CLINICAL AND DIAGNOSTIC EVALUATION OF FINISHED CATTLE EXPOSED TO BETA ADRENERGIC AGONISTS AND PHYSICAL EXERTION

by

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B.S., University of Nebraska-Lincoln 1997
D.V.M., Kansas State University 2003

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Diagnostic Medicine and Pathobiology
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Abstract

The widespread use of beta adrenergic agonists in beef cattle production has been adopted by the beef industry in recent years to improve weight gain and feed efficiency at the end of the feeding period. During this feeding period, anecdotal reports of increased mortality during the timeframe in which beta agonists were being fed to cattle was also reported, and confirmed in epidemiologic studies. Additionally, adverse animal welfare events at abattoirs in cattle fed beta adrenergic agonists were reported in August 2013. The objectives of this dissertation were to investigate physiologic and management factors that may be associated with adverse effects of the use of beta adrenergic agonists in cattle.

Two studies were conducted, one to establish normal Holter monitor registration values and evaluate the electrocardiographic effects of zilpaterol and ractopamine hydrochloride on finishing steers, and one to develop a model to investigate the physiologic effects of forced exercises in finished cattle, which was hypothesized to be a possible factor in reported adverse cattle welfare events in August 2013.

Thirty steers were enrolled to evaluate the effect of ractopamine, zilpaterol or negative control on arrythmia and mean heart rate at 4 different time periods during a 28 day feeding period. Cattle fed ractopamine and zilpaterol had increased heart rate ($P < 0.05$) but no differences in arrythmia rates were found.

Forty steers were enrolled in a study at a commercial feeding facility to develop a model for fatigue in cattle forced to run 1,540 m compared to control cattle walked 1,540 m. Blood lactate, cortisol, rectal temperature, heart rate was increased ($P < 0.05$), blood pH decreased ($P < 0.01$) and to have reduced locomotion, as measured by pedometers, during the 48 hour period

following handling compared to controls. Additionally cattle that were fatter and forced to run had increased lactate (P =0.057) and lower blood pH (P < 0.01) than thinner cohorts.

Cattle handling method is a factor in the health and welfare of cattle and the continued adoption of low stress handling methods throughout the beef industry should be pursued.

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Dedication

I dedicate this Dissertation to my wonderful wife, Julie, and our three children, Noah, Mikaila and Samuel. It is their sacrifices and encouragement that have made this possible.

Chapter 1 - Literature Review of the Physiology and Effects of Beta Adrenergic agonists

Introduction

Beta Adrenergic agonists have been investigated and used in agriculture for the promotion of growth in many species over the last 50 years. The concept of and studies describing the adrenergic receptors upon which these pharmaceuticals act upon was first described in 1948.[1] Prior to this study, the physiologic teaching of that time was that the two classes of adrenergic receptors were either excitatory or inhibitory. However, the same sympathomimetic amines have different potencies for different functions, disproving the physiologic theory of that time and named the two different sympathomimetic receptors identified as *alpha* and *beta* receptors.[2]

Agonists and antagonists of *beta* adrenergic (βA) receptors have been used in human medicine for many purposes, but one of the most common has been the use of short and long acting *beta* adrenergic agonists (βAA) in the treatment of asthma. Other common medical uses include the use of βA antagonists for the treatment of cardiac conditions through the reduction of heart rate to improve cardiac function.[3-5]

The use of these pharmaceuticals has not been without controversy, as the use of βAA have been associated with a host of negative side effects.[6-13] Long-term use of these drugs has been implicated as a risk factor for increased mortality rates in asthmatic individuals[7]. Myocardial effects, including myocardial toxicity, myocardial apoptosis, and increased risk of death in humans and other species have been linked to βAA.[13-16] Cardiac effects commonly seen with the administration of a short acting βAA are tachycardia, tremors, decreased serum

potassium and increased blood glucose.[17] Side effects have not been limited to humans as many species, including rats, dogs, and horses have been shown to have cardiac related side effects.[14,15,18-20]

Pharmacological agonists of these receptors have been used to promote lean muscle tissue growth in food animal production systems in the United States since the approval of ractopamine for swine in 1999.[21] Since that time, ractopamine hydrochloride, primarily a β_1 agonist, has been approved for use in turkeys and cattle. In 2006 zilpaterol hydrochloride , a β_2 agonist, was approved in cattle.[22,23] These βAA's are approved to be fed for improved weight gain and feed efficiency at the end of the feeding period for food producing animals by stimulation of skeletal muscle hypertrophy.[24,25] The use of βAA in food producing animals also had some level of controversy, both in the United States and in Europe.[26-28] The βAAs ractopamine and zilpaterol have been associated with increased death loss during the βAA feeding period and adverse welfare events in the United States.[26,29] Animal welfare is often defined by the principals known as the Five Freedoms. These are 1) Freedom from Hunger or Thirst 2) Freedom from discomfort 3) Freedom from pain or injury. 4) Freedom to express normal behavior 5) Freedom from fear or distress.

Additionally, food safety is a concern for βAA fed to livestock as clenbuterol toxicity in humans who ate liver meat produced by animals illegally fed the βAA clenbuterol in Europe has been reported.[27,28,30]

Cellular and Systemic Physiology

The effects on animal physiology and growth by upregulation of these receptors has been well documented and will be briefly reviewed in this chapter along with the usage of these pharmaceuticals in beef production systems in the United States. A brief list of the general

distribution of major receptor types present on mammalian organs and tissues is listed in Table 1-1.

Beta Receptors

The beta receptor group are seven member G-Protein membrane bound receptors comprised of three main subtypes, β_1, β_2, and β_3 and a mixture of these subtypes is present throughout most mammalian organs, tissues and cells.[31] Ligands for these receptors can be either of endogenous or synthetic in origin as well as either agonistic or antagonistic in activity nature. Additionally, ligands for beta receptors are not exclusive ligands for each receptor but often have a receptor in which they preferentially bind. For example, the endogenous βAA's norepinephrine (NE) and epinephrine (EPI) each have differing affinities for binding to each of the beta receptor subtypes with EPI having $\beta_2 > \beta_1$ affinity when compared to NE. Additionally, physiological response to βAAs is concentration dependent in nature as a higher concentration of the ligand will increase the number of receptors on cell membrane surfaces that bind the ligand and affect the magnitude of the cellular response.[32] Individual βAA have varied pharmacologic properties, such as half-life, oral and parenteral bioavailability, and physiologic response due to specie variations in metabolism, absorption and cellular receptor concentrations.

Membrane concentration of receptors and receptor subtypes vary among organ systems and among species. For example: receptor concentrations vary with age, as both young and old mammals have reduced receptor concentrations, which attenuate response to βAA stimulation.[33,34]

Endogenous catecholamine synthesis

Endogenous production of the βAA epinephrine (EPI) and norepinephrine (NE) occurs in sympathetic neurons and chromaffin cells of the adrenal medulla beginning with hydroxylation

of the amino acid L-tyrosine by the enzyme tyrosine hydroxylase (TH) to form L-DOPA.[35] An alternate route to formation of L-DOPA using phenylalanine can be used by TH in the absence of tyrosine. This is the rate-limiting step of endogenous catecholamine production.[36] Dopamine is then formed from L-DOPA by DOPA decarboxylase, an enzyme that is found in many tissues throughout the body.[37] In cells that produce EPI or NE the next step is the hydroxylation of the β carbon of the side chain of dopamine by dopamine β-decarboxylase to form NE, which will then be stored. Cells that synthesize EPI have one final step in which a methyl group is transferred by Phenylethanolamine *N*-methyltransferase (PMNT) from *S*-adenosylmethionine to NE.[38]

Ligand Binding and Intracellular effects

Following release and distribution of EPI and NE into the blood stream, the molecules bind to the β-adrenergic receptor on the membrane of many different types of cells. The membrane-bound β-receptor is a 7 member G-protein with a central binding site.[25] Ligand binding causes the receptor to phosphorylate cyclic AMP.[25] Increased cyclic AMP (cAMP) concentrations lead to numerous intracellular changes, such as activation of Protein Kinase A (PKA), changes in cAMP gated membrane channels, phosphorylation of multiple cell-type dependent proteins, and changes in protein transcription and translation which will be described in greater detail later.[25,39]

βAA's as Neurotransmitters

The primary actions of endogenous catecholamines are to change the regulatory mechanism of metabolism within affected cells as well as responses that modulate the response to acute stressors. The cells of the adrenal medulla are analogous to post-ganglionic cells of the sympathetic nervous system, which is commonly referred to as the 'fight or flight' system. External stimuli activate the sympathetic nervous system, which in turn causes the adrenal

medulla to release EPI and some NE, rapidly elevating their concentrations in peripheral plasma. The relative plasma concentrations of these two catecholamines vary among species. Norepinephrine is the major neurotransmitter of the sympathetic nervous system and NE is usually two to ten times the concentration of epinephrine in plasma under resting conditions.[35,40,41] Increased plasma concentration of EPI and NE in plasma then systemically act upon cells.

Cardiovascular effects of βAA

All the β-agonists produce significant increases in heart rate.[42] The primary mechanism of acute effects are changes in ion membrane permeability to sodium and potassium, causing a net reduction in the pacemaker threshold, which leads to spontaneous diastolic depolarization.[39] Additionally, increased calcium release from the endoplasmic reticulum leads to increased cardiac myofiber contractility.[4,39,43] This activity increases energy use, and thus cellular oxygen demand by cardiac myocytes. Beta-2 receptor stimulation plays a key role in the feed-forward mechanism of coronary arterial vasodilation, which allows for increased blood flow to supporting the increased oxygen demand by both cardiac and skeletal muscle myocytes during exercise.[44-50]

Epinephrine and NE are key regulators of blood pressure as vasodilation and vasoconstriction of blood vessels is induced by β and α adrenergic stimulation respectively. The vasodilation of major cardiac and skeletal muscle arteries has the effect of reducing peripheral vascular resistance. Lower vascular resistance coupled with increased cardiac output increases blood flow, oxygen, nutrient delivery, and waste removal from cardiac and major skeletal muscle groups.

In normal or acute stress situations the activation of the sympathetic system and catecholinergic effects of EPI and NE are of great benefit to allow short-term stressors or dangers to be overcome. However, long-term stimulation of this system leads to decreased β-receptor expression, and thus reduced effect.[39,51] Additionally, long-term adrenergic stimulation, which occurs in chronic heart failure, has been associated with increased rates of arrhythmias, sudden cardiac death, and cardiac ischemia. Briefly, βAA have been shown to increase risk of cardiac death in patients with chronic cardiac conditions.[5,10,52-54] Pathology due to βAA varies with both agonist and specie. Myocardial necrosis has been shown to occur in many species, with the extent of pathology increased in the presence of ischemia.[14,15,19,42]

These long-term side effects caused by chronic medical conditions are often managed with the use of a receptor antagonist, which have a reversing effect when compared to an agonist and significantly reduce the risk of sudden cardiac death in humans with a myocardial contractile dysfunction.[3]

Respiratory effects of βAA

The bronchodilatory effects of βAA are among the most common uses of βAA as about 1 in 12 people (about 25 million) in the United States and nearly 300 million people worldwide have asthma.[55,56] When used for treatment of respiratory ailments βAA are usually delivered in the form of an aerosolized metered dose inhaler instead of through oral ingestion, as would be common for other routine uses of βAA. The activation of β receptors on respiratory epithelium causes smooth muscle relaxation by PKA phosphorylation of myosin light chain kinase (MLCK), which inactivates the myosin light chain and prevents contraction.[57] The subsequent relaxation causes bronchial dilation, reducing airflow resistance and increases airflow throughout the respiratory tract. The regulation by β_2AA responses involves both cAMP dependent and -

independent mechanisms that may act in concert.[57] These changes may include the changes to the secretory capacity of the epithelium and immune cells.[57]

The use of βAA inhalers for the treatment of asthma has been associated with adverse events such as cardiac arrhythmias, myocardial infarction and sudden cardiac death (SCD).[6-8,10,12,13] The risk of SCD due to the use of inhalers is influenced by multiple factors, including genotypic variation in receptors, βAA used, patient, age, severity of disease, and patient exercise.[7,11,58] However, βAA inhalers are currently the most effective bronchodilators available.

Skeletal Muscle effects of βAA

The hypertrophic effects of βAA on the skeletal muscle traditionally have been the primary focus of their use in production agriculture. At the most basic level, binding βAA to a receptor on a muscle cell produces the same response as it does in other cells: an increase of intracellular cAMP.[59] However, subsequent events lead to large changes in protein synthesis and degradation, which result in significant muscle hypertrophy and increase in muscle fiber diameter.[60-64] Acute stimulation by βAA increases the efficiency of protein synthesis within the muscle fiber without requiring contribution of additional DNA from satellite cells.[33] The muscle growth in response to βAA treatment appears to be a true muscle hypertrophy.[59] However, with longer duration of βAA administration the rate of hypertrophy cannot be maintained without additional DNA and the responsiveness to the βAA is dampened and the accretion of skeletal muscle decreases.[59,65] Additionally, βAA affects Type I and Type II fibers differently as it has been shown to consistently increase the cross-sectional area of the Type II fibers by 10 to 50% in both rats and lambs.[59]

Glycogenolysis is also increased by βAA stimulation of muscle.[66] Glycogenolytic effects are induced by the phosphorylation of glycogen phosphorylase, which converts glycogen, an

intracellular stored form of glucose, to monomeric glucose-6-phosphate. Glucose-6 phosphate is a substrate source for glycolysis, or anaerobic respiration, resulting in the production of ATP and pyruvate. Pyruvate will then enter the Krebs cycle and undergo aerobic respiration, assuming that enough cellular oxygen is available. If the cell is in a hypoxic state, pyruvate will be converted to lactate by lactate dehydrogenase. Lactate will then accumulate within the cell and be removed via blood. The end result of βAA mediated glycogenolysis is to make an energy source stored within the muscle cell rapidly available for production of muscle work.

Effects of βAA on Adipose tissue

Adipose tissue has been shown to have β1 and β_2 receptors. The presence of the less common β_3 subtype has also been shown be present in some species, but not in cattle.[25] The β_3 receptor plays a central role in inhibiting the release of leptin from adipocytes.[67] In adipose cells, βAA promote lipolysis associated with stimulating triacylglycerol hydrolysis and inhibiting lipogenesis stimulated through the β-receptor-G protein-cAMP-PKA pathway.[33,68,69] The βAA-induced increase in cAMP results in the phosphorylation of acetyl-CoA carboxylase, which inhibits the *de novo* biosynthesis of fatty acids. The net response of adipose tissue to βAA is lipolytic and causes a release of fatty acids.[33] This release causes an increase in circulating fatty acids, which may be used as an energy source by conversion to acetyl-CoA.[70] High concentrations of acetyl-CoA can inhibit conversion of pyruvate to acetyl-CoA, forcing pyruvate to be metabolized to lactate by lactate dehydrogenase and potentially increasing metabolic acidosis cases where lactic acidosis is present, such as hypoxic situations.[71]

Immunological Effects

Primary and secondary lymphoid organs are innervated by sympathetic nerve fibers. Macrophages, T and B-lymphocytes, monocytes, eosinophils, mast cells and neutrophils all

possess functional β-receptors.[72] Lymphocytes have been shown to express high-affinity βAA receptors, mainly of the β_2 subclass. Modulation of functions such as cytokine production, lymphocyte proliferation, histamine degranulation and antibody secretion by βAA have been shown *in vitro*.[73] Short-acting and long-acting β_2AA inhibit immune cell function as measured by cell activation, inflammatory mediators, cell recruitment, and cell survival *in vitro* and occasionally in vivo. Anti-inflammatory responses of immune cells to β_2 agonists are additive or synergistic with the increased presence of corticosteroids.[72]

Cytokine production in the spleen, which contains a large sympathetic innervation, is inhibited by β_2-Agonists and helps prevent systemic inflammation.[74] Additionally, sympathetic innervation of spleen and lymph nodes decreases during aging, resulting in decreased immune function.[75]

Liver function & Insulin effects

The liver and pancreas are the major organs responsible for maintenance of blood glucose concentrations.[76] The main effect of βAA in the liver is to stimulate glucose production through glycogenolysis, which is enhanced by cAMP activation of glycogenolytic enzymes.[76] Glycogen is then metabolized to glucagon and then to glucose for release to the blood stream. Increased blood glucose concentration causes increased pancreatic insulin secretion. Pancreatic insulin secretion caused by β_2 agonists is glucose-dependant.[76] Increased circulating insulin promotes glucose uptake by muscles and adipose tissue.[66]

Hypoglycemia is the main physiological factor that increases EPI secretion.[35] Repeated hypoglycemic events, such as those that are commonly associated with diabetes mellitus in humans, decrease receptor sensitivity to EPI.[76] The liver and pancreas are crucial to glucose

regulation, however the effect of β_2AA are of little clinical importance unless the individual is diabetic or pre-diabetic.[76]

βAA use in Cattle Production

There are two FDA approved βAA, ractopamine hydrochloride (RAC) and zilpaterol hydrochloride (ZIL) for use in cattle feeding in the United States.[22,23] These products are fed at the end of the feeding period in commercial feedyards. (Figure 1-1) In 2012 these two βAA were reportedly being fed to 70% of the cattle on the market, although since that time ZIL has been voluntarily withdrawn from the market.[77] The use of RAC and ZIL in cattle increases average daily gain, gain-to-feed ratio, carcass weight and reduced dry matter feed intake, thus greatly increasing efficiency at the end of the feeding period.[22-24,78-80] The magnitude of effect of βAA in ruminants is greater than what has been shown in other species with an increase in carcass protein and decrease in carcass lipid content roughly double, on a relative scale, of that seen in swine.[66]

Ractopamine is approved to be fed at the rate of 8.2 to 24.6 g/ton air dry basis (ADB) for increased rate of weight gain and improved feed efficiency in cattle fed in confinement for slaughter during the last 28 to 42 days on feed. Ractopamine hydrochloride is also approved at a rate of 9.8 to 24.6 g/ton (ADB) for increased rate of weight gain, improved feed efficiency, and increased carcass leanness in cattle fed in confinement for slaughter during the last 28 to 42 days on feed.[23] Additionally, RAC has a label claim to be fed as a top dress feed at the rate of 70 to 400 mg per animal per day in at least one pound of feed with a concentration not to exceed 800 g/ton. Zilpaterol hydrochloride is approved to be fed at a dietary concentration of 6.8 g per ton (7.5 ppm) of zilpaterol hydrochloride/ton of feed to provide 60 to 90 mg zilpaterol hydrochloride per animal per day on a 90% dry matter basis for the last 20 to 40 days on feed with a three day

pre-slaughter withdrawal period.[22] Additionally, in December 2014 a supplemental label for zilpaterol was approved by the FDA. This label allowed zilpaterol to be fed at the rate of 60mg per animal per day and contained the following statement : "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." [81]

Federal regulations prohibit the extra label use of any feed additives. As the approval for ZIL has a label for concentration based (g/ton) feeding only, the total amount of ZIL consumed by each steer is entirely dependent on feed intake. This creates the possibility that individual animals in a pen that have higher intakes may eat more than the upper label limit of 90 mg per animal. Additionally, in commercial situations feed delivery is managed on a pen basis, not an individual animal basis. This creates an environment in which a portion of the population of individuals within a pen may be eating enough feed to consume a dose greater than label, even if the feed delivery to pen would indicate an intake within the label range. When fed to large populations of animals, it could be possible to observe some dose dependent effects, either positive or negative, that would not otherwise have been noted in pre-approval research. One example would be a decrease in feed intake within 24 hours of the introduction of ZIL at the end of the feeding period. Further, cattle that had a greater intake at the time of introduction of ZIL had a greater decrease in feed intake.[82]

Zilpaterol has been shown to increase final body and hot carcass weight by 6.6 kg (and 15.4 kg respectively.[24] The greater increase in carcass weight than live weight is evidence of the significant repartitioning effects of this particular β_2 agonist in finishing cattle as dressing percent, longissimus muscle area are increased 1.657 % and 8.1 cm^2 and backfat thickness decreased .106 cm, respectively.[24] In comparison RAC increases final body weight by a similar

6.5 kg but has a lesser effect on hot carcass weight with 7.4 kg of additional gain, approximately half of the increase seen in cattle fed ZIL.[24]

In addition to increasing performance at the end of the feeding period, both RAC and zilpaterol increase Warner-Bratzler shear force (WBSF), which is a measure of meat tenderness.[24,83,84] One potential explanation of this effect in cattle fed ZIL is the increased muscle fiber diameter due to muscle hypertrophy.[64] The magnitude of effect of RAC, on WBSF in cattle is less with a .31 kg increase compared to a 1.0 kg increase for ZIL.[24] The increased effects of ZIL as compared to RAC could be in part due to the dominant β_2 receptor density in skeletal muscle and adipose tissue of cattle, as ZIL is a primarily β_2 agonist whereas RAC is primarily β_1.[78]

Nutrient Requirements

The effect of βAA on the nutrient requirements of cattle has not been well investigated, as most research has been devoted to the physiologic changes and nutrient partitioning within the animal.[66] However, the βAA cimaterol has been shown to increase nitrogen retention, with no effect on protein digestibility or metabolizability of energy in cattle.[85,86] The metabolizable protein supply provided in rations has been shown to not need to be increased in cattle fed RAC at 200mg per animal per day in heifers.[87]

Impact of βAA on Food Safety

The use of any pharmacologic agent in an animal used for food must be beneficial for the animal, the producer, and have a low likelihood of causing food borne disease or residue remaining in edible tissue in concentrations that may be detrimental to human health. *Salmonella* and *E.coli* O157:H7 are human food borne pathogens which can be carried and transmitted by cattle.[88-90] *Salmonella spp.* have been shown to be present in 38% of cattle

feeding facilities and 5.5% of beef cattle.[89] Prevalence of *E.coli* O157:H7 has been documented in 7.5% of individual cattle and 40.4% of groups at slaughter.[91] In two studies, *E.coli* O157:H7 shedding was reduced in cattle fed ractopamine at 200 mg per head daily. However, prevalence of *Salmonella spp.* tended to increase.[92] The prevalence rate of *E.coli O157:H7* was not affected by the feeding of ractopamine or zilpaterol. However, cattle fed zilpaterol did have greater prevalence rates of *Salmonella spp*. shedding.[93]

Mitigation of residues in edible tissue is also of prime importance, and is a major part of the FDA approval process. Ractopamine and zilpaterol have both been shown to be safe, both compounds having a tolerance in liver, as the residue target tissue, determined by the Food and Drug Administration Center for Veterinary Medicine during the approval process.[22,23] However, feeding of βAA and sale of meat from animals fed βAA is illegal in many countries, including the European Union.[94]

Environmental Impact of βAA in Beef Production

Food production practices have been targeted by individuals and organizations which promote a social and political agenda suggesting that modern food production creates adverse environmental impacts.[95] The use of technology to improve the efficiency of modern beef production has greatly reduced the required resources per unit of beef. The use of βAA technology is one of the changes in production systems during this time that has improved beef production efficiency.

In addition to improved weight gain and feed efficiency, ZIL has been shown to reduce CH_4, N_2O, NH_3 and methanol emissions from cattle fed commercial diets.[96] Reductions in greenhouse gasses emissions and carbon footprint when compared to natural, or no technology, cattle have also been reported in a partial life cycle assessment study.[97]

Economic Impact of βAA in Beef Production

The use of technology in food production is nothing new and does not come without its' own controversy.[26] The macro-economic impacts for both RAC and ZIL have not been fully studied. However, the singular effect of ZIL and the economic impact from cow-calf producer to retail has been investigated. [98] Schroeder and Tonsor (2011) concluded that cow-calf producers gain $2.28 billion, cattle feeders gain $1.48 billion, beef packers lose $1.10 billion, retailers lose $2.34 billion, and consumers gain $2.49 billion over ten years. Impacts of feeding ZIL to cattle for a beef packer include the increase in red meat yield across carcasses at every yield grade.[99] However, it should be noted that this study was performed *ceteris paribus,* as if all things other than ZIL remained the same, which rarely happens in a dynamic marketplace. Potential costs of using this product, such as tracking beef for access to foreign markets, market impact due to changes in packer-feedlot sale and procurement structure, costs of managing ZIL implementation and feed delivery within the feedyard, and changes in beef quality were not considered in this analysis.

The feeding of RAC and ZIL increases saleable yield of the four major primal cuts from beef carcasses.[99] However, this increase is not uniformly distributed across the primal cuts, with the majority of weight gain occurring in the lower-priced cuts of the round and chuck.[100] Valuable subprimal cuts from the hindquarter, such as tenderloin, strip loin, and top sirloin butt, are also increased as a percentage of cold carcass weight from steers fed ZH.[99] Increased response of the lower priced cuts to β-agonists could have economic implications to packers and could have potential changes in the overall economic value of the carcass.[78]

Other potential long-term effects of βAA beyond just supply and demand of beef are public concerns regarding βAA effects on food safety, animal welfare, and beef quality. Food

safety was listed as the biggest concern of consumers, followed by quality and then price.[101] It is important that both of these major market concerns are considered when implementing technologies to minimize the impact of the technology on beef demand and perception.

International acceptance of βAA is not universal. There are 160 countries, including the European Union, either banning or restricting the use of βAA in at least one meat product.[94] This lack of international acceptance, concern for human health, political positioning, and a lack of clear consensus on international maximum residue limits for edible tissues have been major barriers for opening trade markets to cattle and other production species fed βAA.[77] The ban by Russia , the sixth largest export market of U.S. beef, on meat products fed ractopamine is estimated to have cost the U.S. meat industry $500 million.[77] In response to these market pressures the USDA has implemented a Quality Service Verification Program (QSVP) for animals never fed a beta agonist. However, only listed QSVP suppliers currently listed are for pork.[102]

Adverse effects of βAA and food production animals

Cattle welfare is a high priority for the beef industry; therefore research into any production practice or technology that may affect cattle welfare should be pursued.[103] Recently, both ractopamine hydrochloride and zilpaterol hydrochloride have been associated with increased risk of death loss during the late feeding period in feedlot cattle by 91% and 75% respectively.[29] In this same dataset, cattle that were shipped to the abattoir during the months of June through September were at greater risk of death compared to cohorts not fed a βAA, indicating that seasonal factors, such as greater ambient temperature, may be a risk factor as well.[29] In addition to increased death losses, abnormalities in the mobility at abattoirs has gained

considerable attention, with the greatest focus occurring in the fall of 2013, following adverse welfare events at an abattoir.[26,104]

The mobility problems and a series of clinical signs and serum biochemistry abnormalities have become termed Fatigued Cattle Syndrome (FCS) by Thomson *et.al*.[104] Cattle exhibiting FCS have various clinical signs including tachypnea with abdominal breathing, muscle tremors, stiff gait, and reluctance to move, as well as elevated serum lactate and serum creatinine kinase.[104] These clinical signs and serum biochemical abnormalities observed in affected cattle are similar to those observed in pigs with Fatigued Pig Syndrome (FPS) that was described in the late 1990's and early 2000's.[105,106]

The FPS syndrome has been documented to be caused by multiple additive stressors, including animal handling.[105-116] Fatigued Pig Syndrome is been characterized clinically by vocalization, blotchy skin, reluctance or inability to move, and muscle tremors.[105,107] Swine exhibiting FPS have greater blood lactate, decreased blood pH, greater CK, and depleted muscle glycogen.[107] Greater serum lactate concentration has been identified as a consistent characteristic of FPS pigs that become reluctant to move or non-ambulatory.[105,117] The use of RAC has been shown to increase lactate in swine.[118]

To understand the impact of improper handling and stress at the time of slaughter in pigs of heavy body weight, a challenge model was developed to induce FPS.[117] Research using the model has led to FPS mitigation strategies including management changes, such as improvements in handling immediately prior to and during transportation.[105,119]

In addition to stressors, such as handling practices and transportation space, the use of ractopamine hydrochloride in swine has been shown to increase lactate and reduces blood pH in aggressively handled pigs but did not affect pigs handled gently.[120] This is consistent with

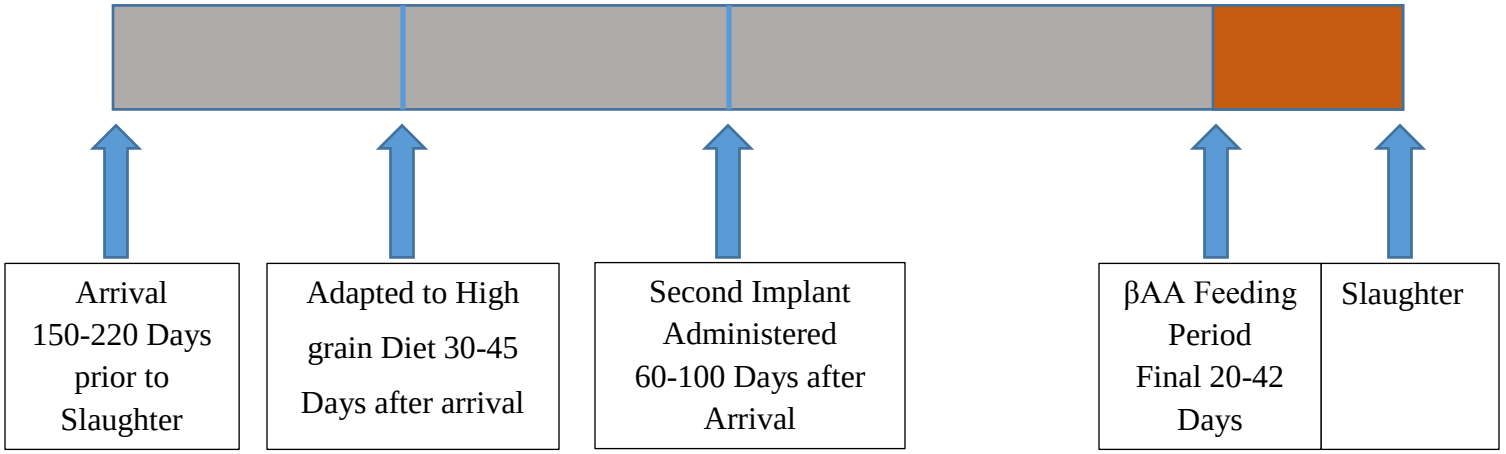
Athayde *et.al*, who reported no affect other than increased creatinine kinase due to RAC administration in properly handled pigs.[121] Additionally, it has been shown that ractopamine is a risk factor for increasing transportation losses if good transportation and handling practices are not followed.[122] Peterson *et.al.* concluded that in addition to aggressive handling, the dose of RAC fed may also be an influencing factor in transport losses as pigs fed 7.5mg/kg RAC had a greater risk of non-ambulatory, non-injured pigs compared to pigs fed 0 or 5mk/kg.[123] The factors that may increase the risk of transportation and late feeding period death losses have not been identified in cattle.

Seasonality has a modifying effect of decreasing feed intake and increasing risk of late day death loss of cattle fed βAA.[29,82] Dry feed intake (DMI) is decreased in cattle fed ZIL starting in as little as one day after the introduction of ZIL to the feed. The magnitude of the decrease is affected by sex, previous DMI, season and feedyard.[82] This suggests that management, environmental factors and physiological factors may influence the biological response to the commercial use of βAA.

Table 1-1 Summary table of physiologic systems, primary β-receptor present and cellular response. Adapted from Textbook of Veterinary Physiology[35]

Target Tissue	Primary Receptor Type	Response
Liver	β_2	Glycogenolysis, lipolysis, gluconeogenesis
Adipose tissue	β_2	Lipolysis
Skeletal muscle	β_2	Glycogenolysis
Pancreas	β_2	Increased insulin secretion
Cardiovascular	β_1	Increased heart rate, increased contractility, increased conduction velocity
	β_2	Vasodilation in skeletal muscle, coronary arteries and all veins
Bronchial Muscle	β_2	Relaxation
Gastrointestinal tract	β_2	Decreased contractility
Urinary Bladder	β_2	Detrusor relaxation
Uterus	β_2	Relaxation
Male sex organs	β_2	Erection
Eye	β_2	Ciliary muscle relaxation
Renin secretion	β_1	Stimulation

Figure 1-1: Timeline of Common Management Events in Commercial Cattle Feedyards from Arrival to Slaughter



*Zilpaterol hydrochloride has a 3 day pre-slaughter withdrawal period.

Chapter 2 - Twenty-four hour Holter monitoring in Finishing Cattle Housed Outdoors

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Running head: Electrocardiogram in beef cattle.

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Introduction

Ambulatory electrocardiogram monitoring, in the form of Holter monitoring, has been used in human and veterinary medicine for decades as an aide in the diagnosis and determination of appropriate therapy for heart rhythm disturbances. Within veterinary medicine, Holter monitors have been primarily used in companion animal species, with little attention has been given to food animal species. Moreover, the heart rhythm in clinically normal cattle fed high concentrate diets and housed outdoors in confined dry-lot facilities has not been previously reported. In order to properly identify pathologic arrhythmias in cattle, the normal rhythm and arrhythmia prevalence in healthy cattle should be defined. Most prior reports of arrhythmia in cattle have been recordings of relatively shorter duration and in animals that were hospitalized or being handled for various reasons.[124-126] Therefore, this study was initiated to describe the normal rhythm and arrhythmia characteristics of this class of cattle.

Animals, materials and methods

All animal handling and animal care practices were reviewed and approved by the Kansas State University Institutional Animal Care and Use Committee (IACUC # 3250). Twenty seven healthy 15 to17-month-old steer cattle were used. Clinical examination, complete blood count and serum biochemical analysis were performed. A lightweight Holter monitor was used in an outdoor environment. The steers (506 +/-5.5kg) were received from a commercial feeding facility in southwest Kansas. Steers were selected from a larger group based on weight uniformity and condition. Steers were adapted to a standard commercial finishing diet prior to shipment from the feeding facility to the research facility. Upon arrival, steers were weighed, identified, and were placed in a pen with *ad libitum* access to grass hay and fresh water, and provided 3.7 kg finish diet per head. Steers were re-acclimated to the finish diet over 10 d, after

which they were placed into dirt floor pens with feed bunks containing an individual animal feeding system.[i] Steers were stratified by weight and randomly assigned to 1 of the 6 pens. Pens were divided into two blocks of three pens with study d 0 separated by 5 calendar days between the 2 blocks. Pens were approximately 18 m x 3.6 m and each contained 5 gated feed bunk gates. Approximately 2.5 m^2 of shade was provided per animal. Steers were provided water ad-libitum from a tank located at the rear of the pen. Steers were individually fed twice daily with the first delivery beginning at 0700 and the second delivery between 0900 to 1000. Cattle were restrained in a hydraulic chute and blood samples for serum chemistry and CBC were collected on all study animals via jugular venipuncture on d -11 and -16 for blocks 1 and 2, respectively. Blood was immediately transferred through the rubber stopper into 3, 10ml serum collection tubes followed by 6-ml EDTA tubes[ii]. All samples were processed within 3 h of sampling. Serum was separated immediately post-centrifugation and stored overnight at 4 to 6^0 C, then submitted to the Kansas State Veterinary Diagnostic Laboratory (KSVDL) for a serum chemistry panel.[iii] Complete blood counts were analyzed using a hematology analyzer.[iv]

Holter Registration

Holter registrations of each monitor were digital. Sample frequency was 180 samples per second. Each registration recorded 3 leads[v]. Silver/Silver Chloride electrodes[vi] were applied to 5 vertically aligned locations just caudal to the forelimbs (Figure 1). Locations were: 1) 3 to 5cm dorsal to the level of the olecranon (left and right), 2) at the level of the dorsal edge of the scapula (left and right) and, 3) dorsal midline. The application sites were shaved with a #40 blade and wiped with 99% isopropyl alcohol soaked gauze sponges. An oil based calcium soap[vii] was applied to the center sponge and cyanoacrylate glue was applied to the outer edge of the electrode prior to placement on the prepared site. The color coded lead harness was attached with

the following lead locations: black- left side location 1, brown- left side location 2, red- dorsal midline, white- location 2 right side, green- location 1 right side (Figure 2-1). Lead wires were snapped onto the electrode and securely attached to the electrode using 2.5cm x 1cm section of adhesive tape across the lead attachment to both edges of the electrode base, but not contacting the animal itself. A custom made 5-cm wide elastic girth strap with Velcro fastener was then placed over all electrodes and wires, with the lead wire harness extending cranially at the dorsal midline. The elastic girth was secured to the animal by encircling the animal twice using 10 cm wide elastic bandage tape[viii] overlapping the cranial and caudal edges of the elastic girth. A custom harness made of polyester webbing was then applied to the steer, consisting of a 10 cm wide girth strap with shoulder straps that attached to the girth strap, crossing between the forelimbs and again over the dorsal aspect of the neck cranial to the point of the shoulder. A crouper strap was looped under the tail, placed along the dorsal midline and attached to the girth strap. A canvas pouch attached to the dorsal aspect of the harness contained the Holter monitor in a protective container with the lead harness inserted through the protective box into the monitor. Final application is shown in Figure 2-2. Following application, cattle were returned to their pen and were allowed to roam freely.

Analysis of Holter registrations was done semi-manually,[ix] with the type and timing of beats manually identified by two of the authors (D.F and J.T.) to verify the software system correctly annotated the identified abnormal complexes. As the software system often incorrectly annotated normal complexes as abnormal, during the inspection the annotated complexes were evaluated and marked appropriately or removed as artifact. The software identified individual heart beats as normal, abnormal or artifact. Portions of the recording marked as artifact were

excluded from the analysis. After evaluation, software output results were compiled into hourly intervals.

Statistical analysis:

Data were compiled and analyzed using a commercial statistical package to perform all statistics and analysis.[x] All data were analyzed using a generalized linear mixed model method using the fixed effects of hour and accounting for repeated measures with steer as the repeated effect. The random effects included were block and pen. Degrees of freedom were calculated using the Kenward-Rogers method. Final model was inspected using Q-Q plots and residual plots versus predicted values.

Results and Discussion

Serum biochemistry analysis and CBC were within normal reference limits on all cattle enrolled in the study. All cattle accepted the Holter monitor and harness after a short adjustment period. The heart rate was calculated every hour with the mean heart rate of 66.8 ± SD 16.4 bpm. Minimum and maximum heart rate ranged from 20 to 102 bpm for an individual beat with a median and mode of 68 bpm. Mean heart rate throughout the day showed an increasing heart rate from 0600 hours and peaking at 0800 hours, which was associated with feeding time. Heart rate decreased following feeding and remained somewhat stable until decreasing into the mid to low 60 bpm range after 2000 hours (Figure 2-3). This is similar to the pattern that has been previously reported in cattle, dogs, cats, horses and humans.[127-132] An example of a normal sinus rhythm for the three channel Holter registration is given in Figure 2-4.

Ventricular premature complexes occurred in four (14.8%) of steers. Rate of VPC occurrence ranged from 0 to 3 complexes per animal per day. Median and mode were 0 VPC/day. A total of 14 VPCs were recorded during this study, of which 9 occurred in a single steer. In this steer, 6

of the nine events were couplets and one event was greater than 2 consecutive beats. Reports of VPCs prevalence in cats, dogs and humans have been reported at 78%, 15-32%, and 29-50% respectively; but are considered rare in horses.[128,131,133,134] This study illustrates that cattle have lower VPCs prevalence rates than common companion animals, but have rates similar to that of horses. An example of a VPC is given in Figure 2-5. Several instances of 2nd degree atrial-ventricular block were also noted in this study and were likely related to hypervagatonia, as cattle have been shown to have a relatively high vagal tone.[135] Atrial premature complexes have been reported to be the most common arrhythmia in cattle and occurred in 23 of 27 (85.2%) of the cattle in this study, of which 86.6% of all events were singlets.[124,136]

In this study, we observed that Holter monitor recordings can be registered with good quality in cattle in open pen housing with properly fitting harness. The cattle used in this experiment were of uniform age and genetic background, which limits to some degree the ability to extrapolate beyond the sample population, as age and gender have been shown to influence ECG recordings.[133,137] Moreover, we purposefully selected steers for uniformity of weight from a larger group, which may have removed some animals in which arrhythmias were overrepresented. The definition of a normal ECG in cattle by Holter monitors is important as heart rate has been used as an indicator of stress in cattle as well as an indicator of cattle welfare[138,139]

In conclusion, VPC's are uncommon in normal finishing cattle, with APC's occurring at a higher rate. Instances of 2nd degree AV block were noted in cattle in this study. Cattle heart rhythm's follow patterns similar to other species with slower rates during the evening and night hours with higher rates in the morning and declining into the afternoon.

Footnotes

[i] Calan Broadbent Feeding system, American Calan, Inc. Northwood NH, USA)

[ii]Greiner Bio One, Monroe NC, USA

[iii] Cobas c501,Roche Diagnostics, Indianapolis IN, USA

[iv] ProCyte Dx, IDEXX Laboratories, Westbrook, ME

[v] DR200/HE, NorthEast Monitoring, Inc. Maynard, MA, USA

[vi] Invivo Quadtrode CV, Philips Medical Systems, Orlando FL, USA

[vii] Luberex, GE Electronics, Rockford IL, USA

[viii]Elastikon, Johnson & Johnson, New Brunswick, NJ

[ix] LX Analysis Software v 5.4,NorthEast Monitoring, Inc. Maynard MA

[x] SAS version 9.3 SAS Institute., Cary NC 27513

Figure 2-1: Electrode application points and elastic strap application on the left side of the steer

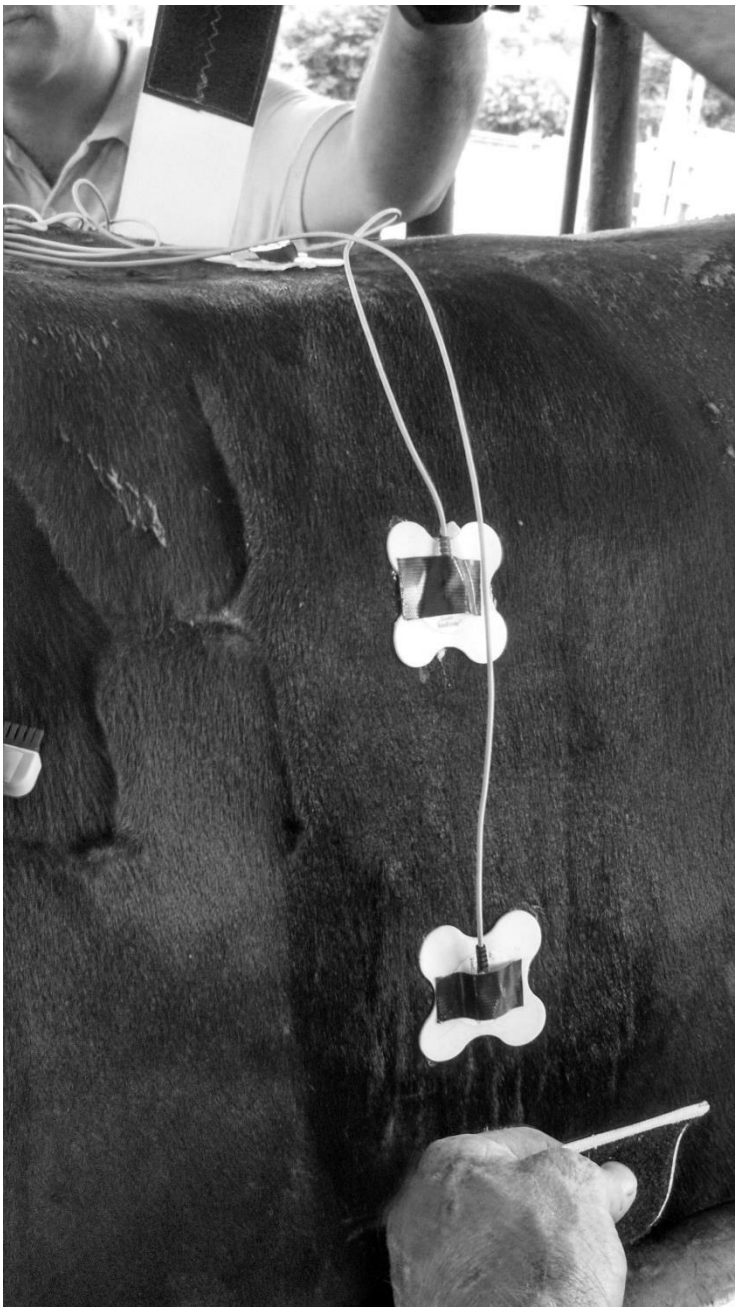


Figure 2-2: Illustration of Holter monitor apparatus applied to steer.

Figure 2-3: Mean heart rate and 95% Confidence Interval over 24 hours in normal finishing steers.

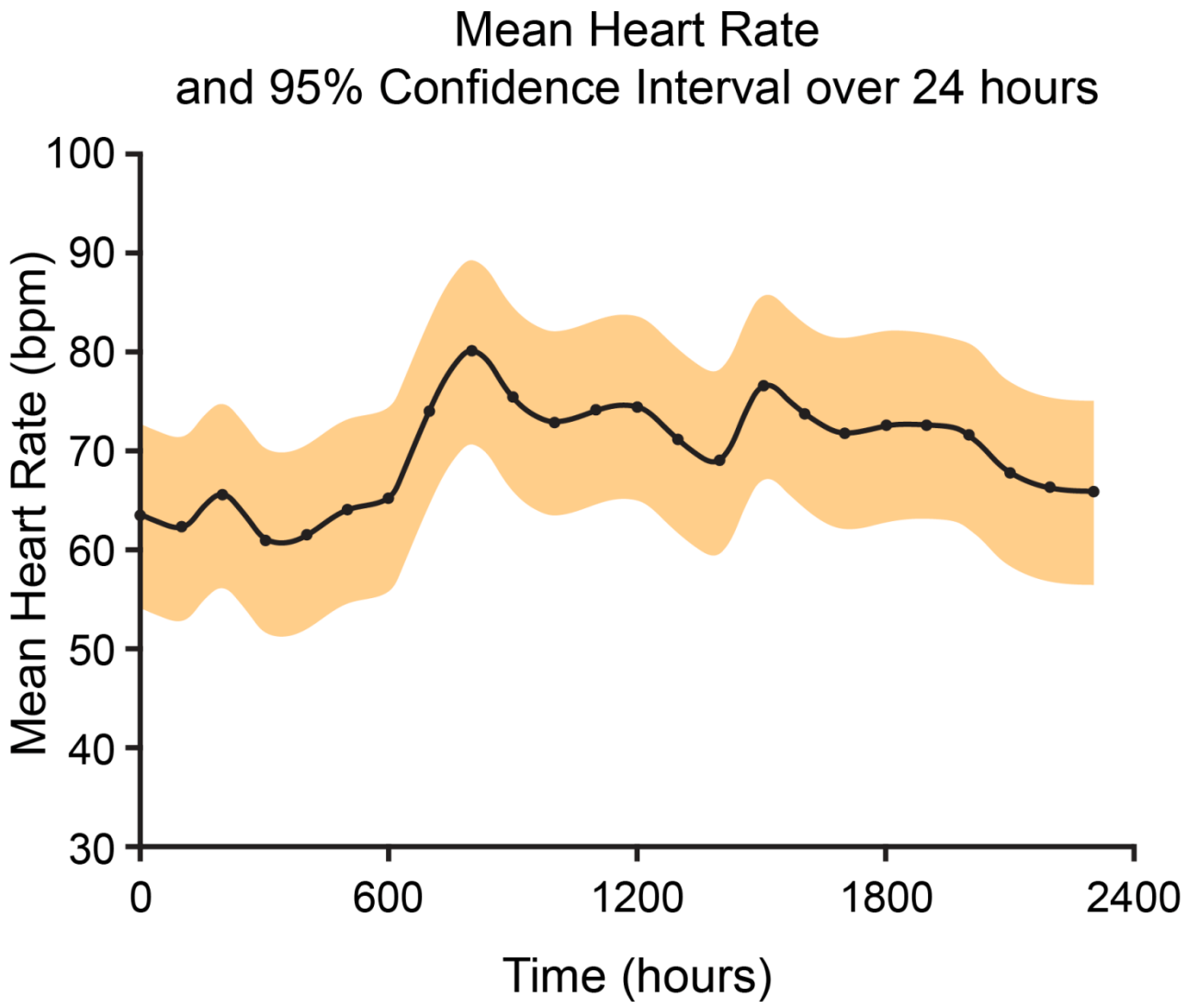


Figure 2-4: Example of Normal Sinus Rhythm for Holter Monitor Registration on three Channels

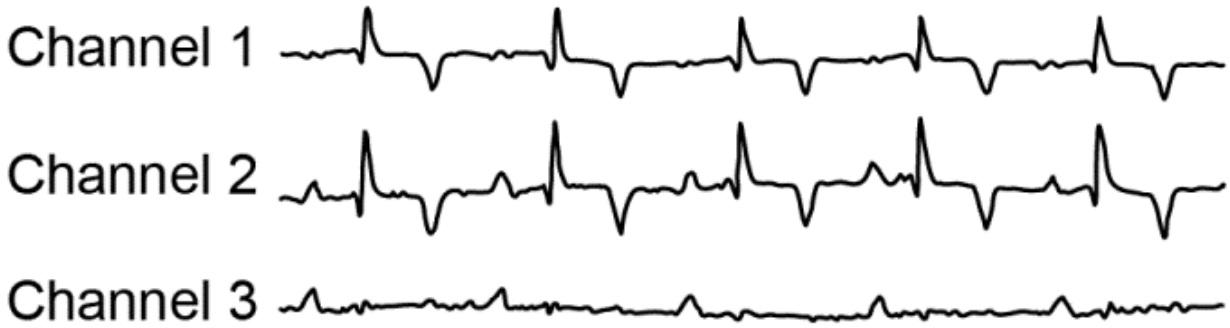
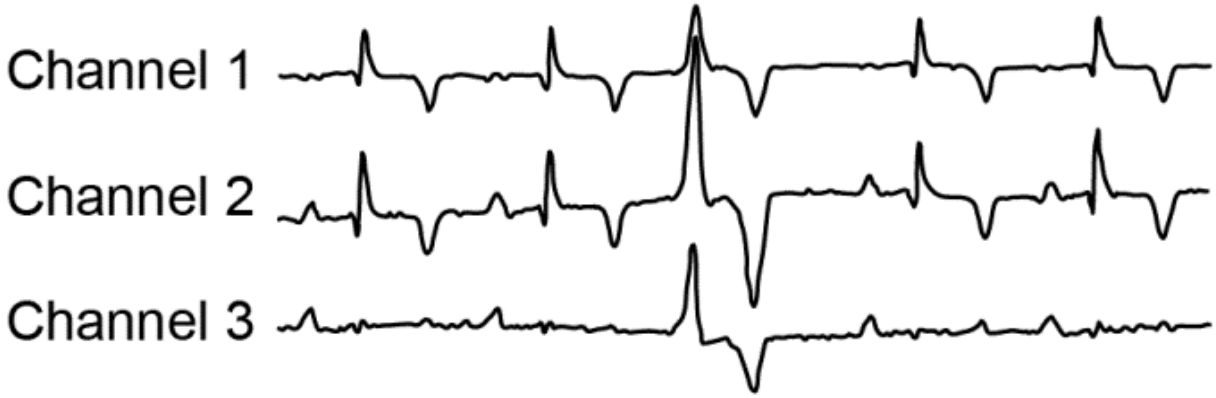


Figure 2-5: Example of a Ventricular Pre-contraction (VPC) recorded by Holter Monitor Registration



Chapter 3 - The Effect of Ractopamine hydrochloride and Zilpaterol hydrochloride on the Cardiac Electrophysiology and Blood Chemistry in Finishing Steers.

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This manuscript represents a portion of a thesis submitted by Dr. Frese to the Kansas State University Department of Diagnostic Medicine and Pathobiology as partial fulfillment of the requirements for a Doctor of Philosophy degree.

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GHL has received consulting fees or honoraria from Elanco Animal Health, Merck Animal Health, and Zoetis.

CDR has received consulting fees or honoraria from Elanco Animal Health, Merck Animal Health, and Zoetis.

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Objective—To investigate the effect of ractopamine hydrochloride and zilpaterol hydrochloride on cardiac electrophysiology, pathology and blood parameters of finishing beef steers

Design—Experimental study

Animals—30 Steers

Procedures-- Cattle were stratified by weight and randomly assigned to 1 of 3 treatment groups and individually fed a diet containing no additive, ractopamine hydrochloride (300 mg·animal^{-1}·d^{-1}), or zilpaterol hydrochloride (8.3 mg/kg of feed DM basis) per FDA label. Ambulatory electrocardiograms were placed on cattle on d -2, 6, 13, and 23 of the trial and recorded continuously for 72, 24, 24, and 96 h, respectively; d 0 was the first day of beta agonist feeding. Blood samples were obtained via jugular venipuncture for complete blood count, serum chemistry and blood lactate analysis at the time of Holter monitor application. Ambulatory electrocardiograms recordings were evaluated for mean heart rate, ventricular, and supraventricular arrhythmias per day.

Results-- Cattle fed zilpaterol hydrochloride and ractopamine hydrochloride had greater mean heart rate than control. Mean heart rates were within normal ranges with the exception of ractopamine on day 14, which was elevated. No differences were observed in VPC, or SVPC between ractopamine hydrochloride or zilpaterol hydrochloride treated cattle and control cattle. No differences were found among treatments in arrhythmia rate when classified as single beat, paired beat, or greater than 2 beats per event.

Conclusions and Clinical Relevance—Results indicated that ractopamine hydrochloride and zilpaterol hydrochloride at FDA approved doses do not increase arrhythmia rates but does increase mean heart rate while mostly remaining within normal physiologic ranges.

Abbreviations

ADG- Average daily gain

βAA –Beta adrenergic agonist

BW- Body weight

CBC-Complete blood count

CHEM- Serum chemistry

CON- Control treatment

CPK- Creatine phosphokinase

d - Day

DM- Dry matter

ECG- Electrocardiogram

FDA- Food and Drug Administration

FFPE- Formalin fixed paraffin embedded

g- Gravity

G:F- Pounds of gain to pounds of feed ratio

h- hour

LM- Longissimus muscle

MBA- Multiple beat arrhythmia

MHR – Mean heart rate

PBA- Paired beat arrhythmia

RAC- Ractopamine hydrochloride treatment

SBA- Single beat arrhythmia

SD- Standard Deviation

SEM- Standard error of the mean

SVPC- Supraventricular precontraction

TD- Treatment day

VPC- Ventricular precontraction

wk- week

ZIL- Zilpaterol hydrochloride treatment

Introduction

The βAA ractopamine hydrochloride and zilpaterol hydrochloride during the last 20 d to 42 d of the feeding period for improved gain and feed efficiency have been used extensively in the cattle feeding industry since their FDA approval in 2003 and 2006, respectively[22,23]. The use of RAC and ZIL in cattle has been shown to increase average daily gain, gain to feed ratio, carcass weight and to reduce dry matter feed intake, thus greatly increasing efficiency at the end of the feeding period.[24,78,79]

Beta adrenergic agonists have been used for decades in human medicine for treatment of respiratory ailments, such as asthma, because of their bronchodilatory effects. However, the physiological response of βAA is not limited to the respiratory tract as βAA receptors are found on nearly every mammalian cell with wide ranging effects including vasodilation, bronchodilation, smooth muscle relaxation and positive inotropic effects.[25]

Despite their documented benefits, use of these pharmaceuticals has occasionally been associated with adverse drug events. Long-term use of these drugs has been implicated in increased mortality rates in asthmatic individuals[7]. Myocardial effects, including myocardial toxicity, myocardial apoptosis and increased risk of death in humans and other species have been linked to βAA [13-16]. Cardiac effects commonly seen with the administration of a short acting βAA are tachycardia, tremors, decreased serum potassium and increased blood glucose.[17] The role of βAA in producing electrocardiographic arrhythmias is varied. In one study ventricular arrhythmias were associated with fenoterol but not albuterol in people.[140] Other studies have found no differences due to βAA in Holter monitor registrations in children.[141]

Physiological responses to βAA vary by dose, drug and specie to which they are administered. For example, ractopamine fed to greyhounds at 1mg/kg, approximately 2x dose commonly fed to cattle, has been reported to cause multiple pathologies.[19] These pathologies include ventricular and supraventricular premature complexes, multiform ventricular premature complexes as well as extensive regions of necrosis of the ventricular walls .[19] However, these effects have not been seen in cattle.[23]

Recently, both RAC and ZIL have been associated with increased risk of death loss during the late feeding period in feedlot cattle by 91% and 75% respectively[29]. In this same dataset, cattle that were shipped to the abattoir during the months of June through September were at higher risk of death, indicating that seasonal factors, such as greater ambient temperature, may be a risk factor as well.[29]

These reports, combined with the documented effects of βAA on cardiac function in other species suggest a hypothesis that βAA may affect the cardiac electrophysiology in feedlot cattle. Data on the cardiac and electrophysiological effects of these two compounds in finishing cattle is limited. Thus, the objective of this study was to determine the effect of RAC and ZIL on the , blood chemistry, pathology, heart and arrhythmia rates measured by the ambulatory electrocardiogram of finishing steers compared to negative controls.

Materials and Methods

The Kansas State University Institutional Animal Care and Use Committee approved all animal handling and animal care practices used in this research under IACUC # 3250.

Cattle and feed

Forty Angus steers (506 +/-5.5kg) were received from a commercial feeding facility in southwest Kansas. Steers were selected from a larger group based on weight uniformity and

condition. Steers were adapted to a standard commercial finishing diet prior to shipment. Upon arrival, steers were individually weighed; identification recorded, placed in a pen with *ad libitum* access to grass hay and fresh water, and provided 3.7 kg per animal of a finishing diet. Finishing diet was a corn/distillers grains base diet with a formulated net energy for maintenance of 2.12 Mcal/kg and net energy for gain of 1.45 Mcal/kg (Table 3-1). Steers were re-acclimated to the finishing diet over 10 d. After 10 d steers were placed into 6 dirt floor pens with individually electronically gated feedbunks.[a] All feed bunk gates were locked open and the cattle were provided *ad libitum* access to feed on a pen basis. On day -43 steers were weighed and 30 steers were selected for calm temperament and previously observed use of the feed bunk gates. Selected steers were stratified by weight by grouping the six heaviest steers and randomly assigning to 1 of 6 treatment-pen-block combinations. This process was repeated for each succeeding weight group of 6 steers until all steers were assigned. Stratification was performed to limit variation due to factors that may affect steers of different weights unequally, such as environmental temperature, and feed consumption. Treatments were: CON no added medication, ZIL [b]; 8.3 mg/kg of feed DM basis, and RAC [c]; 300 mg·animal $^{-1}$ ·d $^{-1}$. Pens were divided into two blocks with treatments equally distributed between blocks. Study d 0 was separated by 5 calendar days between the 2 blocks. Steers were housed in 6 outdoor dirt floor pens approximately 18 m x 3.6 m with 5 individually gated feedbunks in each pen. Feedbunk electronic gate keys were placed around the neck of each steer using a heavy duty plastic chain. Approximately 2.5 m^2 of shade was provided per animal. Steers were provided *ad libitum* access to water from a tank located at the rear of the pen. Randomization was followed by a 43 d feedbunk gate training period to allow steers time to adapt and operate the individually gated feedbunks. Three steers were replaced during training, two from the CON treatment and one

from the ZIL treatment for failure to learn to operate the feed bunk gate and eat without assistance. These steers were replaced from the pool of extra steers with the animal that was the nearest in weight to the weight of the removed animal at the time of randomization.

The CON treatment cattle were fed the base diet which contained higher roughage content than most commercial feedlot diets as a safety factor because of the frequent handling and disruptions to cattle routine. The βAA were mixed into the base diet. Diets containing ractopamine hydrochloride were mixed in batches from the control diet by adding 320 g of a Type B ractopamine hydrochloride medicated article (4410 g/metric ton air dry basis) to 36.3 kg of control diet as fed basis, which provided 300 mg RAC in 7.25 kg as fed basis of diet. Diets were mixed for 5 min in a ribbon mixer. Target drug concentration was verified by taking four samples from a batch of feed and pooling into a single sample, frozen, and then submitted to a commercial lab for analysis. Dosage of RAC used is a commonly fed dose in commercial applications.

Diets containing zilpaterol hydrochloride were mixed in an identical manner as RAC diets with 514 g of Type B ZIL (480 g/ton) medicated article for a target diet concentration of 8.3 mg/kg dry matter basis as per FDA approved label. Three to 4 d of diet were batched per mixing and stored until used for both diets. The mixer was flushed between batches that contained different treatments using compressed air followed by mixing 4.5 kg of control diet for 5 min and repeated once. The second batch of flush diet was randomly sampled and both batches were discarded. Samples of flush diet were submitted to the commercial lab to verify absence of pharmaceutical in the flush feed.

Steers were individually fed twice daily with the first delivery at 0700 with the second delivery between 0900 and1000. Daily feed amount was individually determined by a visual

estimation of feed remaining from the prior days' feed delivery. Remaining feed was not discarded and remained in the feed bunk. On d 12 and 7, for blocks 1 and 2 respectively, feed call protocol was changed and all residual feed was removed from bunks and weighed before being discarded. Cattle fed RAC were fed 7.25 kg as fed basis of diet in the first delivery containing 300 mg of RAC, with the balance of the feed call delivered by CON diet at the second feeding to ensure that the entire dose of the test article was consumed daily. Cattle fed CON and ZIL were fed at both feedings their respective assigned diet. The final day for feeding ZIL was d 24 to meet the FDA specified withdrawal period. Water disappearance for each pen was recorded daily by filling each tank to a designated level and recording volume of water added with a water meter.

Cattle were restrained in a commercial hydraulic chute and weighed. Blood samples for CBC and CHEM were collected on d -16,-11, 6, 13, and 23 via jugular venipuncture into a 60 ml disposable syringe[d]. Baseline blood samples for CBC and CHEM were collected on all study animals on d -11 and -16 for blocks 1 and 2, respectively. Blood was immediately transferred through the rubber stopper into the 3, 10 ml serum collection tubes followed by a single 6 ml EDTA tube[e]. Blood samples for lactate were collected on d -2, 1 6, 13, and 23. On d -2 and 1, blood samples for lactate analysis were collected in a 6 ml collection tube containing sodium heparin. On d 6, 13, and 23 CHEM and lactate samples were collected in an identical manner as previously described for CHEM samples. Following collection, all blood samples were stored in chilled coolers prior to being centrifuged at 3000 × *g* for 15 min. All samples were processed within 3 h of sampling. Serum was separated immediately post-centrifugation and stored overnight at 4 to 6°C. Samples were collected at the time of Holter monitor application for each steer as described later. Blood and serum was submitted to Kansas State University Veterinary

Diagnostic Laboratory for analysis of CHEM panel [f], and blood lactate [g]. Complete blood counts were analyzed using a hematology analyzer,[h] except for block 1 on d 6 which was submitted to Kansas State University Veterinary Diagnostic Laboratory and analyzed[i] due to the previously mentioned analyzer being unavailable on that single date. Laboratory personnel were blinded to sample assignment to treatment.

Electrocardiogram and Blood Chemistry

Holter recordings of each steer were recorded using a commercially available ambulatory digital electrocardiogram recorder.[j] The unit used five lead wires and three recording channels for data collection redundancy. A Silver/Silver Chloride electrode[k] was applied to each of 5 vertically aligned locations just caudal to the forelimbs (Figure 3.1). Locations were: 1) 3 to 5 cm dorsal to the level of the olecranon (left and right), 2) At the level of the dorsal edge of the scapula (left and right) and, 3) Dorsal midline. The application site was shaved with a #40 blade and wiped with 99% isopropyl alcohol soaked 4 × 4 gauze sponges. An oil-based calcium soap[l] was applied to the center sponge and cyanoacrylate glue was applied continuously to the outer edge of the electrode prior to placement on the prepared site. The color-coded lead harness was attached with the following lead locations: Black: left side location 1, Brown: left side location 2, Red: dorsal midline, White: location 2 right side, Green: location 1 right side (Figure 3-1). Lead wires were snapped onto the electrode and securely attached to the electrode using 2.5 cm × 1 cm section of adhesive tape across the lead attachment to either edge of the electrode base, but not contacting the animal itself. A custom made 5 cm wide elastic girth strap with a hook-and-loop fastener was then placed over all electrodes and wires, with the lead wire harness extending cranially at the dorsal midline. The elastic girth was secured to the animal by encircling the animal twice using 10 cm wide elastic bandage tape [m] overlapping the cranial and caudal edges

of the elastic girth. A custom harness made of polyester webbing was then applied to the steer. Harness consisted of a 10 cm wide girth strap with shoulder straps that attached to the girth strap, crossing between the forelimbs and again over the dorsal aspect of the neck cranial to the point of the shoulder. A crouper strap was looped under the tail, placed along the dorsal midline and attached to the girth strap. A canvas pouch attached to the dorsal aspect of the harness contained the Holter monitor in a protective container with the lead harness inserted through the protective box into the monitor (Figure 3-2). Animal identification number was entered into the Holter monitor and ECG signal checked prior to release of the steer from the chute. Each Holter monitor was also assigned its own unique number independent of animal identification. This identification was subsequently assigned to all data files for an individual recording until analysis was complete to maintain blinding. The Holter monitor continuously recorded ECG onto a secure digital data card. Harnesses with monitors were applied between 1600 to 1930 h on experiment days -2, 6, 13, and 23 and removed between 1600 to 1900 h on d 1, 7, 14, and 26. Electrocardiograph data were collected on all or part of experimental d -2 to 1, 6, 7, 13, 14, and 23 to 26. Steers with units that became in need of maintenance during the recording period were removed from their home pen, restrained in a hydraulic chute and necessary maintenance performed.

The Holter data file was downloaded from the secure digital data card into a commercially available analysis program.[m] Two steers in block 2 ZIL treatment on the d -2 Holter monitor application had data files that were invalid and not recoverable, and were omitted from the analysis. The software identified individual heart beats as normal, abnormal, or artifact. Each recording was analyzed in its entirety by a board-certified veterinary cardiologist who was blinded to treatment. Heart beats identified as abnormal were individually classified for

subsequent statistical analysis. After evaluation, software output results were compiled in hourly intervals. Heart rate was determined by measurement of R-R interval time. Mean heart rate was reported by the software as the mean of all R-R intervals not declared artifact within an hour.

Electrocardiogram arrythmia events were summarized within steer into 24-h intervals by setting hour 0 of the day to 0700 h to pair the time of morning feeding with beginning of a TD. An arrythmia event was defined as any ECG rhythm that did not display all P-QRS-T complexes in a normal manner. Arrhythmias were reported as total count of events for each hour of each classification and then summarized by TD. Arrythmia events were classified into SVPC or VPC in origin and as SBA, PBA, or MBA. Data recorded at times associated with animal handling, i.e. prior to 1900 h on day of monitor application and after 1600 h on day of monitor removal were not included in the analysis.

On d 27, steers were transported to a commercial abattoir in southwest Kansas for slaughter. The entire heart, lung, and liver were collected at slaughter from each steer and placed in pre-identified containers for each animal. Immediately following the collections of all organs, subsamples were taken in the following manner. The heart was subsampled by taking a 1 cm cross section through both ventricles and septum halfway between the base and apex of the heart. Lung was subsampled by taking a 200 to 400 g sample from a caudal lung lobe. Liver was sampled by taking a 200 to 400 g sample from a portion of the liver. All organs were visually examined for gross pathologies. Samples from heart, lung, and liver were placed in sealed plastic bags and placed in ice-cooled containers for transportation to the laboratory. Tissue samples were reduced in size approximately 1 to 2 cm^3 and placed in containers with 10% formalin. The histopathologic analysis was done by a board-certified veterinary pathologist who was blinded to treatment. To demonstrate apoptotic cell death, TUNEL staining[o] was performed

on FFPE heart sections from all the treatment groups as per manufacturer's instruction. Canine testes and lymph nodes were used as positive control. Briefly, FFPE tissues were sectioned onto positively charged slides, and heated for 25 min at 60°C. Sections were pre-treated with proteinase k (20 μg/μl) for 15 min at room temperature and 3% peroxide for 5 min. Slides were incubated in working strength Stop/Wash Buffer for 10 min at room temperature to prevent non-specific calcium binding and then incubated with working strength terminal deoxynucleotidyl transferase enzyme (1:50) for 1 h at 37°C. The α-Digoxigenin conjugate was added for 30 min at room temperature and sections were visualized with Diaminobenzidine and counterstained with hematoxylin.

Statistical Analysis

All data were analyzed using the generalized linear mixed model procedure in a commercially available statistical analysis program[p] accounting for repeated measures when appropriate and included the fixed effects of treatment, TD, and their interaction. The random effects included were block and a random intercept by pen. Degrees of freedom were calculated using the Kenward-Rogers method. Repeated measure covariance structure and distribution type were chosen to fit the statistics and variable being tested using Akaike Information Criterion. Arrhythmia classifications were analyzed using a negative binomial distribution with data transformed by adding one to the total count of each category of arrhythmia. The natural logarithm of readable recording hours within TD was used as an offset so results are adjusted to a 24-h period.

Mean heart rate was analyzed using hourly heart rate as a sub-sample within treatment day with random effects of block and repeated measures by steer. Hour intervals with less than 100 readable heart beats were removed from the dataset and treated as missing data. Level of

statistical significance was declared to be $P < 0.05$ for all statistical tests. One steer from the CON treatment was removed on d 14 of the study for severe pododermatitis unrelated to the study and all data collected from this steer was excluded from the analysis. Holter recordings for d 6 to 7 and d 13 to 14 were recorded and analyzed as a single 24 h period and labeled d 7 and d 14 respectively.

Performance data were calculated using BW from d -2 and d 26. Weight on d 26 was shrunk 3% for calculation of final BW, ADG, G:F, and dressing percentage. Carcass identification was maintained at the commercial abattoir by matching the visual identification tag of each animal in order of slaughter and the carcass identification number of each animal assigned by the abattoir. Water disappearance per animal was calculated by taking the total liters delivered daily divided by the number of steers in the pen on that day.

For the primary variables of heart rate and arrhythmias a sample size of 10 experimental units (steer) per treatment group was determined to be necessary to detect a 12.5% difference in between treatment and control groups. Assumptions for calculation included a 10% coefficient of variation, an α of 0.05, and a β of 0.20.

Results

Cattle fed RAC had increased HCW and final live BW in steers compared to CON ($P < 0.05$; Table 3-2) but was not different from ZIL. However, no differences ($P > 0.05$) were noted in ADG, G:F, dressing percentage, LM area, 12^{th}-rib fat thickness, yield grade, or marbling score for cattle regardless of treatment. There was a treatment by week interaction for intake. Intake was greater in RAC cattle ($P < 0.05$) than both ZIL and CON in wk 2 and 3 (Table 3-2). Intake in RAC cattle was greater in wk 2 than in wk 1 ($P < 0.05$) and less in wk 3 and 4 than wk 1 or 2 ($P <0.05$). Intake in CON cattle was less ($P < 0.05$) in wk 3 and 4 than during wk 1 and 2. Intake

in ZIL cattle was less each week ($P < 0.05$). There were no differences detected in daily water disappearance between CON (45.1 ± 6.3 $L \cdot hd^{-1} \cdot day^{-1}$), ZIL (45.4 ± 6.3 $L \cdot hd^{-1} \cdot day^{-1}$) and RAC (46.8 ± 6.5 $L \cdot hd^{-1} \cdot day^{-1}$).

Creatine phosphokinase was elevated on d 13 and 23 in ZIL (Table 3-4) compared to CON and RAC. There were no other differences in other measured blood chemistry parameters among CON, ZIL and RAC treatments (Table 3-3). No differences in blood lactate were found between treatments (Table 3-4).

The mean percentage of the Holter data recording period for each animal during each 24 h collection period of readable quality was 69% with a SD of 24.4%. The most common cause of artifact data was cattle activity such as walking and feeding.

In this study, cattle fed ZIL and RAC had greater MHR than CON ($P < 0.05$). Treatment, day, and the interaction of treatment and treatment day were found to be significant ($P < 0.05$). Mean heart rate was not different ($P > 0.05$) in CON, ZIL and RAC cattle at d -1 and was 71.7 ± 5.3, 68.9 ± 5.9 and 67.4 ± 5.3 bpm respectively. Zilpaterol increased MHR in cattle compared to CON on d 7, 14, and 23, but not on d 0, 24 or 25. Ractopamine increased MHR compared to CON on d 7, 14, and 24 but not on d 0, 23, or 25 (Table 3-7;Figure 3-3). Mean heart rate was decreased in CON cattle on d7 compared to d 0 but not compared to d 14, d 23, d 24, and d 25.

No differences ($P > 0.20$) in arrhythmia events per day of either VPC or SVPC or beats per event were detected between RAC, ZIL and CON (Tables 3.5 and 3.6).

Prevalence of SBA accounted for 84.0% (242/288) of all arrhythmia events. Prevalence of PBA was 11.1% (32/288) with MBA at 4.9% (14/288). It should be noted that 75.3% of all arrythmia events with 72.3% (175/242), 90.6% (29/32) and 92.8% (13/14) of SVA, PBA and MBA, respectively, came from a single animal in the RAC treatment.

No significant histopathologic lesions were detected in lung, liver, or heart of CON, ZIL, and RAC cattle. However, mild myocardial degeneration in the heart and infiltration by small numbers of lymphocytes and plasma cells were seen in liver, kidney and heart of some animals across all the groups. No TUNEL positive cells were seen in any of the CON, RAC and ZIL cattle.

Discussion

This study is to the authors' knowledge, the first study to investigate the ambulatory ECG of cattle using a Holter monitoring system for continuous recording over multiple days as well as the effects of βAA on the Holter monitor recordings of cattle. Previous studies looking at the ECG's in cattle have been done for durations of minutes to a few hours and in a restrained environment of a clinical hospital setting and no population based studies investigating prevalence of arrhythmias or the cardiac effects of βAA in cattle have been performed.

Feed intake, growth performance, and carcass characteristics response to βAA observed in this study are generally in accord with previous reports of effects for both RAC and ZIL.[142,143] Treatments with ZIL and RAC increased ADG about 0.6 kg or 47%; this difference however, was not statistically significant as this study was not designed as a performance study and lack of statistical significance in performance measures should be interpreted in that manner

Normal resting heart rate for individual cattle is 49 to 84 bpm[144]. Cattle fed ZIL and RAC had greater MHR than CON, but all treatment MHR were within the normal range except for RAC on d 14. The magnitude of the MHR difference between ZIL (14.1 bpm), RAC (15.5 bpm), and CON was greatest on d 7 and decreased as the feeding period progressed until no differences were detected on the final TD recorded (Table 3-7; Figure 3-3). This could be due to attenuation of the steers to βAA, which has been previously reported[145]. The reason for the decreased MHR

at d 7 compared to d0 in CON cattle is not readily apparent. Increased MHR by the ingestion of ZIL and RAC is consistent with previous reports in cattle fed ZIL[22] and RAC[146]. The magnitude of the increase in MHR in cattle fed ZIL, with dose calculated to be approximately 0.14mg/kg, is much lower than what has been reported in horses fed 0.17 mg/kg , in which severe tachycardia was reported.[18,147] However, the increase in MHR in ZIL and RAC cattle is somewhat greater than the 9.1 bpm increase reported in a meta-analysis of single dose βAA administration in people.[13] An increased resting heart rate in people has been shown to be a risk factor for mortality independent of physical fitness and other cardiovascular risk factors.[148-150] The degree to which the increased MHR may be a factor for all-causes death in cattle is not known and should be investigated further.

Supraventricular arrhythmias are reported to be more common in cattle than ventricular arrhythmias[125], and are consistent with the results of this study. However, prevalence of supraventricular arrhythmias in cattle is lower than horses, where atrial arrhythmias are common. The most common arrythmia reported in cattle is atrial fibrillation[124] with other supraventricular arrhythmias being rarely reported in cattle[125]. The rates of SVPC reported the current study are higher than previously reported[125] but are also more broadly defined to include more events than a single type of arrhythmia. The most common clinical disorder in cattle subsequently diagnosed with atrial fibrillation was a gastro-intestinal disorder and conversion back to normal sinus rhythm without specific therapy was noted in 60% of the cases reported[124]. In this study, several instances of 2nd degree atrioventricular (AV) block were noted, which is rare in cattle but common in horses, and is considered a benign arrythmia. Other benign events such as bradycardia have been reported in cattle with presurgical fasting[126].

Ventricular arrythmia rates in this study (1.5 to 4.9 events/day) were below VPC rates (>3.0 VPC/h), which have been reported to constitute an increased mortality risk in humans[151]. Rates of VPC greater than 30 VPC/h in healthy people have been shown to increase risk of cardiovascular death and myocardial infarction[152]. However, care should be taken in extrapolation of these risk thresholds in people to other species as the health risk of such events in animals are not well defined. Extrapolation of the results of the current study to the health risk of βAA on cattle should be done with caution as this was not a health risk assessment study.

The gross and histopathological myocardial damage that has been reported in greyhounds were not present in any of the cattle in this study.[19]

The high proportion of arrhythmias observed in this study coming from a single steer (75.3%) warrants further investigation. Though this steer was in the RAC treatment group no inference should be drawn as to the influence of RAC on the arrhythmia rate of this steer, because rate was elevated compared to all other steers at all TD's measured throughout the duration of the study.

Blood lactate concentrations measured in this study were in the general range to slightly higher than previously reported levels (1 to 2 mmol/L) in finishing steers.[153] Creatine phosphokinase levels were increased on d 13 and 23 in ZIL cattle compared to CON and RAC, consistent with previous reports.[22] However, all CPK levels were within normal reference ranges. Increased CPK levels have been reported due to many factors, including myocardial infarction and damage of skeletal muscle. Increased in CPK found in this study may possibly be attributed to increased lean body mass, which has been shown to increase CPK in people and has been shown to increase lean body mass in cattle as measured by reduced USDA yield grade[80,143,154]. However, other possible contributions to increased CPK could also be mild muscle

damage and cannot be ruled out as a potential source. Increased CPK in humans can be an indicator of myocardial infarction, however it has been shown to be misinterpreted as having cardiac origin in cases of acute myocardial infarction in people with increased lean body mass[155].

While there are no differences found in arrhythmia rates or classification among treatments in this study, this does not rule out the potential for RAC and ZIL to be a risk factor for individual animals. This study was designed to evaluated differences among βAA treatments, and not designed to determine arrhythmia rates or events that may be a risk factor for sudden cardiac death or other animal health parameters; results should not be interpreted in that manner.

In conclusion, arrhythmia rate and classification, and blood lactate levels were not influenced by RAC or ZIL compared to CON. Both RAC and ZIL increased MHR, with the greatest magnitude at d 7 and then decreasing throughout the remainder of the feeding period. Mean heart rate was within the normal range reported for cattle in all cases except d 1 Ractopamine MHR. Creatine phosphokinase was increased in ZIL cattle on d 13 and 23 compared to CON and RAC. The rate and classification of cardiac arrhythmias investigated in this study had no changes that can be attributed to ZIL or RAC usage compared to CON. However, in light of rare event health problems observed in the field, more research regarding the relationship of βAA usage as an agent or risk factor that may interact with cardiovascular, environmental and management conditions that may influence late feeding period death losses is warranted.[8]

Footnotes

[a] American Calan; Northwood, NH

[b] Zilmax®; Merck Animal Health, Summit, NJ

[c] Optaflexx®; Elanco Animal Health, Greenfield, IN

[d] Coviden Health Care Products, Dublin, Ireland

[e] Greiner Bio One, Monroe, NC

[h] Cobas c501; Roche Diagnostics, Indianapolis IN

[g] Nova CCX; Nova Biomedical, Waltham, MA

[f] ProCyte Dx; IDEXX Laboratories, Westbrook, ME

[i] Advia 2120i, Siemens Healthcare Diagnostics, Inc., Tarrytown, NY

[j] DR200/HE; NorthEast Monitoring, Inc. Maynard, MA

[k] Invivo Quadtrode CV ; Philips Medical Systems, Orlando, FL

[l] Luberex; GE Electronics, Rockford, IL

[m] Johnson & Johnson, New Brunswick, NJ

[n] Holter LX Analysis Software v.5.4; NorthEast Monitoring, Inc. Maynard, MA

[o] ApopTag; Millipore, Temecula, Cap

[p] SAS 9.3; SAS Institute, Cary, NC

Table 3-1: Composition of experimental diets[1] individually fed to finishing steers

Item	Control Diet[1]
Ingredient	% of DM
Corn grain, cracked	58.5
Dried Distiller's grains plus solubles	21.5
Cottonseed hulls	12.3
Molasses, cane	2.5
Supplement pellet[2]	5.2
Chemical composition, DM basis[3]	
Dry matter, %	86.5
Crude protein, %	13.5
NEm, Mcal/kg	2.12
NEg, Mcal/kg	1.45
Calcium, %	.79
Phosphorus, %	.48
Salt, %	.79

[1] To build the other 2 diets, Type B articles were added to result in diets containing 8.3 mg/kg (dry matter basis) zilpaterol hydrochloride, and 300 mg in 7.25 kg (as fed basis) or 46 mg/kg (dry matter basis) of ractopamine hydrochloride.

[2] Pellet formulated to contain (as fed basis): Crude protein = 14.75%, Calcium = 12.0%, Phosphorus = none added, Potassium = 1.0%, Magnesium = 1.4%, Salt = 5.25%, Vitamin A = 88,000 IU/kg, Vitamin D = 8,800 IU/kg, Vitamin E = 176 IU/kg, Thiamine = 352 IU/kg, Copper = 212 ppm, Zinc = 635 ppm, Monensin = 617 mg/kg, Tylosin = 159 mg/kg.

[3] Analyzed (Servi-Tech Laboratories, Hastings NE 68902).

Table 3-2: Cattle performance, carcass characteristics, and dry matter intake of steers fed control diet or control diet plus zilpaterol (8.3mg/kg dry matter basis) or ractopamine 300 mg·animal^{-1}·d^{-1}

Item	Control		Zilpaterol		Ractopamine	
Live Weight	Mean	SEM	Mean	SEM	Mean	SEM
Initial Wt., kg	581	8.7	583	8.3	599	8.3
Final Wt., kg	616^{a}	11.3	637^{ab}	10.7	652^{b}	10.7
ADG, kg	1.27	0.23	1.91	0.23	1.90	0.23
G:F	0.13	0.02	0.19	0.02	0.18	0.02
HCW, kg	377^{a}	7.3	390^{ab}	7.0	398^{b}	7.0
Dress %	63.8	0.01	63.9	0.01	63.7	0.01
LM Area, cm^2	85.8	4.7	87.1	4.6	94.9	4.6
Backfat, cm	1.18	0.12	1.10	0.12	1.09	0.12
Yield Grade	3.17	0.31	3.03	0.31	2.62	0.31
Marbling Score	521	29.4	531	27.9	535	27.9
Dry Matter Intake, kg/d						
Week						
1	10.0^{a,c}	0.34	10.6^{a,c}	0.33	10.6^{a,c}	0.33
2	9.9^{a,c}	0.34	10.0^{a,d}	0.34	11.2^{b,d}	0.33
3	8.7^{a,d}	0.34	9.4^{a,e}	0.34	10.0^{b,e}	0.33
4	9.1^{a,d}	0.35	9.1^{a,f}	0.34	10.0^{a,e}	0.34

^{a,b} Within rows, means without a common superscript differ ($P < 0.05$)

^{c,d,e,f} Values for week within columns, means without a common superscript differ ($P < 0.05$)

Table 3-3: Selected serum chemistry overall mean concentration results for finishing steers fed control diet or control diet plus zilpaterol (8.3mg/kg dry matter basis) or ractopamine 300 mg·animal^{-1}·d^{-1}

Item	Control		Zilpaterol		Ractopamine		Normal Range[1]	P value*
	Mean	95% CI	Mean	95% CI	Mean	95% CI		
Glucose, mg/dL	67.9	54.8 to 81.1	64.8	50.8 to 78.8	68.2	54.3 to 82.1	29 to73	0.53
Blood urea nitrogen, mg/dL	10.3	6.8 to 15.8	8.5	5.6 to 12.9	10.0	6.7 to 15.3	9 to 24	0.58
Creatinine, mg/dL	1.20	1.12 to 1.30	1.30	1.22 to 1.39	1.24	1.16 to 1.33	.5 to 1.6	0.29
Sodium, mmol/L	143	141 to 144	142	141 to 143	143	141 to 143	131 to 155	0.26
Potassium, mmol/L	4.75	4.60 to 4.91	4.86	4.71 to 5.01	4.8	4.65 to 4.94	4.2 to 6.3	0.59
Chloride, mmol/L	98.5	97.5 to 99.5	97.5	97.5 to 98.5	98.5	97.5 to 99.5	92 to 117	0.39
Bicarbonate, mmol/L	25.4	23.5 to 27.3	25.4	23.4 to 27.3	24.6	22.6 to 26.6	21 to 31	0.63

[1] Normal reference ranges provide by Kansas State University Veterinary Diagnostic Laboratory

* *P* values and means represent overall means for each treatment. No interactions with sample time or treatment were detected. *P* values represent the main effect of treatment

Table 3-4:Blood lactate and creatine phosphokinase concentration in finishing steers fed control diet or control diet plus zilpaterol (8.3mg/kg dry matter basis) or ractopamine 300 mg·animal^{-1}·d^{-1}.

Item	Control		Zilpaterol		Ractopamine		Normal Range
Blood Lactate, mmol/L	Mean	95%CI	Mean	95%CI	Mean	95%CI	
Day							
-2	3.6	2.2 to 5.9	4.7	2.8 to 7.7	3.7	2.2 to 6.1	Normal Range Not Established
1	3.1	1.9 to 5.1	4.3	2.6 to 7.0	4.2	2.5 to 6.8	
6	2.6	1.6 to 4.2	1.8	1.1 to 2.9	2.0	1.2 to 3.4	
13	4.2	2.5 to 7.1	2.8	1.7 to 4.8	3.5	2.1 to 5.8	
23	2.4	1.5 to 4.0	1.7	1.0 to 2.7	1.8	1.1 to 2.9	
Creatine phosphokinase, U/L							
Day							
-11,-16	258^{a,c}	172 to 388	182^{a,c}	122 to 270	189^{a,c}	127 to 282	159 to 332[1]
6	171^{a,d}	114 to 257	196^{a,c}	131 to 291	148^{a,c,d}	99 to 220	
13	111^{a,e}	74 to 167	220^{b,c}	147 to 327	120^{a,c}	81 to 178	
23	132^{a,d,e}	88 to 198	226^{b,c}	152 to 337	135^{a,c,d}	152 to 337	

^{a,b} Within rows, means without a common superscript differ (P < .05)

^{c,d,e} Within columns, means without a common superscript differ (P < .05)

[1] Normal reference ranges provide by Kansas State University Veterinary Diagnostic Laboratory

Table 3-5: Ventricular arrythmia events per steer per day in finishing steers fed control diet or control diet plus zilpaterol (8.3mg/kg dry matter basis) or ractopamine 300 mg·animal^{-1}·d^{-1}

Event*	Control		Zilpaterol		Ractopamine	
	Mean	95%CI	Mean	95%CI	Mean	95%CI
Total ventricular arrhythmias[1]	2	0 to 196	1.5	0 to189	4.9	0 to 787
Single arrhythmia [1]	1.8	0 to 3,707	1.8	0 to 3,395	4.5	0 to 5,204
Paired arrhythmias [1]	1.7	1.3 to 2.2	1.8	1.3 to 2.3	2.0	1.5 to 2.6
>2 Arrhythmias [1]	1.7	1.3 to ~	1.7	1.3 to ~	1.7	1.3 to ~

[1]Events per day.

*Means presented within each row represent an independent statistical model. Means should not be compared across rows.

Table 3-6:Supraventricular arrythmia events per steer per day in finishing steers fed control diet or control diet plus zilpaterol (8.3mg/kg dry matter basis) or ractopamine 300 mg·animal^{-1}·d^{-1}

Event*	Control		Zilpaterol		Ractopamine	
	Mean	95%CI	Mean	95%CI	Mean	95%CI
Total supraventricular arrhythmias[1]	35.8	8.6 to 148.2	40.9	9.5 to 175.5	32.5	7.2 to 108.8
Single arrhythmia	30.5	8.1 to 115.1	35.2	8.9 to 138.4	28	0 to 5204
Paired arrhythmia [1]	3.5	1.8 to 6.6	4.2	2.2 to 7.6	3.9	2.1 to 7.3
>2 arrhythmia [1]	3.4	2.5 to 4.6	3.4	2.5 to 4.5	2.8	2.1 to 3.8

[1]Events per day.

*Means presented within each row represent an independent statistical model. Means should not be compared across rows.

Table 3-7:Mean heart rate in finishing steers fed control diet or control diet plus zilpaterol (8.3mg/kg dry matter basis) or ractopamine 300 mg·animal^{-1}·d^{-1}

Treatment	Mean Heart Rate, beats per minute					
Day	Control		Zilpaterol		Ractopamine	
	Mean	SEM	Mean	SEM	Mean	SEM
Day 0	84.0^{a,c}	5.6	77.8^{a,c,d}	6.3	73.3^{a,c,d}	5.6
Day 7	66.8^{a,d,e,f}	3.6	80.9^{b,c,d}	3.5	82.3^{b,c}	3.5
Day 14	72.3^{a,c,e,f}	4.5	79.6^{a,b,c,d}	3.5	85.1^{b,c}	3.9
Day 23	71.3^{a,d,e}	1.3	76.3^{b,c,d}	1.4	74.7^{a,b,c,d}	1.5
Day 24	73.5^{a,c,e}	1.5	74.6^{a,c}	1.4	79.9^{b,c}	1.4
Day 25	77.5^{a,c,f}	1.5	79.4^{a,d}	1.5	77.3^{a,c,d}	1.4

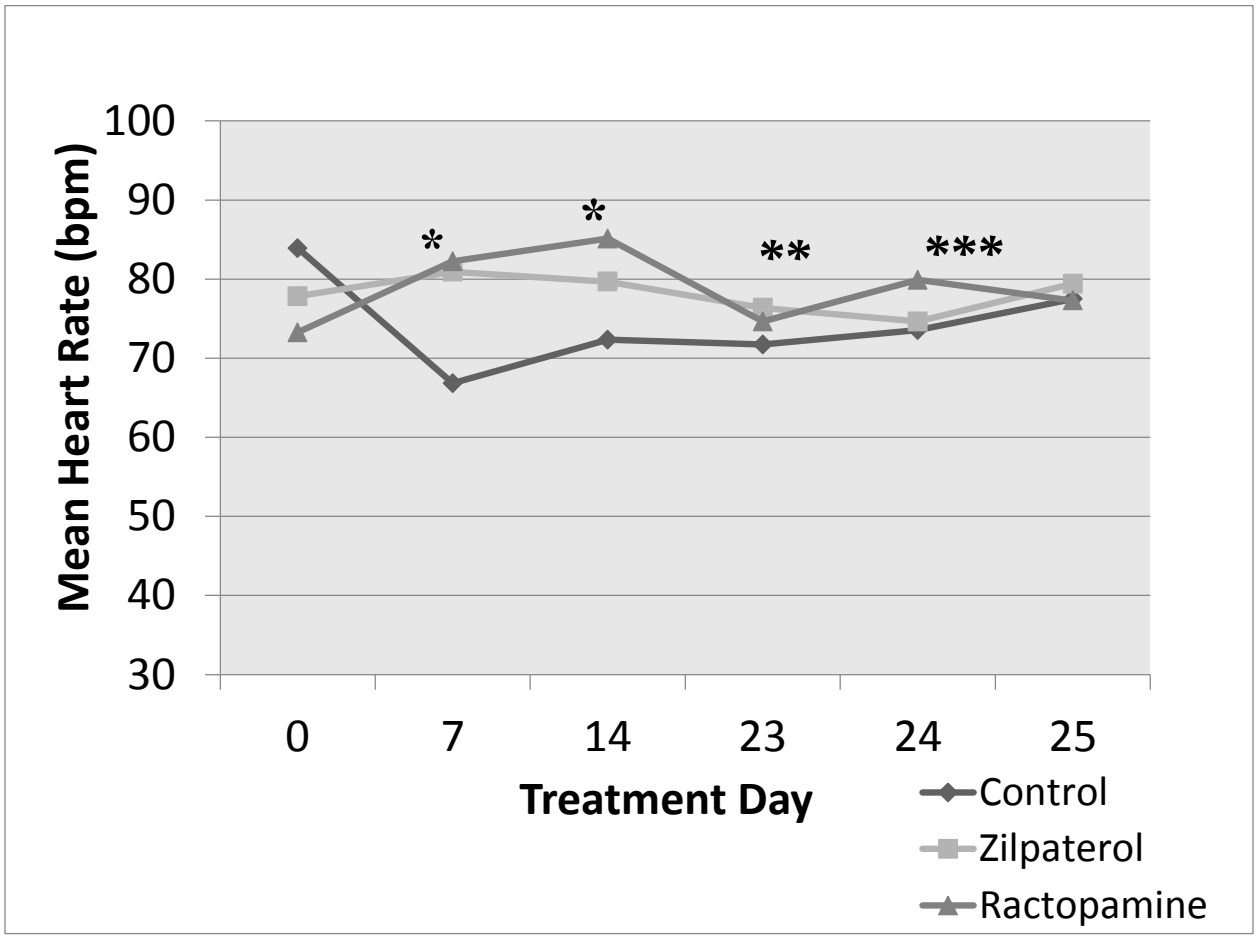
^{a,b} Within rows, means without a common superscript differ ($P < 0.05$)

^{c,d,e,f} Within columns, means without a common superscript differ ($P < 0.05$)

Figure 3-1: Illustration showing location of electrode attachment for Holter monitor application (right side only)

Figure 3-2 Illustration of Applied Holter Monitor and Harness.

Figure 3-3: Mean heart rate in finishing steers fed control diet or control diet plus zilpaterol (8.3mg/kg dry matter basis) or ractopamine 300 mg·animal-1·d-1



* Zilpaterol hydrochloride (ZIL) and Ractopamine hydrochloride differs from control (CON) ($P \leq 0.05$)
** ZIL differs from control CON ($P \leq 0.05$) but not RAC. RAC does not differ from CON
*** RAC differs from ZIL and CON ($P \leq 0.05$)

Chapter 4 - Effect of Cattle Handling Technique on Blood Chemistry Parameters in Finishing Steers not fed a Beta Adrenergic Agonist

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Drs. Hutcheson, Nichols and Corrigan are employed by Merck Animal Health.

Dr. Reinhardt has received consulting fees or honoraria from Elanco Animal Health, Merck Animal Health, and Zoetis.

Objective—To investigate the effects of cattle handling on blood chemistry and clinical symptoms of fatigue in finishing cattle.

Design—Experimental study

Animals—40 Angus-cross steers

Procedures – Cattle were stratified by backfat thickness (BF) and randomly assigned to two treatment groups: 1) low stress handling (LSH) and 2) aggressive handling (AH). Cattle in LSH treatment were walked while AH were ran through an exercise course of 770 m twice. Jugular blood samples were obtained and vital signs recorded at baseline, 770 m, 1540 m and following 2 consecutive 1 h rest periods following exercise. Blood was analyzed for lactate, creatinine kinase (CK), base excess, pH, PvCO2 and P_vO_2.

Results—Lactate was increased in cattle in the thicker BF strata. Aggressively handled cattle had increased lactate, cortisol, P_vO_2, heart rate and rectal temperature and lower blood pH, bicarbonate, base excess, and P_vCO_2 than LSH. Lactate concentrations increased 6 to 10 fold in AH cattle. Creatine kinase concentrations increased at each consecutive sample time in both LSH and AH cattle, but weren't different between treatments. Four AH steers became exhausted and failed to complete the course. Elevated CK, decreased P_vCO_2, and muscle tremors occurred in exhausted steers compared to non-exhausted AH cohorts.

Conclusions—Blood-gases in exhausted cattle indicate altered cardio-pulmonary physiology compared to non-exhausted cattle and warrants further investigation. Cattle in AH treatment had increased lactate, CK, and altered blood-gases during and following exercise. Low stress cattle handling is an important part of cattle management to avoid putting cattle in a compromised physiological state.

ABBREVIATIONS:

AH - Aggressive handling treatment

ATV- All terrain vehicle

BASE- Baseline sample time

BE- Base excess

BF- Backfat

CK- Creatinine kinase

CORT- Serum cortisol

EDTA- Ethylenediaminetetraacetic acid

EXH- Aggressive handling steers that became exhausted and did not complete exercise course

FATBF- Cattle in fatter backfat strata

FCS- Fatigued cattle syndrome

FPS- Fatigued pig syndrome

HCO_3- Blood bicarbonate

HR- Heart Rate in beats per minute

LAC- Plasma lactate

LAP1- Blood sample following 1st time through the alley way

LAP2- Blood sample following 2nd time through the alley way

LMA- Longissimus muscle area

LSH- Low stress handling treatment

NEXH- Non-exhausted aggressively handled cattle

$PvCO_2$- Partial pressure of venous carbon dioxide

PvO_2- Partial pressure of venous oxygen

RR- Respiration rate in breaths per minute

SUBP- Substance P

TCO_2- Total Carbon dioxide

TEMP- Rectal temperature

THINBF- Cattle in thinner backfat strata

1H- Blood sample following 1 h of rest following completion of exercise

2H- Blood sample following 2 h of rest following completion of exercise

Introduction

Cattle welfare is a high priority for the beef industry.[103] Recently, abnormalities in the mobility at abattoirs has gained considerable media and industry attention, with the greatest focus occurring in the fall of 2013.[26] The mobility issues and a series of clinical signs and serum biochemistry abnormalities that has become termed Fatigued Cattle Syndrome by Thomson *et.al*.[104] Cattle exhibiting FCS have various clinical signs including tachypnea with abdominal breathing, muscle tremors, stiff gait, and reluctance to move.[104] Cattle with FCS also had elevated serum lactate and CK.[104] These clinical signs and serum biochemical abnormalities observed in affected cattle were similar to those observed in pigs with Fatigued Pig Syndrome (FPS).[105]

The FPS syndrome has been documented to be caused by multiple additive stressors, including animal handling.[107,108] Fatigued Pig Syndrome is characterized clinically by vocalization, blotchy skin, reluctance or inability to move, muscle tremors.[105,107] Swine exhibiting from FPS have greater blood lactate, decreased blood pH, greater CK, and depleted muscle glycogen.[5] Greater serum lactate concentration has been identified as a consistent characteristic of FPS pigs that become reluctant to move or non-ambulatory.[105,117] To understand the impact of improper handling and stress at the time of slaughter in pigs of heavy body weight, a challenge model was developed to induce FPS.[117] Research using the model has led to FPS mitigation strategies including management changes, such as improvements in handling, transportation[105,119]. The similarities of observations in swine diagnosed with FPS and the affected cattle have led to the hypothesis that high stress handling may be a contributing factor to FCS. This study was designed similarly to the FPS model with the objective to better understand the effects of physical exertion in finished feedlot cattle 17 days prior to shipment on blood and physiological parameters.[117]

Materials and Methods

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

Forty Angus-crossbred steers (563 ± 44 kg) were selected from a single cohort of cattle with 127 days on feed on study day fed at a commercial feeding facility in central Kansas and used to evaluate the effects of 2 handling treatments: 1) LSH cattle were walked approximately 1,540m, and 2) AH cattle were ran the same distance.

The day prior to the study the cattle were weighed and ultrasound performed to determine BF (10.2 ± 2.7mm), and estimated LMA. Ultrasound measurements were made with a 3.5 MHz 10 cm linear probe to capture an image of the sagittal section of the longissimus muscle between the 10^{th} and 13^{th} ribs approximately ½ the distance laterally over the muscle from midline.[a] This image was subsequently analyzed using a software package to estimate LMA, BF, and marbling score.[b] Cattle were then placed into one of two groups based on BF thickness. The first group contained the 20 steers with the lowest BF and the second group contained the 20 steers with the greatest BF. Cattle with the same BF thickness were stratified by weight within BF thickness. Cattle were paired by stratification order, 1 from the greatest BF strata and 1 from the lowest BF strata. Pairs of cattle were then randomly assigned to treatment. After assignment to treatment, each BF pair of steers was randomly assigned to 1 of 5 blocks. Each block each contained 2 pairs of steers from each treatment so that 2 steers from each BF strata half from each treatment were in each block (n = 8). Exercise order of the 5 blocks was determined by random number generator. Treatment exercise order within a block was determined by coin flip. Both treatment groups within a block were exercised consecutively. The study was conducted over 2

consecutive days with processing order of blocks of cattle randomly assigned with the first 3 blocks exercised on day 1 and the final 2 blocks on day 2.

Cattle handling

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

Cattle in the LSH treatment were walked the entire distance with a lead and trail rider each using an ATV. Cattle in the AH treatment were forced to run the entire course distance by 2 people riding ATVs behind the cattle. The amount of time running (excluding sampling time) was between 7 to 8 min for AH cattle. Exercise was stopped on individual cattle meeting exercise stop criteria as described below. These cattle were allowed to recover with their treatment cohort and were sampled at 1H and 2H rest periods with the treatment cohort. Cattle

were sampled and clinical signs recorded following each exercise lap and each of two, 1 hour rest periods in an identical manner as the LSH cattle.

Exercise-Stop Criteria:

To ensure the welfare of the animals involved with the study, exercise-stop criteria were established. Exercise of a steer was to be discontinued if an animal was deemed exhausted by the assigned supervising veterinarian based on the existence of 1 of the following conditions: 1) The steer becomes extremely reluctant to move with a marked decrease in flight zone or becomes recumbent; 2) The steer exhibits open-mouth breathing with excessive salivation; 3) An audible inspiratory or expiratory stridor is present; 4) The steer displays agitation and agonistic behavior toward the handler(s); or 5) The steer becomes lame and presents a lameness score > 1 during the exercise procedure as previously described.[156]

Blood sampling and processing

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

Substance P

Blood samples in a tube coated with potassium EDTA with 100 μL of 100 mM benzimidizine solution were designated for testing for SUBP. Following receipt at the onsite laboratory samples were placed on ice until centrifugation at 3000*g* for 15 minutes at 4ºC.

Following centrifugation, plasma was separated into 2 aliquots and stored in cryovials on dry ice until transfer to permanent storage at -80ºC. Samples were processed as expeditiously as possible with mean time from sample collection to freezing of 45 minutes (±15 minutes). Following transfer to permanent storage 1 sample aliquot was placed on dry ice and shipped via overnight to Iowa State University College of Veterinary Medicine, Department of Biomedical Sciences. Assays were subsequently performed as previously described[157].

iStat Blood Analysis.

Blood samples collected in a 10 ml tube containing coated lithium heparin were analyzed for blood pH, LAC, BE, P_vO_2, P_vCO_2, and HCO_3- using a hand-held analyzer[c] with analytical cartridge.[d] Cartridges were loaded and analysis performed using procedures described by the manufacturer.[c,d] Following confirmation of analysis the tube was centrifuged at 3000*g* for 15 minutes at 4ºC. Following centrifugation plasma was separated, placed in a 5 ml cryovial and stored on dry ice until transfer to permanent storage at -80ºC.

Serum Cortisol

Blood collected in a10 ml coagulation tube were allowed to clot in the tube and then placed in a centrifuge at 3000*g* for 15 min at 4ºC. Serum was separated, placed in a cryovial and stored on dry ice until transfer to permanent storage at -80ºC. Samples were analyzed using a solid-phase, competitive chemiluminescent enzyme immunoassay[e].

Plasma Lactate and Creatinine Kinase

Cryovials containing lithium heparinized plasma stored as previously described were submitted to the Kansas State University Veterinary Diagnostic Laboratory and analyzed for LAC[f] and CK[g].

Statistical Analysis

All data were analyzed using generalized linear mixed models in a commercially available statistical analysis program[h] accounting for repeated measures by steer when appropriate; the model included the fixed effects of treatment, sampling time, and their interactions. Each variable was tested for the effect of BF strata and all their interactions. All interactions found to be non-significant (α=.05) were dropped from the final model. The random effects included were block and day. Degrees of freedom were calculated using the Kenward-Rogers method. Transformation of LAC and CK data were performed by analyzing the natural logarithm of the original data. Model was selected using the Maximum-Likelihood Estimation and Akaike Information Criterion for best fit. Weight and LMA were tested as potential covariates but and were dropped from the final models because they were not significant (α=.05).

One steer from the AH cattle was removed from the analysis as an outlier using Cook's Distance test. Data from LAP2 samples on steers that met exercise-stop criteria prior to sample collection were treated as missing data. Serum Substance P concentrations below assay detection threshold of 5 pg/ml were assigned a value of 2.5 pg/ml.[158]

Within the AH treatment, 4 steers did not finish the course or displayed clinical symptoms of muscle fatigue and exhaustion as per exercise stop criteria. These EXH steers were compared to the 15 remaining NEXH steers using the same procedures described above for each measured parameter. Values of $P \leq 0.05$ were considered significant.

Backfat strata were analyzed within LSH and AH handling for the parameters of LAC, CK and blood pH. Values of $P \leq 0.05$ were considered significant.

Results

Plasma LAC concentrations were greater ($P < 0.05$) in AH cattle compared to LSH cattle at BASE. Plasma LAC concentration at LAP1, LAP2, 1H and 2H were greater ($P < 0.01$) in AH cattle compared to LSH cattle (Tables 4-1 and 4-2). In LSH cattle, a trend was found for greater LAC at LAP 1 ($P = 0.06$) compared to BASE, but no effect of sample time on LAC was detected at other sample times ($P > 0.16$). Base Excess was decreased ($P < 0.05$) in AH cattle compared to LSH cattle at LAP1, LAP2, and 1H but not BASE or 2H. Serum CORT concentrations were greater ($P < 0.05$) in AH cattle compared to LSH at LAP1, LAP2, 1H, and 2H .Compared to BASE CK concentrations, CK concentrations were greater ($P< 0.05$) for LSH cattle at LAP2, 1H and 2H , and at LAP1, LAP2 1H, and 2H for AH cattle (Tables 4-1 and 4-2). However, no differences in CK concentrations were detected between LSH and AH for any sample time.

Heart rate and TEMP were greater ($P< 0.05$) in AH cattle compared to LSH cattle at LAP1, LAP2, and 1H, but not at BASE and 2H. Respiratory rate was not different between LSH and AH ($P > 0.25$) at BASE, LAP2, 1H and 2H rest periods. However, AH cattle displayed a trend for greater RR ($P = 0.06$) at LAP1 compared to LSH. Compared to BASE, TEMP was greater in LSH cattle at 1H and 2H ($P < 0.05$) but not at LAP1 and LAP2. Temperature in LSH cattle was lower at 2H compared to AH cattle. Peak TEMP in both AH and LSH cattle occurred at the final 2H reading. (Table 4-1)

Blood bicarbonate concentration and blood pH were decreased in AH cattle compared to LSH cattle ($P < 0.01$) at LAP1, LAP2, and 1H but were not different between treatments by 2H. Blood gas values for P_vO_2 were greater at LAP1 and LAP2 in AH cattle compared to LSH while P_vCO_2 for AH was decreased at LAP1 and LAP2 compared to LSH.

Blood cortisol concentration was greater ($P < 0.05$) in AH cattle compared to LSH at LAP1, LAP2 and 1H. Cortisol was greater ($P < 0.05$) at LAP2 in AH cattle compared to all other

sample times. (Table 4-1)No differences in SUBP were found ($P > 0.05$) between LSH and AH cattle at the same sample time.

Exhausted steers

Two steers in the AH treatment, with 1 from each BF strata, did not finish the course to the end of LAP2 because they met the exercise-stop criteria. One steer collapsed and then recovered after a brief rest period and the second steer became extremely reluctant to proceed. Both steers completed LAP1. A third steer from the AH treatment, from the thinner BF strata, was observed with apaxial muscle fasiculations at LAP2 sampling and a fourth from the AH treatment thicker BF strata, developed clinical signs consistent with rhabdomyolysis in the alleyway leading to the chute for LAP2 sampling. There was a main effect of EXH cattle for a lower ($P < 0.01$) P_vCO_2 and trends for greater ($P < 0.10$) P_vO_2 and CORT and lower TEMP and SUBP ($P < 0.10$) than NEXH cattle(Table 4-2). However, there were no main effects detected in LAC, CK, pH, BE, HCO_3, RR, and HR for EXH compared to NEXH. All parameters displayed similar changes with sample time as described for AH cattle. Cattle in EXH had greater CK ($P < 0.05$) at 2H compared to NEXH but not at other sample times (Table 4-2). Exhausted cattle had greater ($P < 0.05$) LMA than NEXH cattle. However, no effect of LMA was detected due to between NEXH and EXH on the parameters measured in this study.

Backfat Stratification

A main effect of BF strata ($P < 0.05$) increased LAC concentration by 1.1mmol/L. Backfat strata interacted with sample time for pH and treatment and sample time for HCO_3. Additionally, an interaction of handling treatment and backfat stratification was detected ($P < 0.05$) for LAC, pH, HCO_3 and BE. The magnitude of the effect of these interactions can be

almost entirely accounted for within the main effect of BF strata on LAC and the concurrent effect of increased LAC on pH, HCO_3 and BE

Backfat strata within LSH cattle showed no differences in LAC, blood pH, HCO_3 or BE. Cattle in AH handling group in the FATBF had a higher LAC ($P < 0.05$) at LAP2 and 1H than cattle in THINBF (Table 4-5). Additionally pH was lower at LAP2 and 1H in FATBF AH cattle than THINBFAH ($P < 0.01$) cattle. It should be noted that weight is different ($P \leq 0.05$) between THINBF (528 ± 14.7 kg) compared to the FATBF (581 kg ± 14.7 kg) AH cattle. Backfat stratification tended to place lighter cattle in the THINBF strata although weight between treatments and blocks were not different.

Discussion

Animal welfare is a major concern for all who are involved in the cattle industry.[103] During the summer of 2013, cattle abattoirs throughout the United States reported concern with cattle that were slow or difficult to move.[104] These cattle exhibited reluctance to move, stiff and shortened gait, and lagging behind cohorts. These animals required greater human-animal interaction to initiate movement. Periods of greater environmental temperature, or heat stress, appeared to increase the incidence rates of these slow moving cattle at the abattoir[104]. Upon further investigation it was noted that these cattle had greater serum lactate, CK concentrations, and muscle tremors, in addition to the above mentioned clinical symptoms.

The clinical presentation and blood chemistry results reported here are similar to findings in swine suffering from ‘Fatigued Pig Syndrome ‘or FPS.[105] Heart rates in cattle in this study increased from BASE in both LSH and AH groups, and were greater in the AH cattle compared to LSH. Heart rate peaked in both groups at LAP2 and then decreased, but remained elevated compared to BASE for the entire rest period with the exception of LSH at 1H. Heart rate and

respiratory rate results closely resembled results reported with an increase in heart rate greater in cattle with a greater exercise load.[159,160]

In this study, BASE LAC concentration was greater in AH compared to LSH cattle. The reason for this difference is not apparent. However, LAC concentration of each treatment group was within the normal range reported for LAC concentration in cattle on high grain diets.[153] Cattle in the AH treatment showed significant increases in LAC, which were greater than those previously reported in cattle exercised at trot for 8.8 km even though the distance was much shorter in this study.[161] Concentrations of LAC in AH cattle closely resembled levels and recovery time seen in Hereford calves of lighter weight exercised at a speed of 1.8 m/s. This same study also found peak LAC concentrations were greater with increasing speed of exercise.[160] Blood LAC increase is produced by work load greater than the anaerobic threshold. The anaerobic threshold is a well-documented concept that states blood and muscle lactate concentrations remain relatively stable until a 'work' load is reached at which point a rapid increase in anaerobic metabolism is required to meet energy demands that are not met by aerobic metabolism.[162] In man it has been shown that muscle lactate formation is highly correlated to, and results in, immediate elevations in blood lactate.[163]

Although RR in this study did not differ between treatments, an increase during exercise was followed by a small decrease during the recovery period. However, RR remained elevated during the recovery period compared to BASE, and is consistent with previous research.[159] The lack of difference in RR between AH and LSH cattle was possibly due to an increase in tidal volume which has been reported during exercise in cattle.[159]

Cortisol has long been measured as a physiological response marker for stress in many species, including cattle. In the current study, basal CORT concentrations were higher than

reported in non-stressed cattle, and were significantly greater at the end of exercise and during recovery in AH cattle compared to LSH cattle.[164] The magnitude of the increase (1.5 to 2X) was less than the increase reported in Hereford calves during exercise.[160] Elevation in CORT has not been reported in FPS, but has been reported in other species and man. Increased plasma CORT concentrations have been shown to delay recovery from exhaustive exercise in trout.[165,166] Increased CORT in AH cattle may be a factor in the delayed return of LAC to normal levels of AH cattle.

Substance P has been used as an indicator in livestock species of physiological response to painful procedures, such as dehorning and castration. [167-169] This study showed no difference in SUBP between LSH and AH cattle or NEXH and EXH cattle, but this does not necessarily indicate a lack of pain, as not all studies of painful procedures show a difference in SUBP.[170] Also, recent refinements in assays has led to a change from ELISA to RIA making comparison with results from previous studies that used an ELISA method difficult.

In this study, we induced FCS in cattle with aggressive handling practices but not fed a beta adrenergic agonist. Cattle in this study can be divided into 3 distinct groups: LSH cattle, which showed little physiologic stress and no observed clinical signs of fatigue, AH cattle that completed the course and EXH cattle that did not complete the course or showed clinical symptoms consistent with the description of FCS. Cattle performing draft work walk at slow speeds over a period of time, and may walk a considerable distance during the course of the day. Blood lactate levels are lower in cattle during draft work than reported in the cattle in the current study. However, the cattle in the study by *Zanzinger et.al.* did not produce a metabolic acidosis even when cattle were put under severe strain.[171] This suggests that AH results in greater LAC production and metabolic acidosis than draft work. The results of this study and previous work in

swine and other species show the importance of handling in such a manner as to reduce the possibility of exceeding the anaerobic threshold and incurring possible deleterious effects. The results of LAC and blood gas analysis in LSH cattle are comparable to cattle under a light draft work load.

Exhaustive exercise in this study resulted in greater LAC, BE and CORT and lower blood pH, forming a similar pattern of results in both magnitude and time as has been reported in cattle and other species.[105,161,165,172] The difference in P_vO_2 and P_vCO_2 between EXH cattle and NEXH cattle suggest that a possible cause for exhaustion in cattle could be a difference in efficiency of gas exchange. Numerically greater P_vO_2 and lower P_vC0_2 in EXH cattle compared to NEXH cattle is contradictory to reported physiological responses in cattle to greater exercise and tissue oxygen demand.[160,173] *Kuhlmann et.al.* reported that as speed of exercise increased P_vO_2 decreased and P_vCO_2 increased. In the current study, the P_vO_2 and P_vCO_2 responded in the opposite manner in AG cattle. However, it should be noted that AG cattle in this study were both heavier and exercised at a greater speed than the cattle studied by *Kuhlmann et.al.* These factors could be possible factors in alteration of the response from what has been previously reported. The four EXH steers displayed blood gas changes at 770 m ($P < 0.05$) compared to NEXH steers where differences were not shown until 1540 m. The earlier changes in blood gas concentrations in EXH cattle compared to NEXH are an indicator that the EXH cattle had some differences in blood gas exchange. Possible hypothesis for the changes in blood gas concentration could be 1) inefficient gas exchange in the lungs, 2) inefficient gas exchange at the level of muscle tissue, 3) cardiac insufficiency 4) Increased speed of handling compared to previous research, 5) Increased body weight compared to previous research, or 6) Increased muscle mass. Due to these cattle in this study having increased body weight and were moved at a rate of speed greater than

what has been previously reported it is possible that these two parameters could have been a significant factor in the differences in the observations from this study compared to previous reports. Research in a more controlled environment should be performed in order to further the understanding of blood-gas chemistry of cattle of this size. The degree to which this difference in blood gas concentration contributed to the inability of these 4 steers to finish the course is unknown and data in this study cannot determine the cause of this difference.

The greater CK in EXH cattle at 1H and 2H could originate because of greater muscle damage, greater muscle mass, as indicated by increased LMA, of these four EXH cattle, from cardiac origin, or a combination of these. The CK in NEXH are similar to the CK in LSH, but is elevated in cattle showing signs of FCS, consistent with the findings of FPS in swine.

The difference seen between NEXH and EXH cattle suggest that a small portion of the feedlot cattle population may be at a higher risk for developing detrimental health and welfare problems due to aggressive handling. Research into FPS mitigation strategies led to management changes, such as less aggressive swine handling, greater transportation floor space, and facility design.[107,109-112,114] Animal handling is one of the key components to preventing this multifactorial syndrome in swine.[105,107] Given the similarity in response of AH in cattle and swine it is reasonable to conclude that handling is also a factor in the expression of FPS like clinical signs in cattle and that cattle can undergo 'Fatigued Cattle Syndrome'. Further research to identify specific physiologic risk factors that put cattle at higher risk of adverse events is indicated.

The interaction seen between THINBF and FATBF and AH warrant further investigation into the effect of both backfat and weight on the severity and duration of the metabolic acidosis induced by AH. Lactic acid concentrations were 50% higher in FATBF cattle than THINBF.

Additionally it should be noted that blood pH in THINBF cattle did not decrease between LAP1 and LAP2 although LAC concentrations continued to increase, indicating that these cattle were able to compensate for the increased acid load through other means. The degree to which this effect is due to increased backfat, weight or their interaction should be investigated further.

This study highlights the need for improved and vigilant training for animal handling in commercial feeding operations. Aggressive cattle handling produce significant physiological responses, which can be detrimental to some animals. This emphasizes the need for proper animal care personnel training as low stress handling showed no significant physiologic responses other than a mildly greater CK, HR, and RR following exercise. These data and others show that speed of movement/handling affects the physiologic blood chemistry more significantly than distance moved. It should be noted that a significant difference and high degree of variability exist between and within Zebu based and European based beef breed (Simmental) cattle in blood parameters and performance. Simmental cattle attained higher blood lactate levels, a greater heart rate occurred quicker and were less efficient per unit of work performed than Zebu cattle.[174] It is unknown if these differences would be noticed if exercise were to be performed at a pace that induced anaerobic respiration. Genetic background could be a possible modifier of the effects of handling on the metabolic response of different breeds of cattle and should be investigated further. Of further importance, we have shown that running cattle 700 to 800m at a rapid pace can induce clinical signs of FCS. Cattle movements of these distances can be found commonly in modern commercial cattle facilities and highlight the need for proper movement of the cattle from the pen/housing to the final destination of the cattle within the facility. However, it should be noted that all cattle in this study continued to slaughter without further health and welfare problems.

These results show that EXH cattle have similar LAC, CK, pH ,TEMP, and BE responses as swine displaying signs of FPS.[117] Cattle showing signs of FCS have blood gas values that may indicate these animals are suffering insufficient cardiac function during extreme exercise. While cattle in the fatter BF strata had increased LAC concentrations, the magnitude of increase was small and has little biological effect when compared with the magnitude of cattle handling practices. Further research should pursue further definition of the role cardio-pulmonary function and blood gas exchange may play in this syndrome. Stress of transporting finished cattle is also a consideration in this phenomenon that needs further elucidation on what the potential contribution it has on difficult to move cattle at the abattoir. Also, there is a need for research to be conducted on the management of cattle and design of facilities at the abattoir for further understanding of animal welfare at the packing plant. The beef industry needs to continually improve to ensure that animal welfare is being addressed at every phase of beef production from feedyard to the harvest floor. Investigation into these potential risk factors and mitigation strategies should be pursued to further define the management factors that can increase or decrease the risk for FCS.

Table 4-1 Blood chemistry and vital signs of Aggressive vs. Low Stress handling of finishing during exercise of 1,540m[1]

	BASE[2]		LAP1[3]		LAP2[4]		1 hour rest[5]		2 hour rest[6]		
	LSH[7]	AH[8]	LSH	AH	LSH	AH	LSH	AH	LSH	AH	SEM
Item											
Heart rate (bpm)	76.6^{a}	75.0^{a}	80.8^{a,b}	124.1^{c}	90.1^{b}	140.9^{c}	81.9^{a,b}	105.8^{d}	89.9^{b}	100.6^{d}	3.7
Respiration rate (bpm)	48.0^{a}	46.3^{a}	57.7^{b}	67.8^{b}	72.1^{c}	74.3^{c}	59.1^{b}	65.6^{b}	65.3^{b}	65.4^{b}	3.7
Temperature (C)	39.4^{a}	39.6^{a}	39.7^{b}	39.9^{b}	40.0^{b}	40.6^{c}	39.6^{a}	39.9^{b}	39.6^{a}	39.8^{a}	0.7
Plasma lactate (mmol/L)	3.0^{a}	4.1^{b}	2.3^{a}	16.5^{c}	2.4^{a}	22.3^{c}	2.7^{a}	7.2^{c}	2.5^{a}	4.0^{b}	0.7
Creatinine kinase (U/L)	424^{a,b}	346^{a}	588^{b,c}	544^{b}	681^{c}	648^{c}	829^{c}	1,006^{c}	1,034^{c}	1,430^{c}	1160
pH	7.42^{a}	7.43^{a,e}	7.45^{a,e}	7.25^{c}	7.48^{b,e}	7.19^{d}	7.47^{a}	7.43^{a}	7.46^{a}	7.46^{a}	0.02
P_vC0_2 (mmHg)	46.1^{a}	44.3^{a}	43.1^{a,b}	41.1^{b}	39.3^{c}	30.6^{d}	40.8^{b,c,f}	38.8^{f}	42.3^{b,c}	43.7^{a,b}	1.4
P_v0_2 (mmHg)	30.6^{a}	35.5^{a}	33.5^{a,b}	42.7^{c}	36.6^{b}	51.5^{d}	33.3^{a}	33.8^{a}	33.8^{a}	32.8^{a}	3.7
HCO_3-	30.2^{a}	29.2^{a}	30.2^{a}	17.7^{b}	29.4^{a}	12.3^{c}	29.7^{a}	25.9^{d}	30.3^{a}	30.4^{a}	1.6
Base excess	5.8^{a}	4.9^{a}	6.2^{a}	-9.6^{b}	5.9^{a}	-16.0^{c}	5.9^{a}	1.5^{d}	6.6^{a}	6.7^{a}	.7
Cortisol (nmol/L)	69.6^{a,b}	80.6^{a,c}	58.9^{b}	87.5^{a}	93.1^{c}	144.4^{d}	53.1^{b}	113.5^{d}	72.5^{a}	97.0^{c,d}	15.3
Substance P (pg/ml)	26.4	32.8	22.9	26.1	24.4	24.5	24.6	28.7	24.2	29.9	4.9

$^{a,b,c\ldots}$Values within rows without a common superscript differ *($P \leq 0.05$)*

[1] Angus crossbred steers n = 40; 563 ± 44 kg)

[2] Baseline measurements taken prior to application of treatment

[3] Measurements following a distance of 770 m

[4] Measurements following a distance of 1,540 m

[5] Measurements following a 1 hour of rest following the completion of animal handling

[6] Measurements following a 1 hour of rest following the completion of animal handling

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

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

Table 4-2: Blood chemistry and vital signs of Non-exhausted vs. Exhausted aggressively handled finishing steers during exercise up to 1,540m[1]

	BASE[2]		LAP1[3]		LAP2[4]		1 hour rest[5]		2 hour rest[6]		
	NEXH[7]	EXH[8]	NEXH	EXH	NEXH	EXH	NEXH	EXH	NEXH	EXH	SEM
Item											
Heart rate (bpm)	74.5^{a}	70.3^{a}	124.4^{b}	112.1 ^{a,b}	134.3^{b}	167.3^{c}	114.5^{b,d}	81.9 ^{a,d}	105.3^{b,d}	87.3^{a,d}	5.3
Respiration rate (bpm)	47.3^{a}	46.4^{a}	68.7^{b}	65.7^{b}	76.0^{b}	70.0^{b}	66.9^{b,d}	58.9^{a,c,d}	67.7^{b,d}	56.3^{a,c,d}	3.8
Temperature (C)	39.6^{a}	39.4^{a}	40.0^{b}	39.7^{a,b}	40.8^{c}	40.2^{b}	40.0^{b}	39.7^{a,b}	39.8^{a,b}	40.0^{a,b}	.5
Plasma lactate (mmol/L)	4.1^{a}	4.1^{a}	16.3^{b}	18.5^{b}	21.9^{b}	24.6^{b}	7.2^{c}	7.6^{a,c}	3.9^{a}	4.3^{a}	1.5
Creatinine kinase (U/L)	294^{a}	272 ^{a}	443 ^{a,b}	541 ^{a,c}	522^{b,c}	507 ^{a,c}	777^{c,d}	1145^{d}	1049^{d}	1980^{d}	3414
pH	7.41^{a}	7.48^{a}	7.25^{b}	7.24^{b}	7.18^{c}	7.19 ^{b,c}	7.41^{a}	7.47 ^{a}	7.43 ^{a}	7.50 ^{a}	0.05
P_vC0_2 (mmHg)	45.2 ^{a}	40.2^{a}	42.3 ^{a}	35.3^{b}	31.4^{c}	27.1^{c}	40.1 ^{a,b}	34.2 ^{b}	45.0^{a}	39.3^{a}	2.2
P_v0_2 (mmHg)	35.3^{a,b}	36.0 ^{a}	40.4 ^{b}	51.0^{b}	51.05^{c}	53.2^{c}	32.5 ^{a}	38.5 ^{a}	31.3 ^{a}	38.5 ^{a}	3.3
HCO_3-	29.3^{a}	29.5 ^{a}	18.9^{b}	15.3^{b,c}	12.7^{c}	10.8^{c}	26.0 ^{d}	24.9 ^{a,d}	30.3 ^{a}	29.9 ^{a,d}	1.1
Base excess	4.8 ^{a}	6.0 ^{a}	-8.3 ^{b}	-12.0^{b,c}	-15.6 ^{c}	-17.3 ^{c}	1.3 ^{d}	1.2 ^{d}	6.1 ^{a}	7.0 ^{b}	1.5
Cortisol (nmol/L)	75.6^{a}	90.5^{a,c}	78.4^{a,b}	117.2^{a,c}	140.3^{d}	160.9^{c,d}	110.4^{c}	125.0 ^{c}	88.6 ^{a}	124.8^{c}	19.0
Substance P (pg/ml)	36.5	21.6	28.3	21.3	24.7	19.4	30.1	22.1	31	26.2	4.9

$^{a,b,c,\ldots}$Values within rows without a common superscript differ *($P \leq 0.05$)*

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

[2] Baseline measurements taken prior to application of treatment

[3] Measurements following a distance of 770 m

[4] Measurements following a distance of 1,540 m

[5] Measurements following a 1 hour of rest following the completion of animal handling

[6] Measurements following a 1 hour of rest following the completion of animal handling

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

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

Table 4-3: Comparison of thin and fat BF strata by treatment for selected parameters.

Item	BF STRATA	BASE LSH	BASE AH	800 LSH	800 AH	1600 LSH	1600 AH	1H LSH	1H AH	2H LSH	2H AH	*P* Value Treatment * Backfat
Plasma lactate (mmol/L)	Thin	2.95^{a}	4.70^{a}	2.13^{a,1}	13.6^{b,1}	2.27^{a,1}	18.1^{b,1}	2.87^{a,1}	5.25^{c,1}	2.44^{a,1}	4.45^{a,1}	*P=.057*
	Fat	3.00^{a}	3.63^{a}	2.40^{a,1}	20.0^{b,1}	2.63^{a,1}	27.3^{b,1}	2.47^{a,1}	9.60^{c,2}	2.52^{a,1}	3.52^{a,1}	
Blood pH	Thin	7.42^{a,1}	7.44^{a,1}	7.44^{a,1}	7.28^{b,1}	7.47^{a,1}	7.26^{b,1}	7.45^{a,1}	7.45^{a,1}	7.46^{a,1}	7.46^{1}	$P < 0.01$
	Fat	7.42^{a,1}	7.42^{a,1}	7.45^{a,1}	7.21^{b,2}	7.47^{a,1}	7.10^{c,2}	7.47^{a,1}	7.25^{d,2}	7.45^{a,1}	7.44^{a1}	
HCO_3-	Thin	29.5^{a,1}	28.6^{a,1}	29.9^{a,1}	19.9^{b,1}	29.3^{a,1}	15.0^{c,1}	29.3^{a,1}	27.8^{a,1}	30.2^{a,1}	30.5^{a,1}	$P < 0.01$
	Fat	30.9^{a,1}	30.0^{a,1}	30.3^{a,1}	16.5^{b,2}	29.5^{a,1}	9.6^{c,2}	30.1^{a,1}	24.1^{d,2}	30.4^{a,1}	29.9^{a,1}	
Base excess	Thin	4.9^{a,1}	4.7^{a,1}	5.9^{a,1}	-6.7^{b,1}	5.8^{a,1}	-11.9^{c,1}	6.3^{a,1}	4.0^{d,1}	6.4^{a,1}	6.4^{a,1}	$P < 0.01$
	Fat	6.7^{a,1}	5.5^{a,1}	6.4^{a,1}	-11.2^{a,2}	5.9^{a,1}	-20.0^{c,2}	5.4^{a,1}	-1.0^{d,2}	6.7^{a,1}	6.3^{a,1}	

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

$^{1,2,3.....}$Superscripts within columns with different subscripts differ ($P < 0.05$)

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

[2] Baseline measurements taken prior to application of treatment

[3] Measurements following a distance of 770 m

[4] Measurements following a distance of 1,540 m

[5] Measurements following a 1 hour of rest following the completion of animal handling

[6] Measurements following a 1 hour of rest following the completion of animal handling

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

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

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

Chapter 5 - Effect of Cattle Handling Technique on Voluntary Post Exercise Locomotion Behavior using Pedometers in Finishing Steers not Fed a Beta Adrenergic Agonist.

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This study was funded by Merck Animal Health

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Drs. Hutcheson, Nichols and Corrigan are employed by Merck Animal Health.

Dr. Reinhardt has received consulting fees or honoraria from Elanco Animal Health, Merck Animal Health, and Zoetis.

Objective—To investigate the effects of cattle handling on post exercise activity in finishing cattle as measured by foot pedometers.

Design—Experimental study

Animals—40 Angus-cross steers

Procedures – Forty Cattle were weighed (563 ± 44 kg), stratified by ultrasound backfat thickness and randomly assigned to the following treatment groups: 1) low stress handling (LSH) and 2) aggressive (AH). Cattle in LSH treatment were walked while AH were ran through an exercise course of 1540m following application of a pedometer on the right hind limb just above the metatarsophalangeal joint. Pedometers measured steps taken, standing/lying position changes and count of position changes for 48 hours following completion of handling course.

Results— AH reduced steps taken between 2 to 9 and 18 to 48 hours post handling when compared to hour 0. Cattle handled with LSH methods decreased steps over TREATHOUR, but with a lesser magnitude than AH. STAND time was less (31.7 vs 44.3 ± 1.1 min/h P <0.01) in AH cattle than LSH cattle, but did not interact with BFSTRATA or TREATHOUR. Cattle in FATBF tended to walk more than cattle in THINBF, but did not interact with handling or TREATHOUR

Conclusions— Cattle near slaughter handled with inappropriate handling techniques voluntarily reduce locomotion. This may potentially increase locomotion scores at abattoirs in cattle loaded and transported immediately following inappropriate handling.

ABBREVIATIONS:

ATV- All terrain vehicle

BASE- Baseline sample time

BFSTRATA- Backfat Stratification

FATBF- Fattest backfat strata

FCS- Fatigued cattle syndrome

FPS- Fatigued pig syndrome

AH - Aggressive handling treatment

CLOCKHOUR- Hour of the day

EXH- Aggressive handling steers that became exhausted and did not complete exercise course

HR- Heart Rate in beats per minute

LAC- Plasma lactate

LAP1- Blood sample following 1st time through the course

LAP2- Blood sample following 2nd time through the course

LMA- Longissimus muscle area

LSH- Low stress handling treatment

POS - Number of times an animal changes standing/lying position in a time period

NEXH- Non-exhausted aggressively handled cattle

STAND- Amount of time spend standing during an hour

STEPS- Steps taken during a period

TREATHOUR- Number of hours post exercise

THINBF- Thinnest BF strata.

Introduction

Cattle welfare is a high priority for the beef industry.[103] Recently, abnormalities in the mobility at abattoirs has gained considerable attention, with the greatest focus occurring in the fall of 2013.[26] These mobility issues and a series of clinical signs and serum biochemistry abnormalities have been termed Fatigued Cattle Syndrome (FCS) by Thomson *et.al.*[104] Cattle exhibiting FCS have various clinical signs including tachypnea with abdominal breathing, muscle tremors, stiff gait, and reluctance to move, which is similar to the clinical signs of Fatigued Pig Syndrome.[104]

The Fatigued Pig Syndrome is caused by multiple additive stressors, including animal handling, transportation, facilities and environmental conditions.[105,107-109,175] Fatigued Pig Syndrome is characterized clinically by vocalization, blotchy skin, reluctance or inability to move, and muscle tremors.[105,107] Swine exhibiting FPS have greater blood lactate, decreased blood pH, greater CK, and depleted muscle glycogen.[5] Greater serum lactate concentration has been identified as a consistent characteristic of FPS pigs that become reluctant to move or non-ambulatory.[105,117] To understand the impact of improper handling and stress at the time of slaughter in pigs of heavy BW, a challenge model was developed to induce FPS.[117] Research using the model has led to FPS mitigation strategies including management changes such as improvements in handling, and transportation[105,119]. The similarities between observations in swine diagnosed with FPS and the cattle diagnosed with FCS have led to the hypothesis that high stress handling may be a contributing factor to FCS.

Pedometers have been used to detect and monitor animal lameness, welfare, behavior following painful veterinary procedures such as castration and caesarian section, and behaviors

such as estrus.[176-180] The majority of research using pedometers on cattle has been performed in dairy-type cattle, although some work has been done involving lightweight beef cattle with bovine respiratory disease models. The degree to which handling of cattle affects the locomotion and movement of cattle immediately prior to slaughter has not been documented. This study was designed similarly to the aforementioned FPS model with the objective of better understanding of the effects of animal handling method and physical exertion in finished feedlot cattle 17 d prior to shipment on the cattle mobility as measured by steps taken, lying time and number of lying bouts of cattle near the end of the finishing period.

Materials and Methods

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

Angus-crossbred steers (n=40; BW=563 ± 44 kg) were selected from a single cohort of cattle with 127 days on feed on study day fed at a commercial feeding facility in central Kansas and used to evaluate the effects of 2 handling treatments: 1) Low stress handling cattle were walked approximately 1,540m, and 2) Aggressive handled cattle were ran the same distance.

The day prior to the study the cattle were weighed and ultrasound was performed to determine BF (10.2 ± 2.7mm), and estimated LMA. Ultrasound measurements were made with a 3.5 MHz 10 cm linear probe to capture an image of the sagittal section of the longissimus muscle between the 10th and 13th ribs approximately ½ the distance laterally over the muscle from midline.[a] This image was subsequently analyzed using a software package to estimate LMA, BF, and marbling score[b]. Cattle were then placed into 1 of 2 groups based on BF thickness. The first group contained the 20 steers with the lowest BF and the second group contained the 20 steers with the greatest BF. Cattle with the same BF thickness were stratified by BW within BF

thickness group. Cattle were paired by BF stratification order, 1 from the greatest BF group and 1 from the lowest BF group. Pairs of cattle were then randomly assigned to treatment. After assignment to treatment, each BF pair of steers was randomly assigned to 1 of 5 blocks. Each block contained 2 pairs of steers from each treatment so that 2 steers from each BF strata half from each treatment were in each block (n = 8). Exercise order of the 5 blocks was determined by random number generator. Treatment exercise order within a block was determined by coin flip. Both treatment groups within a block were exercised consecutively. The study was conducted over 2 consecutive days with processing order of blocks of cattle randomly assigned with the first 3 blocks exercised on day 1 and the final 2 blocks on day 2.

Cattle handling

On each study day, cattle were individually restrained in a hydraulic chute, TEMP, RR and HR were recorded using procedures described later, blood was sampled via jugular venipuncture as described later and cattle were sorted into their respective treatment cohorts. Each treatment cohort of 4 steers was moved along a course of approximately 1,540 m including length of cattle chute and alley. The course was shaped generally in a square with the handling facility in the middle of the square, allowing cattle to be moved continuously throughout the course. Cattle were then placed in a nearby pen allowed to rest for two hours. Cattle had *ad libitum* access to water during the rest period.

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

treatment cohort. All cattle were walked back to the processing area after two consecutive 1 hour rest periods.

Exercise-Stop Criteria:

To ensure the welfare of the animals involved with the study, exercise-stop criteria were established. Exercise of a steer was to be discontinued if an animal was deemed exhausted by the assigned supervising veterinarian based on the existence of 1 of the following conditions: 1) The steer becomes extremely reluctant to move with a marked decrease in flight zone or becomes recumbent; 2) The steer exhibits open-mouth breathing with excessive salivation; 3) An audible inspiratory or expiratory stridor is present; 4) The steer displays agitation and agonistic behavior toward the handler(s); or 5) The steer becomes lame and presents a lameness score > 1 during the exercise procedure as previously described.[156]

Pedometer application

A pedometer[i] was secured to the right rear leg just proximal to the metatarsophalangeal joint of the steer using the applicator band with the device attached while the steer was restrained in the hydraulic chute at the time of randomization.

All cattle were shipped to slaughter 17 days following the completion of the experiment.

Data collection

Data was recorded in 15 minute intervals. Individual files were combined and data was truncated to include only the 48 hours immediately following the conclusion of exercise and summarized by the hour. Seven pedometers were excluded from the study because they either failed to stay on the animal or failed to record data. Steps taken and number of lying bouts were recorded as count data and time spent standing was recorded as the percentage of the hour spend

standing. Lying bouts are defined as the number of time an animal changes standing or lying positions.

Statistical Analysis

All data were analyzed using a publically available statistical analysis program[ii]. Analysis of steps taken was performed using a Poisson distribution. The full model included the fixed effects of treatment, TREATHOUR, BF and all of their interactions were considered. CLOCKHOUR was used as a covariate to correct for the effect of time of day as the handling time was different between blocks. All interactions found to be non-significant (α=.05) were dropped from the final model. The random effects included were block and steer within day. Degrees of freedom were calculated using the Kenward-Rogers method. Model was selected using Akaike Information Criterion for best fit. Seven steers were removed from the analysis, 3 from the AH treatment and 4 from the LSH treatment, due to pedometers failing to record data, or pedometers failed to remain on the leg. Values of $P \leq 0.05$ were considered significant. Data for STEP were analyzed by adding 1 to the total number of steps taken using a Poisson distribution. Analysis of STAND was performed using a binomial probability distribution. Analysis of POS was performed using a Poisson distribution and Bonferroni method of adjustment for multiple comparisons.

Within the AH treatment, 4 steers did not finish the course or displayed clinical symptoms of muscle fatigue and exhaustion as per exercise stop criteria. One EXH and two NEXH steer were removed due to failed pedometers. These three EXH steers were compared to the 14 remaining NEXH steers using the same procedures described above for each measured parameter. Values of $P \leq 0.05$ were considered significant.

Results

An interaction of cattle handling, and TIMEHOUR ($P < 0.05$) interactions was observed. There was a trend for cattle of the fatter BF to take more steps than thinner BF ($P < 0.07$). There was no interaction of BF with any other factor. Cattle in AH walked less than LSH (49.8 vs 68.9 steps/h; $P < 0.01$). Cattle in AH were not decreased in steps taken until 20 h post handling when compared to LSH ($P < 0.05$; Figures 1 and 2). Briefly, Cattle in LSH took fewer steps at TREATHOURS 2, 4, 16, 22, 39, 40 and 45 to 48 after handling ($P < 0.05$). Cattle in AH took fewer steps in 9 of the first 24 hours and all time points except hours 36 and 40 after 24 hours ($P < 0.05$). There was a main effect of TREATHOUR on EXH or NEXH cattle but no interactions of TREATHOUR with handling or BF were detected ($P > 0.36$).

Briefly, STAND was decreased for AH cattle compared to LSH cattle (31.7 vs 44.3 ± 1.1 min/h $P < 0.01$;Table 5-1) and a main effect of TREATHOUR was observed ($P < 0.01$) but there were no handling by TREATHOUR interaction. There was no effect of BF or its' interactions with TREATHOUR or handling on STAND ($P > 0.26$).

There was no difference in POS (0.63 vs 0.61 changes/h;Table 4) due to handling, BF or their interaction ($P > 0.22$). The range of POS was 0 to 4 and 0 to 3 changes in position per hour with a median of 1 for LSH and AH respectively. POS was increased by 15% numerically but not statistically in EXH ($P > 0.74$) cattle compared to NEXH (0.68 vs 0.59 changes/h).

Discussion

Cattle in AH spent more time lying down than cattle in LSH, although they did not change standing/lying positions more often. Lying behavior has been shown to be a measure of cattle comfort.[181] Additionally, restlessness as measured by steps taken and weight shifting has been shown to be a useful indicator of comfort in dairy cattle which are forced to stand.[182]

However, in this study, it seems more likely that the increased time resting is an indicator of fatigue rather than of increased comfort.

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

The reduction in steps is periodic, as there is a period of reduction from 2 to 9 hours post-handling for nearly the entire period from 18 to 48 hours post-handling in AH cattle. Cattle in LSH also showed a decrease in steps at 2 and 4 hours post-handling.

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

The average distance fed cattle are shipped direct to slaughter from feedyards in the United States is 276 ± 15 km (166 mi), which would likely mean an approximately 3 h transit time [183]. Voluntary reduction of movement in this study began starting 2 h post-handling, regardless of handling method and would coincide with arrival time at an abattoir for many fed cattle. Given this change in voluntary behavior in both LSH and AH cattle and the arrival time

lead to a hypothesis that it is possible that distance that cattle have to travel to a load out could play a role in the incidence of FCS at abattoirs and should be investigated further.

Swine and cattle suffering from FPS and FCS have been documented to clinically recover if allowed to rest quietly [104,117,175]. The results of this study would suggest that, from the standpoint of animal locomotion, following a rest period in cattle showing clinical signs of FCS at abattoirs, that a time between 9 and 18 h post-handling could be a time at which cattle might be moved with greater ease. However, cattle that have been handled improperly have markedly reduced movement beyond 18 h post handling. Additionally, finished cattle that are handled improperly and transported greater than 18 h to slaughter may be at greater risk for mobility problems upon arrival at the abattoirs.

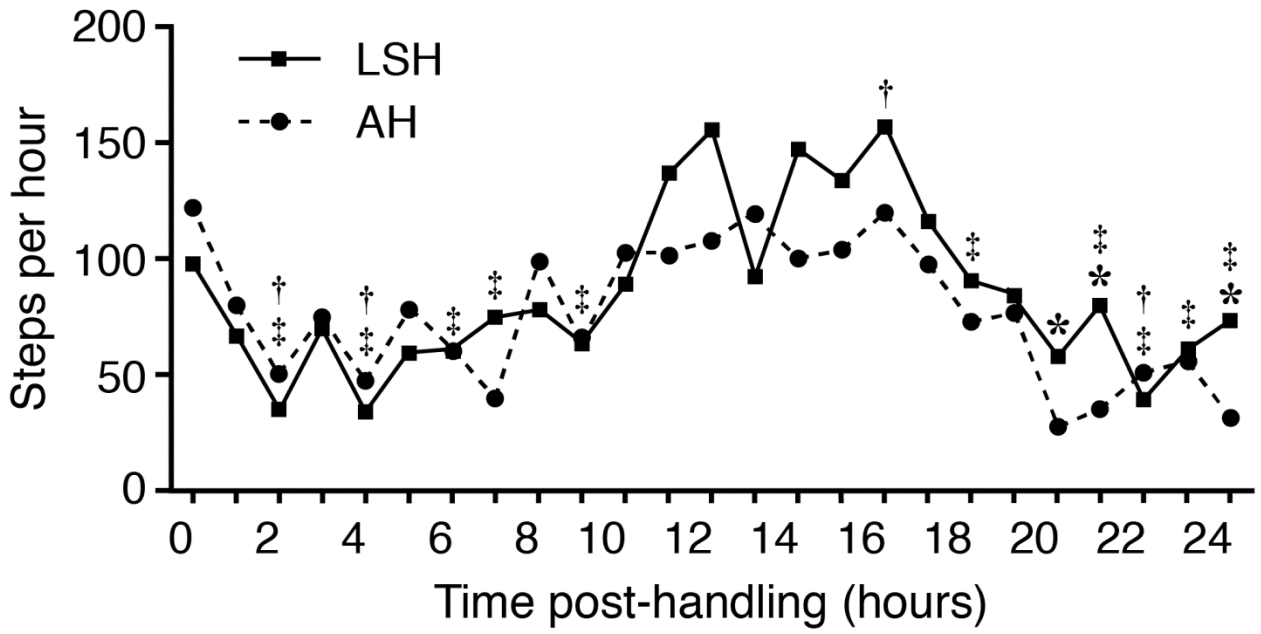
In conclusion, cattle handling can affect voluntary cattle movement for up to 48 hours following forced movement of 1,540 m. Cattle that are handled inappropriately and are moved a distance at considerable speed are affected to a greater degree and sooner following handling. This study reinforces the need for proper animal handling training in low stress handling techniques to reduce risk of locomotion and ambulatory problems.

Table 5-1:Effect of Aggressive vs. Low Stress handling of finishing during exercise of up to 1,540m on standing time and position changes for 48 hours post-handling.[1]

Item	**LSH[2]**	**AH[3]**	**SEM**	P value
Standing Time (min/h)	31.7	44.3	1.10	*P < 0.01*
Position Changes (changes/h)	0.63	0.61	0.20	*P* =0.51

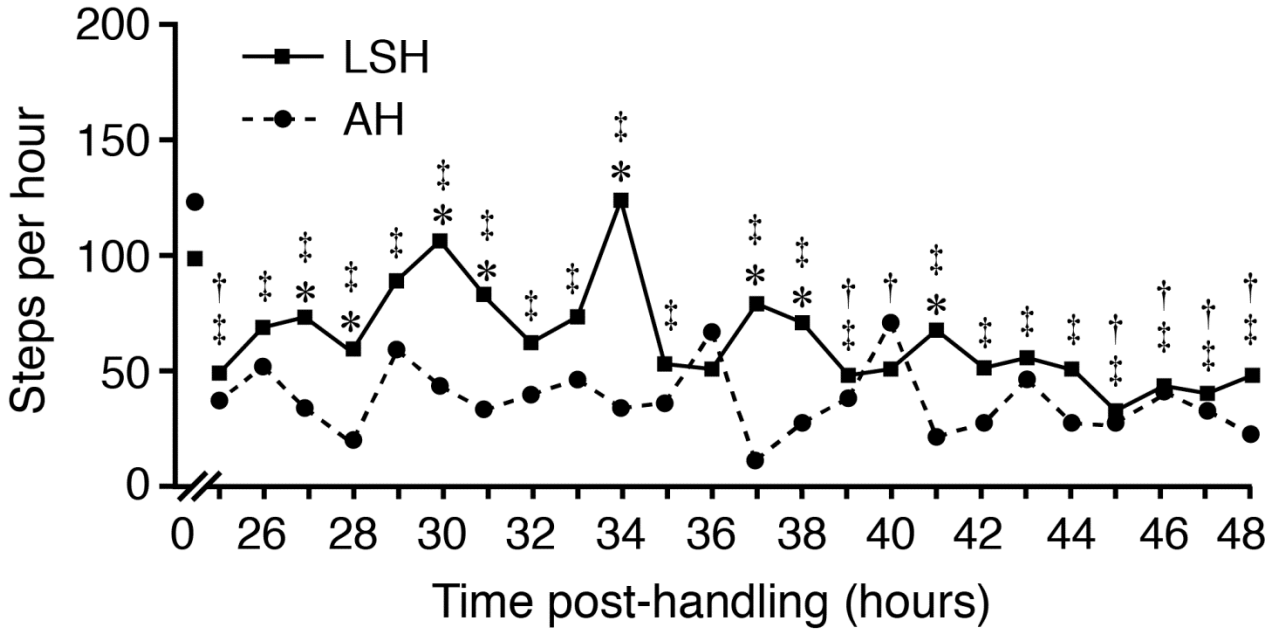
[1] Angus crossbred steers (n = 40; 563 ± 44 kg)
[2] Low stress animal handling methods. Cattle were walked with a lead rider.
[3] Aggressive animal handling methods. Cattle were ran without a lead rider up to the course distance of 1,540 m in 7 to 8 min.

Figure 5-1: Effect of Aggressive vs. Low Stress handling of finishing steers during exercise of up to 1,540m on the number of steps taken 0 to 24 hours post handling.[1]



[1] Angus crossbred steers (n = 40; 563 ± 44 kg)
[2] Low stress animal handling methods. Cattle were walked with a lead rider.
[3] Aggressive animal handling methods. Cattle were ran without a lead rider up to the course distance of 1,540 m in 7 to 8 min.
[*] Denotes difference between LSH and AH ($P \leq 0.05$)
[†] Denotes difference between steps at hour 0 and the hour indicated for LSH.
[‡] Denotes difference between steps at hour 0 and the hour indicated for AH.

Figure 5-2: Effect of Aggressive vs. Low Stress handling of finishing steers during exercise of up to 1,540m on the number of steps taken 24 to 48 hours post handling.[1]



[1] Angus crossbred steers (n = 40; 563 ± 44 kg)
[2] Low stress animal handling methods. Cattle were walked with a lead rider.
[3] Aggressive animal handling methods. Cattle were ran without a lead rider up to the course distance of 1,540 m in 7 to 8 min.
[*] Denotes difference between LSH and AH ($P \leq 0.05$)
[†] Denotes difference between steps at hour 0 and the hour indicated for LSH.
[‡] Denotes difference between steps at hour 0 and the hour indicated for AH.

Footnotes

[i] IceTag™ IceRobotics Ltd. Edinburgh, United Kingdom

[ii] SAS 9.3, SAS Institute, Cary NC United States

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