximately 1 s per impulse) on the hip: once while in the alley before restraint for post-handling sampling and once during loading onto semi-trailers at the feedlot. The HI was the whole plot 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 ($P < 0.05$).

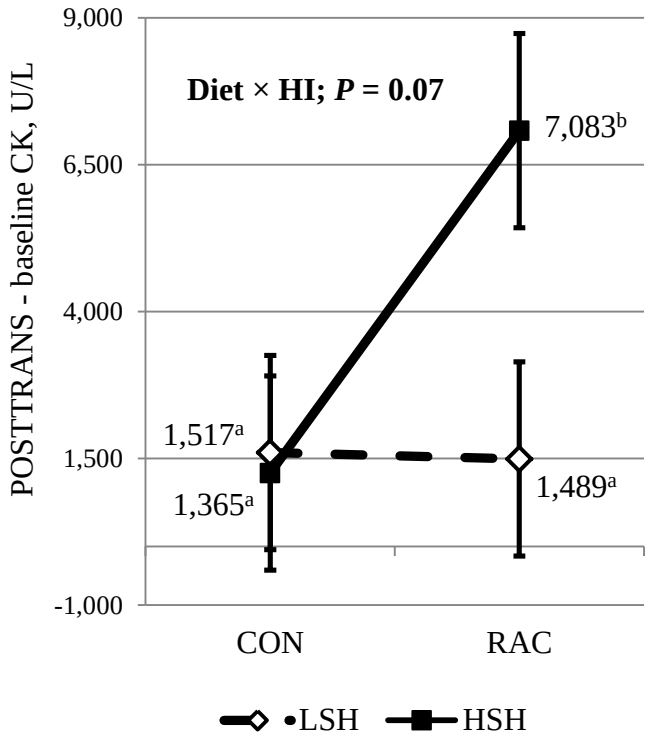

Figure 5-4. Diet × handling intensity (HI) interaction for the change in creatine kinase (CK) concentrations from baseline to POSTTRANS. Treatments were assigned in a 2 × 2 factorial arrangement in a split-plot design with the following treatments: 1) Diet: CON – no β-agonist vs. RAC – 400 mg·animal^{-1}·d^{-1} ractopamine hydrochloride for 28 d and 2) HI on the day of slaughter: LSH – cattle moved at a walking pace through a 1,500 m handling course with a lead rider and no electric prod use vs. HSH – cattle moved at a minimum of trot through a 1,500 m handling course with no lead rider and an electric prod applied twice (approximately 1 s per impulse) on the hip: once while in the alley before restraint for post-handling sampling and once during loading onto semi-trailers at the feedlot. The HI was the whole plot 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 ($P < 0.05$).

Chapter 6 - References

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Appendix A - Vital parameters and blood variables of cattle at each time point (Chapter 5)

Table 6-1. Least squares means for the effects of ractopamine hydrochloride (RAC) and handling intensity (HI) on POSTHAND vital parameters and blood variables of beef cattle on the day of slaughter.[1]

	Diet[2]				HI[2]			
Variable	CON	RAC	SEM	*P*-value[3]	LSH	HSH	SEM	*P*-value[3]
No. of observations[4]	16	16	-	-	8	8	-	-
Vital parameters								
Respiratory Rate, rpm[5]	52.0	52.7	1.4	0.59	51.1	53.6	1.4	0.10
Heart Rate, bpm[5]	133	123	2.9	0.02	116	139	3.3	< 0.01
Rectal Temperature, ºC	39.8	39.8	0.06	0.27	39.8	39.8	0.07	0.77
Blood variables								
Lactate, mmol/L	7.6	7.1	0.6	0.50	3.1	11.6	0.7	< 0.0001
pH	7.43	7.42	0.01	0.20	7.49	7.36	0.01	< 0.001
HCO_3, mmol/L[6]	21.6	21.7	0.6	0.89	25.6	17.8	0.7	< 0.0001
pCO_2, mmHg[6]	37.2	38.0	0.7	0.24	39.2	36.1	0.7	< 0.01
TCO_2, mmol/L[6]	26.2	26.4	0.7	0.76	31.1	21.6	0.8	< 0.0001
pO_2, mmHg[6]	37.8	36.6	1.3	0.38	36.4	38.0	1.6	0.51
sO_2, %[6]	71.3	67.7	1.8	0.09	71.8	67.3	2.1	0.18
Base excess, mmol/L	1.0	0.7	0.9	0.79	6.5	- 4.8	1.0	< 0.0001
Epinephrine, pg/mL	1,127	1,132	103	0.97	741	1,517	104	< 0.001
Norepinephrine, pg/mL	1,164	1,329	119	0.03	973	1,520	121	< 0.001
Cortisol, ng/mL	40.9	41.3	2.1	0.87	35.6	46.6	2.1	0.001
Creatine kinase, U/L[7]	472	570	104	0.25	499	538	99.0	0.74
Glucose, mg/dL	148.3	148.3	6.9	0.99	96.6	200.1	7.7	< 0.0001
Potassium, mmol/L	5.0	5.2	0.08	< 0.01	5.1	5.1	0.08	0.54

[1]POSTHAND procedures were performed immediately following the application of HI treatments over a 1,500 m handling course.

[2]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) HI on the day of slaughter: Low-Stress Handling (LSH) – cattle moved at a walking pace through a 1,500 m handling course with a lead rider and no electric prod use vs. High-Stress Handling (HSH) – cattle moved at a minimum of trot through a 1,500 m handling course with no lead rider and an electric prod (Miller Manufacturing Company, Glencoe, MN) applied twice (approximately 1 s per impulse) on the hip: once while in the alley prior to restraint for post-handling sampling and once during loading onto semi-trailers at the feedlot. The HI was the whole plot and diet was the subplot.

[3]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]No. of observations = number of experimental units used to calculate treatment means. In this case, experimental unit = subgroups of 4 animals for diet (Subplot); Experiment unit = pens of 8 animals for HI (whole plot).

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

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

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

Table 6-2. Least squares means for the effects of ractopamine hydrochloride (RAC) and handling intensity (HI) on POSTTRANS rectal temperature and blood variables of beef cattle harvested on study d 28.[1]

	Diet[2]				HI[2]			
Variable	CON	RAC	SEM	*P*-value[3]	LSH	HSH	SEM	*P*-value[3]
No. of observations[4]	8	8	-	-	4	4	-	-
Vital parameters								
Rectal Temperature, ºC	39.6	39.7	0.21	0.65	39.5	39.7	0.21	0.42
Blood variables								
Lactate, mmol/L	6.8	5.9	0.9	0.11	5.4	7.3	1.1	0.26
pH	7.44	7.45	0.02	0.64	7.44	7.44	0.02	0.77
HCO_3, mmol/L[5]	26.7	26.5	0.5	0.71	26.9	26.3	0.5	0.41
pCO_2, mmHg[5]	43.1	42.6	1.5	0.76	43.2	42.5	1.6	0.70
TCO_2, mmol/L[5]	30.1	30.6	0.50	0.38	30.8	29.9	0.58	0.32
pO_2, mmHg[5]	32.7	31.1	0.7	0.09	31.4	32.4	0.8	0.41
sO_2, %[5]	63.9	61.3	1.4	0.21	61.8	63.4	1.4	0.47
Base excess, mmol/L	4.57	5.20	0.72	0.36	5.44	4.33	0.77	0.28
Epinephrine, pg/mL	1,166	786	173	0.11	945	1,008	195	0.83
Norepinephrine, pg/mL	1,361	1,095	238	0.03	1,215	1,241	238	0.81
Cortisol, ng/mL	28.5	34.2	2.0	0.02	27.0	35.7	2.5	0.09
Creatine kinase, U/L[6]	1,782	3,675	1,970	0.12	1,773	3,694	1,973	0.17
Glucose, mg/dL	108.9	105.7	3.1	0.47	106.7	107.9	3.3	0.81
Potassium, mmol/L	4.8	4.8	0.08	0.79	4.9	4.7	0.08	0.24

[1]POSTTRANS procedures were performed on cattle slaughtered on study d 28 (n = 64) and was completed in a side-chute in the lairage area at the slaughter facility following unloading from semi-trailers.

[2]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) HI on the day of slaughter: Low-Stress Handling (LSH) – cattle moved at a walking pace through a 1,500 m handling course with a lead rider and no electric prod use vs. High-Stress Handling (HSH) – cattle moved at a minimum of trot through a 1,500 m handling course with no lead rider and an electric prod (Miller Manufacturing Company, Glencoe, MN) applied twice (approximately 1 s per impulse) on the hip: once while in the alley prior to restraint for post-handling sampling and once during loading onto semi-trailers at the feedlot. The HI was the whole plot and diet was the subplot.

[3]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]No. of observations = number of experimental units used to calculate treatment means. In this case, experimental unit = subgroups of 4 animals for diet (Subplot); Experiment unit = pens of 8 animals for HI (whole plot).

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

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

Table 6-3. Least squares means for the effects of ractopamine hydrochloride (RAC) and handling intensity (HI) on slaughter blood variables of beef cattle.[1]

	Diet[2]				HI[2]			
Variable	CON	RAC	SEM	*P*-value[3]	LSH	HSH	SEM	*P*-value[3]
No. of observations[4]	16	16	-	-	8	8	-	-
Blood variables								
Lactate, mmol/L	11.7	11.1	0.36	0.12	11.1	11.7	0.4	0.11
HCO_3, mmol/L[5]	26.0	27.4	0.4	< 0.01	26.9	26.4	0.4	0.30
Epinephrine, pg/mL	7,998	7,845	308	0.58	7,838	8,004	308	0.55
Norepinephrine, pg/mL	8,678	8,618	448	0.93	8,331	8,965	448	0.35
Cortisol, ng/mL	50.5	51.4	2.0	0.77	50.3	51.6	2.0	0.66
Creatine kinase, U/L[6]	2,650	4,670	1,279	< 0.001	2,260	5,242	1,195	< 0.01
Glucose, mg/dL	175.2	155.4	8.9	0.09	172.3	157.7	8.9	0.20
Potassium, mmol/L	7.1	7.2	0.15	0.80	7.2	7.1	0.15	0.25

[1]Slaughter blood samples were collected using 50 mL fecal cups during exsanguination inside the slaughter facility. Blood gas nalyses were not performed on samples obtained during exsanguination due to exposure to atmospheric gases during collection.
[2]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) HI on the day of slaughter: Low-Stress Handling (LSH) – cattle moved at a walking pace through a 1,500 m handling course with a lead rider and no electric prod use vs. High-Stress Handling (HSH) – cattle moved at a minimum of trot through a 1,500 m handling course with no lead rider and an electric prod (Miller Manufacturing Company, Glencoe, MN) applied twice (approximately 1 s per impulse) on the hip: once while in the alley prior to restraint for post-handling sampling and once during loading onto semi-trailers at the feedlot. The HI was the whole plot and diet was the subplot.
[3]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]No. of observations = number of experimental units used to calculate treatment means. In this case, experimental unit = subgroups of 4 animals for diet (Subplot); Experiment unit = pens of 8 animals for HI (whole plot).
[5]HCO_3 = bicarbonate.
[6]Statistical analysis was conducted on log transformed values and treatment estimates were back-transformed for reporting purposes.