RELATIONSHIP BETWEEN CURRENT TEMPERAMENT MEASURES AND PHYSIOLOGICAL RESPONSES TO HANDLING OF FEEDLOT CATTLE

by

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ABSTRACT

Temperament in beef cattle has become a research focus due to increasing consumer awareness of animal welfare. Researchers have defined temperament as behavioral responses to a perceived stressful event. “Fight-or-Flight” response is influenced by environmental and genetic factors including age, sex, and breed. Subjective chute scoring systems have been used by many researchers. Due to the subjectivity and associated variability among observers, chute scores have been questioned for repeatability and consistency. An alternative measurement currently used, is exit velocity or speed at which an animal exits a chute, and is recognized as the most practical objective measure for assessing temperament. Physiological markers used to evaluate temperament are increased blood cortisol and lactate concentrations. The first objective of this study was to compare temperament differences between feedlot steers and heifers. The second objective was to evaluate physiological measures taken chute side as potential markers for defining an animal’s temperament and potential predictor. Pulse, oxygen saturation, rectal temperature, blood lactate, blood glucose and lactate concentrations, salivary cortisol, and serum cortisol were measured on mixed breed and sex feedlot cattle (n = 197), significant difference $\alpha = P < 0.05$. Exit velocities were used to classify animals as fast, medium, and slow, plasma lactate was significantly different between all classes. Exit velocity and physiological measures differed between sex. Heifers had higher exit velocities ($P = 0.003$), plasma lactate concentrations ($p = 0.03$), and cortisol concentrations ($P = 0.001$). Simple correlations among these variables showed rectal temperature (heifers $r = 0.44$, $P < 0.0001$; steers 0.45 $P < .0001$), plasma lactate (heifers $r = 0.52$ $P < 0.0001$; steers $r = 0.63$ $P < 0.0001$), serum lactate (heifers $r = 0.53$ $P < 0.001$; steers $r = 0.59$ $P < 0.001$) and glucose (heifers $r = 0.54$ $P < 0.001$; steers $r = 0.32$ $P < 0.003$) were all correlated to exit velocity in both sexes. Cortisol measures were not correlated to exit velocity in steers but were in heifers. Linear models were constructed and evaluated using Akaike information criterion, the top candidate model was plasma lactate in combination with body temperature to predict exit velocity.

INTRODUCTION

Temperament in beef cattle has had renewed interest by researchers because of increased concerns for animal welfare by consumers within the United States (Lyles and Calvo-Lorenzo, 2014). Temperament has been defined as the way in which an individual reacts to a novel or challenging situation (Fordyce et al., 1988a; Grandin, 1998; Reale et al., 2000; Curley et al., 2006; Ferguson et al., 2006; Cafe et al., 2011). Furthermore, it has been suggested that increased excitability or extreme temperament results in fearful and, in some cases, aggressive behavior during handling. There is clear evidence that handling excitable cattle during, increases the risk of injury to both themselves and handlers, and may inflict damage to handling facilities (Grandin, 1993; Waiblinger et al., 2006; Vetters et al., 2013). Identifying and removing excitable animals may reduce the need for forceful handling techniques, as well as improve the safety of animals and producers alike.

Temperament is influenced by environmental (e.g. previous experiences, predator interaction) and genetic factors such as age, sex, and animal breed (Fordyce et al., 1988a; Cooke, 2014). Environmental factors can contribute to a negative experience in cattle during handling which results in storage of negative memories in the brain (Grandin, 1993; Medina et al., 2002; Tsigos and Chrousos, 2002). Researchers have indicated that "bad" experiences with human interaction or predators have resulted in a higher stress response during subsequent exposure and repeated exposure resulted in a longer time needed to return to both mental and physiologic homeostasis, which is defined as the

dynamic internal equilibrium (Tsigos and Chrousos, 2002; Devine, 2006; Cooke et al., 2013).

Temperament has a direct impact on feedlot performance, carcass quality, and meat quality (Voisinet et al., 1997a; Voisinet et al., 1997b; Ferguson et al., 2006; Grandin, 2006; Cafe et al., 2011; Hall and Berg, 2011; Boles et al., 2015). Francisco et al. (2012) concluded that *Bos taurus* cattle reared on rangeland with excitable temperaments had decreased weaning weights compared to less excitable cohorts and subsequently resulted in decreased carcass weight. Results from numerous studies, have indicated that cattle with excitable temperaments, as determined by chute score or exit velocity, have decreased average daily gains as well as decreased carcass weights (Ferguson et al., 2006; Cafe et al., 2011; Turner et al., 2011; Francisco et al., 2012). Furthermore, meat quality aspects such as tenderness and color are negatively affected by excitable temperaments (Voisinet et al., 1997a; Boles et al., 2015). From an economic standpoint, temperament can have a large impact in the feedlot industry. A 1% increase in the nutritional efficiency of feedlot cattle would translate to an estimated increase of $23 million dollars a year to the feedlot industry (Huntington et al., 2006).

Temperament affects the degree of the response to perceived stress, with excessively negative temperaments having a strong impact on neuroendocrine function and concomitant response to stress (Matteri et al., 2000; Burdick et al., 2009). Fell et al. (1999) and Curley et al. (2008) reported that excitable cattle had greater basal concentrations of the stress hormone cortisol, ultimately leading to decreased growth, poor immune function, and negatively impacting carcass traits. When the animal

perceives something in the environment to be “hazardous,” this stimulates the hypothalamic-pituitary-adrenal axis (HPA), initiating a cascade of endocrine reactions, in an attempt to maintain homeostasis (Curley et al., 2008; Carroll and Sanchez, 2014). These reactions involve neurons in the periventricular nucleus of the hypothalamus synthesizing and secreting corticotrophin-releasing hormone (CRH) and vasopressin in response to a stress stimulus (Aguilera, 1998; Matteri et al., 2000). Increases in vasopressin and CRH secretion subsequently alter the anterior pituitary gland secretion of adrenocorticotrophic hormone (ACTH) which, in turn, stimulates the adrenal gland to increase synthesis and secretion of glucocorticoids (Aguilera, 1998; Matteri et al., 2000; Curley et al., 2008). Glucocorticoids are steroid hormones that play a major role in the catabolism of fats, proteins, and glycogen to increase systemic glucose concentration providing energy necessary for the brain and other organ systems to maintain homeostasis (Norris et al., 2014).

Another physiologically important response to stress is activation of the sympathetic nervous system, involving adrenergic neurons in the brain and postganglionic sympathetic neurons that release epinephrine and norepinephrine into the circulatory system. This system is often activated in parallel with or prior to activation of the HPA (Burdick et al., 2011b). The heart and associated blood vessels subsequently respond to the release of epinephrine and norepinephrine by increasing heart and respiration rate, blood pressure, and oxygen to the peripheral tissues (Levy, 1971; Burdick et al., 2011b). Furthermore, epinephrine has a direct influence on the brain centers responsible for responding to adverse or negative emotions associated with stress

stimulus resulting in fear behavior and stimulating the hippocampus to encode a negative memory (Tsigos and Chrousos, 2002).

Increased stress response can alter muscle metabolic pathways to switch from aerobic to anaerobic metabolism which leads to an increased lactate outpur. Limited amounts of lactate can be utilized in the muscle, but most is transported out of the muscle into the blood via the monocarboxylate transporters MCT1 and MCT4 (Coles et al., 2004; Baird et al., 2007; Thomas et al., 2012). Earlier studies clearly indicated hat blood lactate concentrations were related to elevated neuroendocrine response (cortisol) in "flightly" cattle (Holmes et al., 1972).

Temperament has been evaluated using a variety of tests that objectively or subjectively measure an animal's response to human interaction. Currently, exit velocity, defined as the speed at which an animal exits a chute, is recognized as the most practical objective measure for assessing temperament (Cafe et al., 2011). Exit velocity is measured as the time to travel 1.83 m upon exiting the chute (Curley et al., 2006; Burdick et al., 2011a; Vetters et al., 2013). Subjective chute scoring systems have also been used by many researchers (Fordyce et al., 1982; Fordyce et al., 1988a; Voisinet et al., 1997b; Fell et al., 1999; Kadel et al., 2006; King et al., 2006; Hall and Berg, 2011; Francisco et al., 2012). Due to the subjectivity and associated variability among researchers, chute scores have been questioned for repeatability and consistency as a measure of temperament. In studies investigating biomarkers as potential indicator of stress, blood lactate, was found to be significantly correlated with chute score and exit velocity (Boles

et al., 2015). Moreover, Curley et al. (2006) reported that cortisol, measure in blood, was correlated to exit velocity in Brahman bulls.

Feedlot cattle are exposed to human interaction frequently (Grandin, 1993; Grandin, 1998). The human interaction can induce a fear or stress response which may have a lifetime effect on how an animal perceives and reacts to an adverse stimuli (Francisco et al., 2012; Cooke, 2014; Lyles and Calvo-Lorenzo, 2014). Evaluating feedlot cattle may provide significant indicators for quantifying temperament using physiological markers (Hall and Berg, 2011; Cooke, 2014). Therefore, the purpose of this study was to compare chute scores and exit velocities to physiological biomarkers that may result in a more objective and practical measure for temperament.

LITERATURE REVIEW

Perception of Environment

Animal behavior depends on the way in which individual animals perceive their environment. Simple alterations in the environment can cause livestock to negatively respond to such a change, commonly this is referred to as the "Flight or Fight" response. Basic senses play a key role in livestock behavior and how they interact within the environment. Visual, olfactory, auditory, and tactile stimuli all contribute to livestock behavior (Waynert et al., 1999; Lanier et al., 2000). Over the last thirty years, extensive behavior research on cattle has been conducted in the beef industry to better understand how these cattle perceive their environment.

Due to grazing behavior, where cattle spend large quantities of time with their head down, the auditory system is relied upon heavily to perceive changes within their environment. It has been shown that reducing excessively loud sounds and vocalization while handling cattle reduced their agitated behavior (Grandin, 1993; Grandin, 1998; Waynert et al., 1999; Lanier et al., 2000). Incorporating increased levels of human vocalizaitons and the clanging of metal to stimulate handling facilities, Waynert et al. (1999) evaluated minute-by-minute changes in heart rate and movement of yearling beef heifers exposed to different sounds. They concluded that heart rate and movement concomitantly increased with human-associated noise, indicating that an auditory stimulus has a strong influence on an animal's physiological stress responses in cattle.

Visual perception is also a major factor that determines how cattle respond to their environment and perceive external stressors. Although cattle have 360º panoramic vision and 25º to 50º binocular vision they possess very poor depth perception (Grandin, 1980). Due to their poor depth perception, Grandin (1980) concluded that management of light and minimizing extreme changes in light, from shadows to "light beams", would encourage calmness and compliance in cattle during handling (Grandin, 1998). Later research by Lanier et al. (2000), concluded that gross visual stimuli, such as movement of flags combined with yelling elicited a rapid movement away from the stimuli. In summary, these findings indicate that attention to environmental influences would improve the behavior of cattle during handling while reducing the need for human intervention.

In addition to auditory and visual stimuli, olfactory stimuli can also have an acute impact on behavior. Grandin (1975) found that cattle had a great aversion to the smell of blood, similarly to what would be sensed when cattle enter a stun box, and supported by cattle balking and refusing to enter a hydraulic squeeze chute when previously restrained cattle were castrated. The perception of olfactory environment was later expanded by Pfister et al. (1990) who investigated predator fecal odors and the impact of predator fecal odors on feeding behavior of cattle and sheep. These researchers concluded that feed preference was altered and, in some cases, refusal of feed was noted due to the presence of predator odor. Interestingly, individual animals differed in their response to the presence of predator fecal odors. Some animals would take much longer before they would go to the through and consume feed in the presence of the odors. This negative

response to odor could be attributed to the way in which the animal perceives their environment and can also come from previous exposure to predators that would cause a change in behavior.

Negative prior experience was modeled in wolf exposed compared to wolf-naïve Angus crossbred cows, by evaluating vaginal temperatures, plasma cortisol concentrations, and temperament (Cooke et al., 2013). Under a simulated-wolf presence, cows previously exposed to wolves had increased excitability as indicated by elevated chute score and temperament score. Additionally, cows previously exposed to wolves had an almost doubled fear-elicited cortisol concentration compared to cows not previously exposed to wolves.

In summary, the basis of behavioral changes due to temperament and stress response begins with the manner by which an animal perceives its environment. The auditory, olfactory, and visual senses initiate the physiological and behavioral changes necessary to respond properly to environmental stimuli. These are then encoded in amemory system, perhaps the hippocampus. Thereafter, memory of these stimuli determines how the animal will behave in response to a variety of subsequent stimuli. Stimuli identification initiates a complex cascade of neuroendocrine signals allowing animals to change metabolic and physiological processes to maintain homeostasis (Aguilera, 1994).

Neuroendocrine Factors

In order to survive, mammals must maintain a constant state of homeostasis. Preservation of homeostatic conditions requires continuous behavioral, endocrine, and autonomic adaptions in order to monitor and respond to external and internal stressors. The stress response cascade is primarily driven by the hypothalamic-pituitary-adrenal (HPA) axis. Neuroendocrine secretions cascade from the brain to the adrenal gland resulting in the synthesis and secretion of glucocorticoids and catecholamines from the adrenal gland. Curley et al., (2008) reported that heifers with differing temperaments had different reactions to exogenous HPA (CRH, ACTH) hormones. Additionally, limbic system and amygdala involvement determine speed and severity of the stress response which is ultimately determined by prior experiences stored in the hippocampus (Medina et al., 2002; Cooke et al., 2013).

Hypothalamus

In early research, the hypothalamus was referred to as the "Master Gland" because of its involvement maintaining homeostasis throughout the body (Senger, 2012). The hypothalamus is the basal portion of the diencephalon which lies below the thalamus, it makes up the third ventricle of the brain including the optic chiasm, infundibulum, tuber cinereum, and the mammillary bodies (Hadley and Levine, 2006). Within the lower portion of the tuber cinereum lies the median eminence which is highly vascularized with blood vessels draining into the pituitary stalk. Another important vascular contributor in the hypothalamus is the hypophysial portal system which links the median eminence to

the pituitary gland (Hadley and Levine, 2006). Within the walls of the hypothalamus, there are numerous symmetrically positioned clusters of neurons, hypothalamic nuclei. Two of the hypothalamic nuclei, paraventricular nuclei (PVN) and supraoptic nuclei, possess axons that innervate into the median eminence and extend into the neurohypophysis, forming the supraopticoparaventriculohypophysial tract (Aguilera, 1998; Hadley and Levine, 2006). These nuclei and associated axons are vital in the secretion and action of CRH, ACTH and vasopressin during a stress response.

Corticotropin-Releasing Hormone.

Corticotropin-releasing hormone (CRH), the first neurohormone identified from the hypothalamus, initiates the start of the neuroendocrine-endocrine cascade involve in the physiological response to stress that ultimately restores the homeostatic condition of the animal via down-stream secretion of glucocorticoids (Aguilera, 1998; Cook, 2004; Hadley and Levine, 2006). Corticotropin-releasing hormone is a highly potent and conserved 41-amino acid peptide synthesized and secreted within the paraventricular nucleus (PVN), serving as the chemical signal for synthesis and secretion of ACTH within the pituitary gland. Axons from the PVN extend onto the primary capillary plex of the hypophyseal portal sytem on the external portion of the median eminence which acts on the anterior portion of the pituitary gland to stimulate the synthesis and secretion of ACTH (Aguilera, 1998; Cook, 2004; Hadley and Levine, 2006). Furthermore, CRH neurons distributed in dorsal, lateral, and medial-ventral parvicellular system and act on the sympathoadrenal system which stimulates glucocorticoid secretion from the adrenal gland (Aguilera, 1998; Cook, 2004).

Studies using elector-chemical probe technology have allowed researchers to rapidly measure the CRH response within the brain during a stressful event. Cook (2002) demonstrated that as stress increased in sheep CRH active neurosecretory cell activiity in the amygdala also increased, indicating that repetitive stress potentiated responsiveness of CRH release from the amygdala to a novel stressor. More importantly, a unique feedback mechanism was elucidated involving circulating glucocorticoids acting upon the synthesis and secretion of CRH in the PVN (Aguilera, 1998; Cook, 2002, 2004). Additionally, glucocorticoids sensitize the amygdala to preemptive response to stressors, ultimately, reflecting the role of CRH on the pituitary gland and the release of ACTH(Cook, 2002).

Pituitary Gland

The pituitary gland is composed of two distinct lobes, anterior and posterior. Superior and inferior hypophyseal arteries supply blood to the pituitary gland. The superior hypophyseal artery has two branches, anterior and posterior, which enter the hypophyseal stalk and the hypothalamus, respectively (Hadley and Levine, 2006; Senger, 2012). The hypothalamo-hypophyseal portal which arises from capillary beds in the median eminence provides a vasclular link between the central nervous system and pituitary gland. When CRH is secreted into this portal, cells within the anterior pituitary called cortiotropes of the pars distalis portion of the anterior pituitary synthesize and secrete ACTH, the smallest peptide secreted from the anterior pituitary (Page, 1982; Hadley and Levine, 2006). ACTH subsequently enters the adenohypophyseal limbs of the pituitary vein and is systemically released into the peripheral blood system. ACTH

stimulates the adrenal glands to secrete glucocorticoids, mainly cortisol in response to a stress stimulus.

Cortisol.

Steroidogenic cells found in the adrenal cortex are important for many different biological and metabolic processes. Lipid droplets fill these cells and act as the supply of cholesterol for the synthesis of cortisol (Hadley and Levine, 2006). Under homeostatic conditions, cortisol follows a circadian rhythm in cattle with the highest concentration of serum cortisol released around 0800 and decreasing into the evening until it starts to rise again around midnight (Gatti et al., 2009). However, when an animal perceives a stimulus as threatening, the systemic concentration of cortisol quickly rises to alter physiological and metabolic conditions in preparation of the "Flight or Fight" response (Cook, 2004; Gatti et al., 2009).

The ability of an animal to respond to a stressful stimulus relies heavily on cortisol. When CRH and ACTH are secreted, the systemic concentration of cortisol rises, which in turn increases synthesis of both glycolytic and gluconeogenic enzymes in the liver providing key energy for brain and muscle function (Gatti et al., 2009; Cooke, 2014). Whereas, short-term release of cortisol is beneficial to provide energy needed to respond to a stressor, long-term stress causes a catabolic effect on skeletal muscle and adipose tissue (Gatti et al., 2009; Fazio et al., 2012; Cooke, 2014). Following proteolysis and lipolysis, the subsequent amino acids and free fatty acids become gluconeogenic factors within the liver and thus produce glucose that is necessary for bodily functions (Gatti et al., 2009).

In cattle production, limiting constant long-term stress is important for increasing efficiency of growth and carcass quality. Curley et al. (2006) evaluated the association between current temperament measures and serum cortisol in Brahman bulls. They concluded that serum concentration of cortisol were positively correlated with exit velocity and pen scores, indicating a concomitant increase in exit velocity, pen score, and serum cortisol in response to handling

In other livestock species, similar responses have been observed. In sheep exposed to canine predator stress, Corticotropin-releasing hormone and cortisol concentrations increased in the amygdala and blood, respectively (Cook, 2002). Cook (2002) incorporating, immunosensor-based microdialysis probes in the amygdala, showed that in response to predator stress, CRH secretion had two peaks. The first peak of CRH in the amygdala corresponded with predator stress; the second peak occured following peak cortisol concentration in the amygdala. Interestingly, Cook (2002), observed an inverse change in the peaks of CRH in the amygdala with increased exposure to predator stress. In a subsequent phase of the study, Cook (2002) documented that sheep under a predator stress and exposed to an additional novel stress (electrical shock) had exaggerated peaks of CRH and cortisol; reflecting a fear conditioning response.

In summary, high systemic cortisol concentrations may have damaging effects on the growth and development, especially if the temperament of an animal becomes more flighty, and/or the presence of a conditioned fear response exists, there is a costly metabolic shift to supply energy. Short-term, secretion of cortisol is vital for homeostasis, however excessive secretion results in catabolism of muscle and adipose tissue.

Therefore, identifying and sorting cattle based on their degree of temperament may result in the opportunity to minimize the stress response and handling challenges and subsequently enhancing the growth efficiency and carcass quality of cattle.

Autonomic Stress Response

While the HPA axis is absolutely necessary to provide an animal with metabolic and physiological changes, the systemic increase in cortisol takes minutes, "Flight or Fight" is immediately derived from physiological response through the sympathetic and parasympathetic nervous system (Aguilera, 1998; Hadley and Levine, 2006; Ulrich-Lai and Herman, 2009). When cattle are confronted with stress, the brain triggers sympathetic and parasympathetic neurons that subsequently alter vital organs to extensively alter metabolic, physiologic, and psychologic function (Ulrich-Lai and Herman, 2009).

To initiate " Flight or Fight" response, sympathetic post paravertebral ganglia terminate at the chromaffin cells within the medulla of the adrenal gland, systemically releasing epinephrine as the animal is exposed to a stressor (Ulrich-Lai and Herman, 2009; Burdick et al., 2011b; Lawrence et al., 2012). Originally referred to as adrenalin and noradrenalin, Cannon (1914) first characterized the epinephrine response, noting that, under "Flight or Fight" response, there was a concomitant increase in vasoconstriction and heart rate, which Cannon hypothesized would result in a subsequent increase in glycolytic activity. Epinephrine increases lipolysis and hepatic glycogenolysis, increasing systemic free fatty acids and glucose concentrations, respectively (Jones and Marchello,

1983; Lawrence et al., 2012). In addition, epinephrine increases skeletal and cardiac muscle contractility while uniquely relaxing bronchial smooth muscle to allow for higher respiration rates (Lawrence et al., 2012).

With constant exposure to stress in cattle, excessive catabolic effects brought about by high systemic concentrations of catecholamines, results in negative effects on growth, marbling, and carcass quality (Jones and Marchello, 1983; Forkman et al., 2007). As previously described, the enhanced cardiac response measuring heart rate may provide a model to assess an animal's stress-response. This is supported by research indicating that heart rate increased exponentially during transport, with an immediate return to resting heart rate after the cattle were unloaded, reflective of the action of the autonomic nervous system in response to a stress or fearful event (Bulitta et al. (2011). Furthermore, Burdick et al. (2011b) noted that when exit velocity, and pen score increased, circulating concentrations of epinephrine and cortisol increased. Additionally, bulls with excitable temperaments had higher rectal temperatures than calm bulls, corroborating that excitable temperaments have a higher degree of stress response.

Therefore, measurements of heart rate and rectal temperature may be significant biomarkers for assessing temperament in cattle. In addition, from a handling standpoint, these indicators are much easier and quicker to obtain, than measuring circulating epinephrine due to the quick clearance and half-life (Hadley and Levine, 2006). Heart rate and rectal temperatures are also less invasive than venipuncture, the need for trained technicians is reduced, resulting in easier implementation if a significant association exists between temperament and these indicators.

Gluconeogenesis

Efficiency of cattle is gained by their ability to retain large amounts of feed-stuff. In cattle, the rumen is the largest compartment of their digestive system composing over 70% of the total digestive system and containing large populations of mostly anaerobic bacteria including cellulolytic, xylanolytic, and pectinolytic bacteria highly adapted to break down the cells of forage consumed by cattle (Ellis and Hill, 2005; Furness et al., 2015). Carbohydrates are consumed by bacteria and very little are absorbed or passed through to caudal portions of the digestive system causing the active glucose pathway to be very different than omnivores. The small intestine of ruminant species contains the active site of absorption for glucose; however, due to microbial dependency of carbohydrates, results in limited glucose absorption in the small intestine (5-20%).Glucose needs are met by a process called gluconeogenesis (75 – 90%). Microbial population in the rumen produces: methane, carbon dioxide, and acetic, propionic, and butyric acids (Bergman, 1990; Nafikov and Beitz, 2007). Volatile fatty acids (VFA) of acetic, propionic and butyric are absorbed through the rumen wall and through caudal portions of the digestive tract, either metabolized intraepithelially into ketone bodies or lactate, or directly entering the blood stream (Bergman, 1990; Nafikov and Beitz, 2007; Furness et al., 2015). Glucose can then be synthesized by the liver using lactate or propionate through gluconeogenesis (Exton, 1972; Yost and Young, 1977; Deckardt et al., 2013).

Lactate

In reaction to a stressor or a perceived threat to survival, metabolism reverses to anaerobic mechanism. Consequently, the increase in anaerobic glycolysis, results in an excessive amount of lactic acid being produced (Stryer, 1981; Juel and Halestrap, 1999). This is supported by prior research which indicated a 60% increase in systemic lactate concentration following an epinephrine injection after a period of exercise (Holmes et al., 1972). Endocrine and heightened lactate response has also been observed in other species exposed to both soft and rough handling practices for extended periods of time (Chloupek et al., 2011). During this accelerated rate of glycolysis, cellular pH decreases, disrupting homeostasis (Stryer, 1981; Juel and Halestrap, 1999; Philp et al., 2005). Proton-linked monocarboxylate transporters (MCT) are then utilized to transport lactate from cell to blood stream (Juel and Halestrap, 1999; Philp et al., 2005).

Recent research has assessed the use of blood lactate concentration as an objective measure of temperament. Evaluating exit velocity, chute score and blood lactate, in Angus x Simmental steers. Utilizing a hand held lactate analyzer commonly used in human research and validated by Burfeind and Heuwieser (2012) in dairy cattle for use in whole blood, results indicate exit velocity, chute score, and blood lactate were significantly associated. Lactate concentrations increased with adverse temperament. These findings as well as research of others (Holmes et al. (1972); Chloupek et al. (2011)), strongly suggest that blood lactate could be an effective objective measure to assess temperament and subsequent stress response to handling in beef cattle.

Temperament Measures and Effect of Temperament on Growth and Carcass

Temperament is defined as the way in which an individual reacts to a novel or challenging situation (Grandin, 1998; Reale et al., 2000; Ferguson et al., 2006). Behavioral responses to proper/improper handling and restraint in chutes is reflected in an animal's temperament (Kadel et al., 2006). Therefore, since temperament may influence growth performance, carcass grading, and meat quality, it would seem logical to quantify temperament in cattle from weaning to slaughter.

Temperament Measures.

Early temperament measures consisted of subjectively scoring an animal in a pen or squeeze chute. Tulloh (1961) quantified temperament through the use of chute scores. Tulloh based his system on a Likert scale ranging from 1 (animal stands quietly) to 6 (the animal is aggressive and struggles violently. Later work of Grandin (1993), followed with a five point chute score system where one defined a calm animal with no movement in the chute score of five in which the animal is rearing, twisting violently in the chute. Additionally, findings also indicated an inverse relationship between proper handling and negative temperament, resulting in a greater voluntary compliance during handling in cattle.

Another widely used objective measure is exit velocity. Originally referred to as flight distance, as technology developed, the term exit velocity was created to define the rate in which an animal travels a standard distance (Fordyce et al., 1982; Ferguson et al., 2006). Later work by Fordyce et al. (1988a) utilizing exit velocity in Brahman cross and Shorthorn bullocks and cows, reported that horned cattle tended to have lower

temperament scores than hornless cattle, suggesting that *Bos taurus* Shorthorn cattle were more docile than *Bos indicus* Brahman cattle. The increased excitable temperament of *Bos indicus* cattle has been documented by numerous researchers, when compared to *Bos taurus* cattle (Fordyce et al., 1988a; Voisinet et al., 1997b; Curley et al., 2006; Ferguson et al., 2006; Kadel et al., 2006; Burdick et al., 2009; Burdick et al., 2011a; Cafe et al., 2011).

Using chute and pen score, in combination with exit velocity, Curley et al. (2006) compared these techniques over multiple observations, as well as investigating the relationship between temperament and serum cortisol concentrations. Results indicated that only exit velocity was positively correlated with cortisol concentrations across all observations. Concluding that exit velocity was a valuable tool for assessment of cattle temperament and a possible predictor of stress response to future handling events.

Temperament and Average Daily Gain.

Beyond assessing associations between temperament measures, many researchers have assessed an animal's temperament and subsequent effect on growth performance, carcass grade, and meat quality traits (Ferguson et al., 2006). Some studies have included other subjective measures like exit score, where an observer assigns a score to the speed in which the animal leaves the squeeze chute, while other researchers have included second objective measures like blood lactate to assess an animal's temperament (Petherick et al., 2009b; Vetters et al., 2013; Boles et al., 2015).

In beef production, efficient growth or average daily gain (ADG) of an animal is vitally important for economic viability. Burrow and Dillon (1997) observed an inverse

relationship between exit velocity and ADG when evaluating *Bos indicus* and *Bos taurus* heifers, indicating reduced growth in animals leaving the chute faster. Voisinet et al. (1997b), evaluating *Bos taurus* and, *Bos taurus* x *Bos indicus* steers and heifers for chute scores on 194-213 days on feed also reported a similar correlation with chute scores and ADG. Later, Fell et al. (1999) evaluated Angus x Hereford cattle with the same 5- point scale chute score, and exit velocity to classify the animals into two groups: calm and nervous and compared them with a non-classified group. The cattle were fed for a period of 85 days with the calm group having higher ADG than non-classified cohorts and significantly higher ADG then the nervous group. Similarly, Petherick and colleagues (2002) used exit velocity to classify 120 *Bos indicus* x *Bos taurus* steers into three temperament groups: poor, mixed, and good, following a 101day feeding period, reported that animals classified with a poor temperament had significantly lower ADG than the mixed temperament group. Animals classified as good temperament had significantly higher ADG than mixed and poor temperament groups. Recently, Francisco et al. (2012) assessed 260 Angus x Hereford feeder at weaning, mid-weigh through growing, and then at finishing, for temperament using both chute score (1-5) and exit velocity, classifying an animal's temperament as adequate or excitable. Findings indicate that body weight of calves with excitable temperaments were lower than those of adequate temperaments at weaning. No statistical differences were found for ADG during pre-conditioning, growing, or finishing phases. Nonetheless, excitable animals tended to have decreased hot carcass weights than calves with adequate temperaments. In contrast, Boles et al. (2015) found that cattle with excitable temperaments had higher ADG than those with

calm temperaments. Possible influences for the differences may be that cattle were held at a commercial feedlot, rather than an experimental station, and the calm animals may have tended to be "shy-feeders" which would account for the dissimilarities in results.

Collectively, these studies indicate that cattle with excitable or poor temperaments have a lower ADG than cattle with an adequate or calm temperament. The differences observed in ADG can be influenced by a multitude of factors and mechanisms. This would include, excitable animals exhibit an increase in movement and expense of energy (Burrow and Dillon, 1997), animals with excitable temperaments have decreased feed conversion efficiency which results in decreased ADG and growth of the animal (Petherick et al., 2002). Decreased potential for growth because of stressed/temperamental animals reduces gains and efficiency, impacting the industry. Identification and selection of animals with adequate temperaments is important to the sustainability of the feedlot industry.

Temperament Influence on Carcass and Meat Quality

Lastly, research has indicated that temperament has an impact on carcass and meat quality. Researchers have shown that as temperament of an animal becomes more aggressive and excitable, there is higher incidence of bruising and dark cutting (Fordyce et al., 1988b; Voisinet et al., 1997a; Petherick et al., 2002; Vogel et al., 2011). Additionally, temperament influences the quality grade, and tenderness (Gruber, 2010; Cafe et al., 2011; Turner et al., 2011; Boles et al., 2015). Gruber (2010) evaluated heart rate, rectal temperature, and serum cortisol in *Bos taurus* steers and heifers for stress

associated with physical handling and chute restraint. Findings indicating that steaks with increased Warner-Bratzler shear force (WBS) came from animals exhibiting higher stress response. Behrends et al. (2009), evaluating feeder cattle at weaning using exit velocity, chute score, and pen score to assess temperament, reported exit velocity and weaning were highly associated with feedlot ADG, and carcass and meat quality, Ribeye area (REA), and yield grade (YG), and WBS. As the exit velocity increased, reflecting a more excitable temperament, the carcass quality decreased and subsequently WBS increased.

Consumer perception of animal welfare and their demand for a more consistent and higher quality of product makes it imperative to study the anti- and postmortem factors that may influence the behavior and growth of beef cattle. Understanding the stress response of an animal and its effects on animal's behavior during handling would be a vital piece to the puzzle. The objective of this study was to improve or replace current temperament measures with simple objective measures that could be applied chute side to enhance identification of temperament in feedlot cattle. The hypothesis of this study was that objective measurements of body temperature, heart rate, blood oxygen saturation, plasma lactate as measured by a hand-held meter, serum lactate, glucose, non-esterified fatty acids, salivary cortisol, and serum cortisol will be associated to exit velocity and chute score. Secondly, an objective measure or combination of measures could be used to classify an animal's temperament similar to exit velocity and chute score.

Materials and Methods

Cattle Selection and Management

Research was conducted under animal care protocol (2014-AA09) approved by the Montana State University Agricultural Animal Care and Use committee. One-hundred and ninety-seven feedlot cattle were sampled from a commercial, certified Beef Quality Assurance feedlot in Chappell Nebraska.

Diet Composition

Animals were fed a standard concentrate feedlot diet, consisting of 94% concentrate feed composed of rolled corn, beet pulp, dried distillers grains, protein supplement, corn silage and ground hay: dry matter basis 14.58% CP and 0.61 Mcal/lb net energy for gain, three times daily. Diets fed to mixed sex pens included melengestrol acetate (MGA), 0.5 mg/day, to suppress estrous in females.

Temperament Measures

Feedlot. The feedlot operator was requested to select pens of cattle with excitable temperaments and pens with calm temperaments to increase the variation for sampling.

Exit Velocity. A Polaris timer system (Farmtek Inc., Wylie Texas) was used to measure exit velocity with photo-transmitters placed 1.83 and 3.65 meters in front of chute.

Chute Score. Chute score as described in Table 1 was assigned by the same individual for each sampling period (Federation, 2011). Chute scores were assigned according to behavior throughout handling process, i.e. entering hydraulic squeeze chute, while measures were being collected, and exit from the squeeze chute.

Table 1. Chute score as recommended by Beef Improvement Federation

Score		Description
1	Docile	Mild disposition. Gentle and easily handled. Stands and moves slowly during processing. Undisturbed, settled, somewhat dull. Does not pull on headgate when in chute. Exits chute calmly.
2	Restless	Quieter than average, but may be stubborn during processing. May try to back out of chute or pull back on headgate. Some flicking of tail. Exits chute promptly.
3	Nervous	Typical temperament is manageable, but nervous and impatient. A moderate amount of struggling, movement and tail flicking. Repeated pushing and pulling on headgate. Exits chute briskly.
4	Flighty (Wild)	Jumpy and out of control, quivers and struggles violently. May bellow and froth at the mouth. Continuous tail flicking. Defecates and urinates during processing. Frantically runs fence line and may jump when penned individually. Exhibits long flight distance and exits chute wildly.
5	Aggressive	May be similar to Score 4, but with added aggressive behavior, fearfulness, extreme agitation, and continuous movement which may include jumping and bellowing while in chute. Exits chute frantically and may exhibit attack behavior when handled alone.
6	Very Aggressive	Extremely aggressive temperament. Thrashes about or attacks wildly when confined in small, tight places. Pronounced attack behavior

http://beefimprovement.org/content/uploads/2015/04/REVISED-Master-Edition-BIF-Guidelines-Final-4-2015.pdf

Blood Collection and Preparation

Samples were drawn from the jugular vein using 1.25 mm x 30 mm Vacuette ® needles (Greiner Bio-One, Kremsmünster, Austria) while the animals were restrained in a hydraulic squeeze chute (Moly Manufacturing, Lorraine KS, USA). Two different Vacutainer® tubes (Becton, Dickinson and Company, Franklin Lakes NJ, USA) were used for blood collection, silicone coated tubes to collect blood for the purpose of

measuring non-esterified fatty acids, lactate and cortisol, and sodium fluoride potassium oxalate coated tubes for collecting blood to measure glucose. The sodium fluoride potassium oxalate Vacutainer® tubes were stored on ice during collection and were centrifuged at 2,700 rpm for 30 minutes using a Cole-Parmer VS-3400 centrifuge (Cole-Parmer, Vernon-Hills IL, USA). Blood samples were centrifuged within four hours from collection to prevent enzymatic breakdown of glucose (Mikesh and Bruns, 2008). After samples were centrifuged, serum was decanted from cellular components and stored in 8 ml Nalgene® General Long-Term Storage Cryogenic Tubes (Thermo Fisher Scientific Inc., Waltham NC, USA) and stored at -20º C until analyzed. Silicone coated serum Vacutainer® tubes were used for collection of blood samples for the purpose of measuring non-esterified fatty acids, lactate, and cortisol. Vacutainer® tubes were stored on ice during collection and stored at 1ºC for twenty-four to thirty-six hours after collection at the feedlot. Serum tubes were centrifuged either using Thermo Scientific Sorvall Legend RT+ or Cole-Parmer VS-3400 centrifuge at 2,700 rpm for 30 minutes. For the serum tubes that were centrifuged using the Sorvall Legend RT+ the temperature of the centrifuge was regulated at 4ºC. After samples were centrifuged, serum was decanted from cellular components and stored in 8 mL Nalgene® General Long-Term Storage Cryogenic Tubes and stored at -80°C until analyzed.

Non-Esterified Fatty Acids

To analyze non-esterified fatty acids, HR series NEFA (lot #KK053) (Wako Life Sciences, Inc., Mountain View CA, USA) micro-plate assay procedure was used. Serum was brought to room temperature and placed on a Baxter Multi-Tube Vortexer for 60

seconds on speed setting 4. Standards, controls, and samples were run in triplicates. Greiner Bio-one Flat-bottom 96 well microplates (lot#E130309T) were prepared by pipetting 5µL of standards, control, and serum into each well using a Eppendorf Xplorer 10 µL single channel electronic micropipette. Standards including blank wells prepared for each with serial dilution of standards using phosphate buffer saline (0.01 M pH 7.2). Standard concentrations of non-esterified fatty acids were as follows: 0, 0.03125, 0.0625, 0.125, 0.25, 0.5, and 1 mEq. Blank wells contained phosphate buffer saline. Two-hundred microliters of HR series NEFA-HR color Reagent A (lot #KK053) (Wako Life Sciences, Inc., Mountain View CA, USA) was added to each well using an Eppendorf microchannel pipette. After the addition of Reagent A, the plate was mixed on Roto-Mix for 1 minute at speed setting 4.5 and then placed in Thelco incubator for 10 minutes at 37°C. Once the plate was incubated with color Reagent A, 100 µL of HR series NEFA-HR color Reagent B was added to each well using an Eppendorf Research multichannel pipette. Following the addition of color Reagent B, the plate was mixed on Roto-Mix for 1 minute at speed setting 4.5 and then incubated in a Thelco incubator for 10 minutes at 37°C. A three-minute cooling period at room temperature (21° C) followed incubation. The plate was read at 550 nm on an Epoch™ Microplate Spectrophotometer (BioTek) and Gen5™ microplate data collection and analysis software (BioTek) was used for data collection and reduction with concentration of serum non-esterified fatty acids being reported in mEq. Means, standard deviations, and the coefficient of variation were calculated for all samples. Any sample with a coefficient of variation greater than 12% was repeated. Intra and Inter coefficient of variation for bovine serum containing

0.05mEq was 5.1 % and 5.6 % respectively, and for a serum sample containing 0.2 mEq was 4.6% and 11.1% respectively.

Glucose

To analyze serum concentration of glucose, Infinity™ Glucose (lot # 241531) (Thermo Scientfic) micro-plate assay procedure was used. Serum was brought to room temperature and placed on a Baxter Multi-Tube Vortexer (Baxter Diagnostics Inc, Deerfield IL, USA) for 60 seconds on speed setting 4. Greiner Bio-one Flat-bottom 96 well microplates (lot #E130309T) were prepared by pipetting 5µL of standards (0, 10, 20, 40, 80, 160, 320 mg/dL), controls and serum into appropriate wells. All standards, controls, and samples were run in duplicate. Two-hundred microliters of Infinity™ Glucose (lot # 241531) (Thermo Scientfic) was pipetted into all wells using an Eppendorf Research Multi-channel pipette (Eppendorf AG, Hamburg, Germany). Plates were covered with Eppendorf adhesive PCR Film (lot #A030211H) and placed on a Thermolyne Roto-Mix (Barnstead/Thermolyne, Dubuque IA, USA) for one-minute at speed setting four. After one-minute on mixer the plates were incubated in a Thelco (GCA/Precision Scientific, Chicago IL, USA) incubator for 10 minutes at 37º C. Plates were cooled at room temperature (21 ºC) for 30 minutes and then read at 340 nm on an Epoch™ Microplate Spectrophotometer (BioTek Instruments Inc., Winooski VT, USA). Gen5™ microplate data collection and analysis software (BioTek) was used for data collection and reduction with concentration of serum glucose being reported in mg/dL. Means, standard deviations, and the coefficient of variation were calculated for all samples. Any sample with a coefficient of variation greater than 12% was repeated. Intra

and inter coefficient of variation for bovine serum containing 173.67 mg/dL was 6.02 and 5.4% respectively, and for serum containing 176.74 mg/dL was 7.25% and 4.4% respectively.

Lactate

Plasma. Plasma blood lactate was measured using a < 2 µL of blood from jugular venipuncture using a Lactate Pro® meter (Akray Inc. Minami-ku, Kyoto Japan) and reported as mmol/L.

Serum. Lactate Assay Kit II (Biovision Inc, Milpitas CA, USA) micro-plate assay procedure was used to analyze serum concentration of lactate. Frozen serum samples were placed in a tepid water bath to bring samples to room temperature and mixed on Baxter Multi-Tube Vortexer for 1 minute on speed setting 4. A 1:10 dilution of the samples was used and samples were run in triplicate. Sample dilution was prepared by placing 20 µL of serum and 180 µL phosphate buffer saline (0.01 M pH 7.2) into a 96 well plate covered with Eppendorf PCR film and mixed on Roto-Mix for 5 minutes on speed setting 4. After the plate was mixed 5 µL of diluted serum was placed into a Greiner Bio-one Flat-bottom 96 well microplate (lot#E130309T) using an Eppendorf Research plus multichannel pipette. Forty-five microliters of Lactate buffer from the Lactate Assay Kit II (Biovision Inc, Milpitas CA, USA) was added to each well using an Eppendorf multichannel pipette. A lactate enzyme mix was prepared following assay protocols provided by Biovision. Fifty microliters of enzyme mix was pipetted into each well using an Eppendorf multichannel pipette. Following the addition of the enzyme mix,

the plate was placed on the Roto-Mix for 5 minutes on speed setting 4. Including the time on the mixer the plate was incubated at room temperature for 30 minutes. The plate was read on read at 450 nm on an Epoch™ Microplate Spectrophotometer (BioTek) and Gen5™ microplate data collection and analysis software (BioTek) was used for data collection and reduction with concentration being reported as nmol. The following equation was used to report actual concentrations of lactate: nmol/5µL x Dilution Factor = nmol/µL. Means, standard deviations, and the coefficient of variation were calculated for all samples. Any sample with a coefficient of variation greater than 12% was repeated. The intra and inter coefficient of variation for serum lactate for bovine serum containing 3.5 mM was 6.14% and 14.8% respectively, and for serum containing 0.78 mM wsa 10.2% and 17.9% respectively.

Cortisol

Salivary Cortisol. Two salivary samples were collected from each animal using Salivette tubes (Sarstedt AG). Salivette tubes were kept on ice during collection and were quickly placed on dry ice (~ -79°C) for transportation back to Montana State University. Salivette tubes were placed on lab bench for 20 minutes at 21°C to thaw. To collect saliva, Salivette tubes were placed in a Thermo Scientific Sorvall Legend RT+ centrifuge and were centrifuged for 4 minutes at 1,000 rpm at 8°C. Once saliva was separated from internal cotton swab, the swabs were discarded and the saliva for each animal was combined into one tube. After combining, samples were stored at -20°C until analyzed.

An Expanded Range High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit from Salimetrics® (Salimetrics, State College PA, USA) was used to analyze saliva for cortisol levels. Two non-specific binding wells were used in the provided 96 well plates (lot# 1505508). The following were added to other well within the plate; 25 µL of provided standards, and controls as well as salivary samples. Standards, controls and salivary samples were run in triplicates. Twenty-five microliters of assay diluent was placed into the two non-specific binding wells as well as the "zero" wells for the standards. Two-hundred microliters of enzyme mix, enzyme conjugate and assay diluent prepared following Salimetrics® protocol, was placed into each well. The plate was placed on Roto-Mix for 5 minutes at speed setting 4 and then incubated at room temperature (21°C) for an hour.

After incubation the plate was washed with diluted wash buffer (10x dilution). Plate was washed by adding 300 µL of diluted wash buffer into each well four times. Provided TMB substrate solution was added at 200µL per well and then plate was placed on Roto-Mix for 5 mins at speed setting 4 making sure plates were covered and in the dark. The plate was incubated at room temperature (21°C) for an additional 25 minutes covered. Fifty microliters of provided TMB stop solution was added to each well and plate was mixed for 3 minutes on Roto-Mix at speed setting 4. If green color was still visible in any well the plate was mixed for an additional 3 minutes. The plate was read at 450 nm on an Epoch™ Microplate Spectrophotometer (BioTek) and Gen5™ microplate data collection and analysis software (BioTek) was used for data collection and reduction with concentration being reported as µg/dL. Means, standard deviations, and the

coefficient of variation were calculated for all samples. Any sample with a coefficient of variation greater than 12% was repeated. The intra and inter coefficient of variation for salivary cortisol for pooled bovine saliva containing 0.12 µg/dL was 9.8% and 14.4% respectively, and for pooled saliva containing 1.0 µg/dL was 4.9% and 13% respectively.

Serum Cortisol. Cortisol was measured using MP Biomedicals Cortisol Antibody Coated Tube radioimmunoassy kit. The kit was validated for cattle serum. Serum samples were randomized and thawed at 27° C. Samples were run in duplicates with 25 µL of serum in each tube. After the serum was pipetted into tubes, 1 mL of I^{125} was added to the tubes. Samples were then shaken and put in an incubator at 22.5° C for 18 hours. All tubes were decanted, except for the total count tubes, and placed on a Perkin Elmer 2470 Automatic Gamma Counter (Waltham, Massachusetts). The inter- and intra- assay coefficient of variation were 4.5% and 9.3%, respectively.

Chute Side Measures

Body Temperature. Rectal temperature was taken using a Jorgensen Laboratories Digital Thermometer fitted with a rectal probe (Jorgensen Laboratories, Loveland CO, USA) and recorded in Fahrenheit (F^o) and later converted to Celsius by the following equation (F^o - 32) x 5/9.

Heart Rate. A Mediaid Veterinary pulse oximeter (Mediaid Inc., Cerritos CA, USA) fitted with rectal probe was used to determine heart rate as measured by absorption of light. A minimum of 3 readable pulses, light absorbency differences from volume of

blood systematically changing with each pulse phase. Heart rate was displayed as beats per minute.

Oxygen Saturation. A Mediaid Veterinary pulse oximeter fitted with rectal probe was used to determine percent oxygen saturation (%SpO_2). Red and infrared light absorption differences in oxyheamoglobin and deoxyheanoglobin are measured and displayed as a percent oxygen saturation.

Statistical Analysis

The MEAN procedure of SAS (Statistical analysis software, SAS Institute Inc., version 9.4) was used for descriptive statistics with sample size, means, standard deviation, minimum and maximum being reported for all animals, and then by sex. The General Linear Model and Least Square Means procedure of SAS (SAS 9.4, 2014) was used to analyze differences of physiological and temperament measures, where the class variable was exit velocity classification and sex. The dependent variables were chute score, pulse, oxygen saturation, rectal temperature, plasma lactate meter (Lactate Pro® meter), individual exit velocity, glucose, non-esterified fatty acids, serum lactate, salivary cortisol, and serum cortisol. Because there was a significant ($P \leq 0.04$) difference between sexes, Pearson product-moment correlations were calculated by sex. Linear models were analyzed (R-Studio version 2.15.1) where exit velocity was compared to the variables and combination of variables of plasma lactate, rectal temperature, glucose, salivary cortisol, and serum cortisol to determine which measure or measures could possibly be used as an objective temperament measure similar to exit velocity. An Akaike

information criterion (AIC) was used to analyze the quality of the models (Akaike, 1974). The lowest AIC values are reported here for steers and heifers indicating the best candidate linear models to predict exit velocity. All data were considered significant when the *P*-value was less than 0.05.

RESULTS AND DISCUSSION

Descriptive Statistics

The purpose of this study was to compare chute scores and exit velocities to physiological responses of heart rate, body temperature, blood oxygen saturation, metabolites and hormones, to potentially find a biomarker which could improve temperament classifications as an objective measure.

Table 2. Descriptive statistics for body weight (WT), chute scores (CS), heart rate (HR), oxygen saturation (O_2), rectal temperature (TEMP), blood lactate as measured by the handheld meter (PLAC), exit velocity (EV), serum glucose (GLUC), non-esterified fatty acids (NEFA), serum lactate (SLAC), and cortisol salivary (SCORT) or serum (BCORT) for all animals sampled

	N	Mean	SD	Range
WT (kg)	197	411.17	81.81	220-582
CS[1]	197	3.1	0.60	2-5
EV (m/s)	194	2.56	1.32	0.31-7.12
HR (beats/min)	191	62.79	19.07	25-138
O_2 (SpO_2%)	191	77.62	13.28	51-100
TEMP (ºC)	196	39.86	0.49	38.3-41.9
GLUC (mg/dL)	193	108.21	33.64	63.1-305.1
NEFA (mEq)	189	0.23	0.12	BDL[2]-0.99
PLAC (mM)	192	3.96	2.75	BDL[2]-13.6
SLAC (mM)	193	5.86	3.99	0.16-20.44
SCORT (µg/dL)	191	0.22	0.15	0.05-0.87
BCORT (µg/dL)	197	1.90	1.03	0.10-5.03

[1]Chute Scores – 1 = Docile, 2 = Restless, 3 = Nervous, 4 = Flighty (Wild) 5= Aggressive, 6 = Very Aggressive.
[2] NEFA – Below detectable limit (BDL) of 0.01 mEq, Lactate Pro® Meter BDL of 0.08 mM

The average cute score assigned to animals was 3.1 with a range of 2 to 5 (Table 2 and 3). These values are similar to those reported by Boles et al. (2015). It should be noted that there was only one animal, a heifer, that was assigned a chute score of 5. Addtionally, individual mean exit velocity was 2.56 with a range of 0.31-7.12 m/s. The mean exit velocities reported here for steers and heifers were similar to exit velocities

reported by Curley et al. (2006) evaluating Brahman bulls. Curley et al. (2006) reported excitable bulls had mean exit velocities of 4.04 m/s ± 0.16. Burdick et al. (2011a) also measured exit velocities of Brahman calves. These researchers reported that after 56 days postweaning the mean exit velocity was 2.12 m/s ± 0.05. Both of these studies evaluated *Bos indicus* animals which have been noted to have a more excitable temperament than *Bos taurus* cattle (Burrow and Dillon, 1997; Ferguson et al., 2006; Sant'anna et al., 2012). In mixed breed steers of *Bos taurus* parentage (n = 1,181), Vetters et al. (2013) measured exit velocities twice reporting mean exit velocities of 2.9 ±0.88 m/s and 3.0 ±0.87 m/s respectively. Furthermore, Boles et al. (2015) evaluated exit velocities of 154 Angus X Simmental steers, reporting mean exit velocity of 2.88 ± 0.77 m/s supporting the data presented here.

The average heart rate of the animals measured was 62.17 with a range of 25 to 138 beats per minute with the mean heart rate higher in steers than heifers (Table 2 and 3). The Merck Veterinary Manual (2010) lists the average basal heart rate in dairy cows to be between 48-84 BPM. The very low range reported here suggests some animals were not healthy but this was not the case, all animals visually appeared not to be struggling with breathing or movement. This suggests there may have been a problem with the meter used to measure the heart rate. The pulse oximeter used is designed to be used in sedated and fasted animals for surgery. The animals in this study were awake not fasted and only held in a hydraulic squeeze chute. This could be why some of the values were

Table 3. Descriptive statistics for body weight (WT), chute scores (CS), heart rate (HR), oxygen saturation (O2), rectal temperature (TEMP), blood lactate as measured by the handheld meter (PLAC), exit velocity (EV), serum glucose (GLUC), non-esterified fatty acids (NEFA), serum lactate (SLAC), and cortisol salivary (SCORT) or serum (BCORT) for steers and heifers

STEERS	N	Mean	SD	Range
WT (kg)	82	402.35	84.55	227-581.8
CS[1]	82	2.9	0.56	2.0-4.5
EV (m/s)	81	2.21	1.25	0.31-6.02
HR	80	63.2	19.8	32-110
O_2 (SpO_2%)	80	77.35	15.20	51-100
TEMP (ºC)	82	39.79	0.45	38.8-41.9
GLUC (mg/dL)	82	104.73	33.47	65.6-305.1
NEFA[3] (mEq)	79	0.27	0.12	0.07-0.87
PLAC[4] (mM)	78	3.47	2.61	BDL[2]-13.6
SLAC (mM)	82	5.43	4.26	0.16-20.44
SCORT (µg/dL)	79	0.18	0.14	0.10-4.9
BCORT (µg/dL)	82	1.64	0.94	0.05-0.85
HEIFERS				
WT (kg)	109	414.2	79.83	220.0-580.9
CS[1]	109	3.22	0.59	2-5
EV (m/s)	108	2.76	1.37	0.31-7.12
HR (beats/min)	105	62.17	18.46	25.0-138
O_2 (SpO_2%)	105	77.27	11.75	51-99
TEMP (ºC)	108	39.93	0.53	38.3-41.5
GLUC (mg/dL)	105	112.04	34.16	63.1-295
NEFA[3] (mEq)	105	0.21	0.10	BDL[2]-0.99
PLAC[4] (mM)	108	4.32	2.83	BDL[2]-13.0
SLAC (mM)	105	6.06	3.79	0.38-19.67
SCORT (µg/dL)	107	0.26	0.15	0.05-0.87
BCORT (µg/dL)	109	2.13	1.07	0.15-5.03

[1]Chute Scores – 1 = Docile, 2 = Restless, 3 = Nervous, 4 = Flighty (Wild) 5= Aggressive, 6 = Very Aggressive
[2] NEFA – Below detectable limit (BDL) of 0.01 mEq, Lactate Pro® Meter BDL of 0.08 mM
[3]NEFA –Non-esterified fatty acid.
[4]Lactate Pro® Meter

lower than what is reported for the basal hear rate. The basal heart rate reported is a large range and doesn't indicate the upper limit expected with a stress response. Additionally, dairy cattle have a different metabolic rate than crossbred feedlot cattle (Baird et al., 2007). Important to animal welfare and temperament however when Bulitta et al. (2011) evaluated heart rate of heifers and cows before, during, and after transportion. They

reported the mean resting heart rate to be 80 ± 6 BPM for heifers and the average peak heart rate to be 136 ± 35 BPM. Comparing the mean heart rates for steers and heifers presented here to data presented in Bulitta et al. (2011) suggests that the animals were near resting heart rate while in the chute. Moreover, data presented here illustrates that heart rate of indivuduals was near resting heart rate and any with eleveated heart rate would represent the temperament of the animal and not an additive effect from handling stress.

Mean measures for plasma lactate using a Lactate Pro® meter and serum lactate concentrations were 3.86 mM and 5.86 mM respectively with the range of below detectable limits for the meter to 13.6 and 0.16 to 20.44 respectively. These mean dissimilarities are attributed to differences in sensitivity of measures and techiniques used. Two microliters of whole blood is measured in less than sixty-seconds in the Lactate Pro® meter with serum concentrations being measured with a sensitive ELISA assay. Nonetheless, there is a strong correlation (Table 5 and 6) between the two measures. This strong correlation between a hand held meter and serum has also been reported by Burfeind and Heuwieser (2012) where a similar handheld lactate meter was correlated to serum with $r = 0.75$ ($p < 0.01$) in lactating cows and $r = 0.98$ in calves ($p < 0.01$). Additionally, Boles et al. (2015) reported a significant association between blood lactate measured with Lactate Pro® meter and chute scores and exit velocities.

Tables 2 and 3 show mean salivary and serum cortisol concentrations to be 0.22 and 1.9 with a range of 0.05 to 0.87 and 0.10 to 5.03 respectively. Similar to mean measures for Lactate Pro® meter and serum lactate concentrations, mean measures for

salivary and serum cortisol were different. Hellhammer et al. (2009) assessed the reliability and relationship of salivary cortisol to the unbound cortisol in blood. Reporting that salivary cortisol is dissociated from the stress response due to the complexity of various factors that modulate the HPA axis, therefore the reported measures would vary between salivary and serum cortisol concentrations. Nonetheless, Hellhammer et al. (2009) concluded that salivary cortisol was the strongest and most reliable measure for cortisol, when compared to other bodily excretions like milk, urine, and feces with a moderate highly significant correlation between the two measures ($r = 0.52$ $p < 0.0001$).

Mean values for non-esterified fatty acids are reported in Tables 2 and 3. Average NEFA concentration for the animals in this study was 0.23 mEq with a range of BDL (0.01 mEq) to 0.99 mEq. Values for steers were singificantly higher than NEFA values for heifers.

Little information has been published on using oxygen saturation, glucose, and non-esterified fatty acids (NEFA) to quantify stress response in beef cattle. We elected to measure oxygen saturation to capture the increased cardiovascular rate due to the activation of HPA and autonomic nervous system. Likewise, glucose was measured because of the amplified rate of glycogenolysis caused by the stress hormone cascade and therefore, increased systemic glucose should be present in more excitable animals.

Figure 1. Frequency plot of chute scores for both sexes assigned by a single observer

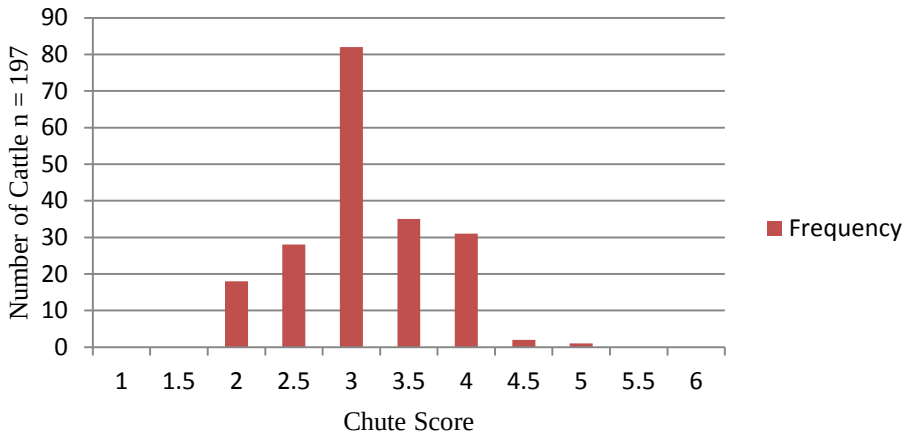

Figure 1 shows a frequency plot of chute scores for all animals as assigned by a single observer. Majority of the chute scores were 3 and 3.5 even though exit velocities and physiological responses suggest greater variation in cattle sampled. Because of the use of the hydraulic squeeze chute, descriptors of the chute scores and subjective observation, there is a question as to the ability of chute score to identify differences that are not extreme. For this research, the feedlot operator was asked to select two pens of "calm" animals and two pens of "excitable" animals as determined by previous handling experience of animals (i.e. entry into feedlot) to achieve extremes in the measurements. As Figure 1 shows, there were no extreme animals and there was a tendency for chute scores to be toward the middle with the mean chute score of 3.1 with a SD of 0.60, even when extremes were anticipated. This tendency to use the center of the scale reduces the variation in the data complicating interpretation and analysis. The chute scores were therefore not included in the statistical analysis with the objective measures. This data

suggests the chute scores may be more effective at identifying animals that are “violent” but not those that are stressed but not responding overtly.

Furthermore, the use of the chute scoring system developed in the early 90’s has been effective at removing more volatile animals but may not be precise enough to identify stressed animals that may subsequently influence feedlot performance (Grandin, 1993; Ferguson et al., 2006). Other researchers have also questioned chute scores as a temperament measure because of the subjectivity and repeatability between observers (Baszczak et al., 2006; Curley et al., 2006; Benhajali et al., 2010; Boles et al., 2015). Our data confirms that within a score, the description is too broad causing animals classed under the same score to have different behavioral (exit velocity) and physiological responses. Using chute scores only allows observers to separate extreme animals while temperament differences still exist. This will not allow for further progress through selection. With the use of chute scores and temperament scores being implemented by breed associations to calculate expected progeny differences (EPD) there may be mixed results (American Angus Association, 2010; Hyde, 2010; Federation, 2011). As our chute scores show, animals that were classified by the feedlot operator as excitable were not separated by chute scores. This means that if producers were to select heavily for docility or temperament using the chute scoring system, there would be animals that are truly excitable classified as moderate.

Relationship Between Physiological and Temperament Measures and Sex

Animals were placed into three different groups based on exit velocity (fast, medium, and slow). The means of individual exit velocity (Table 4) were significantly different between each classification ($P \leq 0.0001$), indicating that the classification was succesful in grouping animals that left the chute at different speeds and would suggest temperament classifications were sucessful. Additionally, as exit velocitity increased the physiological measure of plasma lactate as measured by the Lactate Pro® meter, also increased (Table 4), indicating it could also group animals in classes similar to exit velocity. Other phsyiologic measures of body temperature, systemic glucose concentration, serum lactate concentrations, and serum cortisol concentrations were significantly different ($P \leq 0.01$) between fast exit velocities and the other two classes. This indicated that in the animals classified as fast there was a significant increase in the physiolgical response corresponding to faster exit velocities. Lastly, salivary cortisol means were significantly higher ($P \leq 0.0001$) in the fast classification than in either the medium or slow classes, also showing an increased stress response in the animals classed as fast.

Table 4. Classification of animals by exit velocity for body weight (WT), chute scores (CS) heart rate (HR), oxygen saturation (O2), rectal temperature (TEMP), blood lactate as measured by the handheld meter (PLAC), exit velocity (EV), serum glucose (GLUC), non-esterified fatty acids (NEFA), serum lactate (SLAC), and cortisol salivary (SCORT) or serum (BCORT). Exit velocities were separated by thirds with fastest exit velocities being classified as fast, slowest exit velocities as slow and the middle one-third classed as medium.

	Exit velocity class				
Item	FAST	MEDIUM	SLOW	SEM	*P*-value
n = 197					
WT (kg)	452.8^{a}	402.9^{b}	374.7^{c}	10.8	****
CS	3.4^{a}	3.1^{b}	2.8^{c}	0.07	****
HR (beats/min)	65.8	63.2	59.6	2.7	n.s.
O_2 (%SpO_2)	79.8^{a}	74.0^{b}	78.6^{ab}	1.9	*
TEMP (^{o}C)	40.15^{a}	39.78^{b}	39.64^{b}	0.06	****
EV (m/s)	4.10^{a}	2.56^{b}	1.06^{c}	0.07	****
GLUC (mg/dL)	129.68^{a}	101.32^{b}	94.63^{b}	4.3	****
NEFA[2] (mEq)	0.23	0.24	0.24	0.02	n.s.
PLAC[3] (mM)	6.4^{a}	3.2^{b}	2.4^{c}	0.3	****
SLAC (mM)	9.28^{a}	4.76^{b}	3.86^{b}	0.48	****
SCORT (µg/dL)	0.27^{a}	0.21^{b}	0.17^{b}	0.02	****
BCORT (µg/dL)	2.25^{a}	1.74^{b}	1.69^{b}	0.14	**

Significance = *$P < 0.05$ **$P \leq 0.01$, ***$P \leq 0.001$, **** $P \leq 0.0001$

^{a,b,c} Means within a row that have a different superscript letter differ ($P < 0.05$)

[1]Chute Scores – 1 = Docile, 2 = Restless, 3 = Nervous, 4 = Flighty (Wild) 5= Aggressive, 6 = Very Aggressive.

[2] NEFA –Non-esterified fatty acid.

[3]Lactate Pro® Meter

Similar classification systems have been reported in Burdick et al. (2011a) where temperament was classed by exit velocites as, calm, intermediate, and temperamental. Brahman calves, classed as calm or intermediate were more cooperative and less excitable in subsequent handling events. Additionally, Boles et al. (2015) used exit velocities to class Angus X Simmental steers as fast, medium, and slow, reporting similar results to Burdick et al. (2011a). Exit velocities decreased with subseqent handling. Animals within this experiment had been exposed at least once to the handling facility,

suggesting that exit velocities and classification of animals respresent the temperament of the animal, not a response to a novel situation. In summary, using exit velocities to classifying animals showed that means of physiologic measures were higher in animals classified as fast than animals classed as medium or slow, eliciting that excitable temperaments have a greater stress response to hanlding. Furthermore, plasma lactate as measured by Lactate Pro® meter, can sort animals into comparable classifications as exit velocity making it a candidate for an objective measure of temperament similar to exit velocity.

Table 5. Least square means for body weight (WT), chute scores (CS), heart rate (HR), oxygen saturation (O2), rectal temperature (TEMP), blood lactate as measured by the handheld meter (PLAC), exit velocity (EV), serum glucose (GLUC), non-esterified fatty acids (NEFA), serum lactate (SLAC), and cortisol salivary (SCORT) or serum (BCORT) classed by sex.

Item	STEERS	SEM	HEIFERS	SEM	*P*-Value
n	87		109		
WT (kg)	426.85	9.04	425.33	7.84	n.s.
CS[1]	2.94	2.9	3.24	3.2	***
EV (m/s)	2.24	0.14	2.80	0.12	**
HR(beats/min)	63.2	2.1	62.2	1.9	n.s.
O_2 (SpO_2%)	77.35	1.5	77.26	1.3	n.s.
TEMP (ºC)	39.78	0.05	39.93	0.05	*
GLUC (mg/dL)	104.72	3.74	112.04	3.30	n.s.
NEFA[2] (mEq)	0.26	0.01	0.20	0.01	***
PLAC[3] (mM)	3.45	0.31	4.35	0.26	*
SLAC (mM)	5.43	0.44	6.05	0.39	n.s.
SCORT (µg/dL)	0.18	0.02	0.26	0.01	***
BCORT (µg/dL)	1.64	0.11	2.13	0.09	***

Significance = $*P < 0.05$ $**P \leq 0.01$, $***P \leq 0.001$, **** $P \leq 0.0001$
Values are Least Square Means. Significantly different $P < 0.05$.
[1]Chute Scores – 1 = Docile, 2 = Restless, 3 = Nervous, 4 = Flighty(Wild) 5= Aggressive, 6 = Very Aggressive
[3]NEFA –Non-esterified fatty acid.
[4]Lactate Pro® Meter

Sex had a significant affect on many of the physiological and temperament measures (Table 5).Weight was not significantly different between steers and heifers which indicated that the two sexes were at equal stages in the feeding period. However, chute score, exit velocity, body temperature, NEFA, plasma lactate and cortisol measures were all significantly different ($P \leq 0.05$). The significant difference in chute score ($P \leq 0.001$), and exit velocity ($P \leq 0.01$) between steers and heifers indicate heifers were more excitable than steers. Data for steers was similar to exit velocity data reported by Behrends et al. (2009); Cooke (2014), and Boles et al. (2015). Comparable results for sex differences in exit velocities have been reported in Hoppe et al. (2010), where chute scores and exit velocities were evaluated in Angus, Charolais, Hereford, Limousin, and German Simmental calves, reporting that heifers had higher chute scores and flight speeds than steers. Similar temperament differences have been reported in heifers and steers. Voisinet et al. (1997b) reported heifers to have higher chute scores with more excitable temperaments then steers. Additionally, Gruber (2010) reported *Bos taurus* heifers to be more excitable during pen behavior scoring and more reactive to being confined in a chute than steers. In contrast, sex did not have an effect on exit velocities in Burdick et al. (2009); (2011a), and Francisco et al. (2012). Discrepancy in data reported here compared to Burdick et al. (2009); Burdick et al. (2011b); Francisco et al. (2012) could be due to the fact the animals were measured in the middle of the feeding phase which means the handling facility was not a novel experience. Researchers have reported that cattle become less agitated and there is a decrease in excitable temperaments as the animals become accustomed to the handling facility (Hall and Berg, 2011; Lyles and

Calvo-Lorenzo, 2014). Moreover, cattle management and proper handling early and throughout the lifespan of cattle is important for a reduced stress response to handling (Grandin, 1993; Petherick et al., 2009a; Hall and Berg, 2011). Measuring crossbred feedlot cattle with no previous knowledge of their handling experience contributes to the differences reported here.

Furthermore, rectal temperatures were significantly higher in heifers ($P < 0.05$) compared to steers. Burdick et al. (2011b) evaluated rectal temperatures of bulls classified as calm, intermediate, and excitable, prior to and during a lipopolysaccharide (LPS) challenge. The lowest average baseline rectal temperature was reported in the calm bulls, slightly above 38.5 °C (SE ± 0.20) and the highest average baseline rectal temperature was in the excitable bulls with rectal temperature near 39.0 °C (SE ± 0.15). Comparing data to Burdick et al. (2011b) our average rectal temperature for steers and heifers was higher than bulls classified as excitable. After LPS challenge, Burdick et al. (2011b), reported an increase in all rectal temperatures with excitable bulls having the lowest increase in rectal temperatures compared to calm bulls. Interestingly, when epeniphrine was measured prior too and after LPS administration, excitable bulls had the highest baseline average concentration of epeniphrine and following the LPS challenge epeniphrine concentrations decreased in exctiable bulls but peaked 1 hr after LPS administration in calm bulls. Despite the decrease in epinephrine concentrations after LPS challenge, excitable bulls maintained the highest concentration of epienphrine compared to calm bulls. This peak in epinephrine in calms bulls coincided with the rise in rectal temperature suggesting that rectal temperature is a strong indicator of a stress

response. Evaluating rectal temperatures of feedlot cattle can aid in the classification of temperament. Our data along with Burdick et al. (2011b), supports that rectal temperature rises in response to handling. Elevated rectal temperatures reported in this study could be due to handling but also reflects temperament differences.

Lastly, salivary and serum cortisol concentrations were significantly different ($P \leq 0.001$) between steers and heifers, with heifers, having higher circulating salivary and serum cortisol levels than steers. Cooke (2014) reported mean circulating plasma concentrations of cortisol in *Bos taurus* heifers with adequate temperaments to be 3.21 µg/dL and excitable temperaments to be 4.18 µg/dL. Comparing our data to that of Cooke (2014) suggests that serum cortisol of heifers was well below heifers catagorized by Cooke (2014) as having adequate temperament. No cortisol means for *Bos taurus* steers were reported by Cooke (2014), however cortisol means for *Bos indicus* steers were reported to be 1.67 µg/dL for adequate temperament and 1.96 µg/dL for exctiable temperaments. Mean cortisol concentrations reported here are similar to mean cortisol concentrations reported by Cooke (2014) for *Bos indicus* steers having adequate temperament. Furthermore, means for cortisol concentrations reported by Cooke (2014) were much lower for *Bos indicus* steers than *Bos taurus* heifers. This is similar to data reported here with steers having lower cortisol response to handling than heifers. However, part of the difference seen in Cooke (2014) could be attributed to the differences in response to handling between *Bos taurus* and *Bos indicus* and differing basal concentrations of serum cortisol (Burrow and Dillon, 1997; Ferguson et al., 2006; Kadel et al., 2006).

Table 6. Pearson correlation coefficients among pulse, oxygen saturation (O_2), rectal temperature (TEMP), blood lactate as measured by the handheld meter (BLAC), exit velocity (EV), serum glucose (GLUC), non-esterified fatty acids (NEFA), serum lactate (SLAC), and cortisol salivary (SCORT) or serum (BCORT) for Heifers

	PULSE	O_2	TEMP	BLAC	EV	GLUC	NEFA	SLAC	SCORT	BCORT
PULSE	1									
O_2	-0.121	1								
TEMP	-0.105	-0.152	1							
BLAC	-0.203 **	-0.168	0.398 ****	1						
EV	-0.208 *	-0.056	0.443 ****	0.529 ****	1					
GLUC	-0.093	-0.291 **	0.419 ****	0.644 ****	0.537 ****	1				
NEFA	-0.135	0.193	0.102	-0.019	-0.068	-0.100	1			
SLAC	-0.162	-0.164	0.387 ****	0.828 ****	0.534 ****	0.720 ****	-0.072	1		
SCORT	-0.090	-0.153	0.568 ****	0.375 ****	0.362 ****	0.382 ****	0.048	0.341 ***	1	
BCORT	-0.052	0.028	0.417 ****	0.330 ***	0.218 *	0.272 **	0.108	0.468 ****	0.520 ****	1

Significance = *$P < 0.05$ **$P \leq 0.01$, ***$P \leq 0.001$, **** $P \leq 0.0001$

Pearson correlations are reported for both sexes (Tables 6 and 7) because of the significant differences found between heifers and steers. Pulse and oxygen saturation, were significantly but lowly correlated in steers (r = 0.28, $P = 0.01$) but were not related in heifers (r = -0.12, $P = 0.22$). Pulse and oxygen saturation were not significantly correlated to any other measures evaluated in this study. Similarly, in both sexes, NEFA was not correlated to chute side measure or metabolites, nor hormones. Rectal temperatures were moderately correlated to blood lactate in both heifers and steers however the correlation was stronger in steers (r = 0.497, $P < 0.0001$) than in heifers (r = 0.398, $P < 0.0001$). Additionally, rectal temperatures were correlated to metabolites and exit velocity with moderate highly significant correlation, to glucose, serum lactate,

salivary cortisol, and serum lactate. Interestingly, heifers had the highest correlation between rectal temperature and salivary cortisol concentrations (r = 0.567, $P < 0.0001$). Gruber (2010) also found a positive correlation between rectal temperature and serum lactate concentrations however it was a much lower correlation (r = 0.14). Additionally they reported a positive correlation between rectal temperature and serum cortisol (r = 0.33).

Table 7. Pearson correlation coefficients among pulse, oxygen saturation (O_2), rectal temperature (TEMP), blood lactate as measured by the handheld meter (BLAC), exit velocity (EV), serum glucose (GLUC), non-esterified fatty acids (NEFA), serum lactate (SLAC), and cortisol salivary (SCORT) or serum (BCORT) for Steers

	PULSE	O_2	TEMP	BLAC	EV	GLUC	NEFA	SLAC	SCORT	BCORT
PULSE	1									
O_2	0.281 **	1								
TEMP	0.024	-0.197	1							
BLAC	-0.244 *	-0.262 *	0.498 ****	1						
EV	-0.133	-0.053	0.447 ****	0.631 ****	1					
GLUC	-0.128	-0.204	0.540 ****	0.517 ****	0.322 ***	1				
NEFA	-0.198	-0.047	-0.141	-0.089	-0.008	-0.123	1			
SLAC	-0.024	0.135	0.477 ****	0.781 ****	0.591 ****	0.483 ****	-0.100	1		
SCORT	0.088	-0.269 *	0.445 ****	0.127	0.162	0.214	-0.025	0.105	1	
BCORT	0.107	-0.122	0.445 ****	0.344 **	0.159	0.372 ***	0.115	0.393 ***	0.524 ****	1

Significance = *$P < 0.05$ **$P \leq 0.01$, ***$P \leq 0.001$, **** $P \leq 0.0001$

Plasma lactate was also significantly correlated to metabolite and hormone measures. Importantly, plasma lactate as measured by the Lactate Pro® meter was highly correlated to serum lactate in both sexes. Indicating that the Lactate Pro® meter reflected the systemic concentration of lactate. This agrees with the validation study of Burfeind

and Heuwieser (2012) where they found a strong correlation (r = 0.98) between measures from the blood lactate meter and serum lactate measured in a laboratory.

The data presented here shows that as exit velocity increased both lactate measures increased. These results agree with Coombes et al. (2014) who showed that as flight speed increased plasma and muscle lactate increased. Additionally, Gruber (2010) found that animals that adversely reacted to handling and chute restraint had higher plasma lactate at slaughter. Additionally, lactate was moderate to highly correlated (r = 0.64) to systemic glucose concentrations. These findings combined with the finding from Gruber (2010) and Coombes et al. (2014) demonstrated that excitable animals mobilized glucose through glycogenolysis due to increased energy demand in response to stress in the muscle, resulting in elevated lactate and glucose being transported into the blood.

Furthermore, salivary cortisol was correlated to serum cortisol in both sexes with almost equal r-values (r = 0.52) and equal significance. Evaluating the relationship of cortisol to exit velocity and metabolites, exit velocity was not correlated to serum or salivary cortisol measures in steers but in heifers there was a positive correlation to both cortisol measures. Curley et al. (2006) evaluated the relationship of temperament measures and cortisol at three different observation times using yearling Brahman bulls. Curley et al. (2006) reported a positive correlation on day zero (r = 0.26, $P = 0.04$) and on day 120 (r = 0.44, $P < 0.01$) between exit velocity and serum cortisol. Although in this study, there was no significant correlation between cortisol measures and exit velocity in steers, correlations reported here in heifers were similar to data reported by Curley et al. (2006).

Additionally, in heifers, both cortisol measures were moderately correlated to lactate. Interestingly, in steers, only serum cortisol not salivary cortisol was moderately correlated to lactate. Both cortisol measures were moderately correlated to glucose in heifers, while in steers, serum cortisol not salivary was moderately correlated with glucose. In pigs, Choe and Kim (2014) evaluated the relationship of blood glucose and blood lactate to serum cortisol levels at exsanguination. They reported moderate correlation coefficients between blood lactate and serum cortisol ($r = 0.55$, $P < 0.0001$) and blood glucose to serum cortisol ($r = 0.43$ $P < 0.0001$). Our data in conjunction with Choe and Kim (2014) and Curley et al. (2006) supports the indication that as temperament increases (excitable) the HPA axis is activated, cortisol increases and therefore glycogen is converted quickly in the muscle and the lactate produced is shuttled into the blood (Silbernagl and Despopoulos, 2009; Coombes et al., 2014). Simultaneously, the increased cortisol secretion causes an increased rate of glycogenolysis and therefore a detectible increase in systemic glucose concentrations.

The major objective of this study was to identify if a simple physiological chute side measure or combination of measures correlate to an animal's temperament defined by exit velocity and chute score. Chute scores were not included in any of the comparison statistics because there was central tendency for use of the scale resulting in very little variation, therefore physiological measures were compared to exit velocity. The strongest correlations to exit velocity were rectal temperature, blood lactate meter measured by Lactate Pro®, serum glucose, and serum lactate. In order to better the United States beef industry, identifying animals with excitable temperaments entering the feedlots would be

helpful. Including temperament as a sort criterion on cattle entering the feedlot would improve our ability to efficiently finish the cattle. For that reason, implementing easy to use, chute side measures would allow producers to objectively categorize their animals for temperament and improvement in ADG and meat quality (tenderness, color) would be achieved. However, in order to implement these objective measures, models need to be constructed and validated to see if one or combination of measures can predict exit velocity. If these measures cannot be used as a predictor, how well can they be used as secondary support with exit velocity for determining an animal's temperament?

Modeling for Exit Velocity Using Chute Side Measures

Due to the differences found between chute side measures and exit velocity in steers and heifers the models were classed by sex. Data was randomized and eighty percent of the data was used for analysis of the model. Normality of exit velocity was plotted for sexes using histogram and QQplot in R (R-studio version 0.98.1028); additionally Shapiro test was used to analyze normality. No transformation was used on exit velocity (Shapiro and Wilk, 1965) because exit velocity was normally distributed. Generalized additive models were used to evaluate non-linear relationships, and the models were plotted using gamma plots to visualize response. For both sexes, the chute side measures appeared to have a linear relationship to exit velocity. Salivary cortisol was also included in the analysis because the collection of saliva requires little training and no specialty equipment for collection. In both sexes salivary cortisol appeared to have a near quadratic response to exit velocity with the asymptote near the mean because of this and

the secretion and known dilution of cortisol in saliva this model was not analyzed (Hellhammer et al., 2009).

Linear models were constructed comparing the chute side measures of heart rate, oxygen saturation, body temperature, and plasma lactate as measured by the Lactate Pro® meter to replace exit velocity. An Akaike information criterion was used to evaluate the strength of candidacy of these chute side measures to replace exit velocity. The AIC data is presented in Table 7. In steers, the combination of plasma lactate meter measures and rectal temperature had the strongest AIC weight and therefore represents the best fit model. However, in heifers, plasma lactate meter and rectal temperature do not have the same strength as in steers. As shown in Table 7 the AIC for just plasma lactate meter could also be a candidate and removed strength from the combination of plasma lactate meter and rectal temperature. However, in heifers the AIC assumption of delta AIC having a difference of at least two between top and second candidate models was violated. Nonetheless, due to the strength of plasma lactate meter candidacy and its inclusion in the top model the linear model of plasma lactate meter and rectal temperature is still accepted as the top candidate model.

Table 8. AIC values for chute side measures: blood lactate meter (BLM), rectal temperature (TEMP), pulse, oxygen saturation (O_2) to exit velocity (EXIT) for steers and heifers

STEERS	AICc	Δ AICc	AICcWt	Cum.WT	LL
BLM + TEMP	187.30	0.00	0.84	0.84	-89.30
BLM	190.64	3.34	0.16	1.00	-92.11
TEMP	200.26	12.95	0.00	1.00	-96.93
PULSE	205.64	18.33	0.00	1.00	-99.61
O_2	207.10	19.79	0.00	1.00	-100.34
Null EXIT	212.93	25.63	0.00	1.00	-104.37
HEIFERS					
BLM + TEMP	243.20	0.00	0.65	0.65	-117.33
BLM	244.43	1.23	0.35	1.00	-119.06
PULSE	259.18	15.98	0.00	1.00	-126.43
TEMP	260.37	17.17	0.00	1.00	-127.02
O_2	260.92	17.72	0.00	1.00	-127.30
Null EXIT	272.70	29.50	0.00	1.00	-134.27

In summary, the AIC concluded the top candidate model to be plasma lactate meter measures in combination with rectal temperature to predict exit velocities. The strength of the candidacy of the plasma lactate meter along with the ability of the meter to sort animals into three categories similar to exit velocity suggests the plasma lactate meter could be used as an objective measure of temperament in cattle. Further studies are needed to determine plasma lactate levels that correspond to exit velocity classifications.

Conclusion

Temperament has a direct impact on efficiency and perception of beef cattle production in the United States. Previous efforts have laid a foundation of measures in which we can identify excitable animals. However, there is still a need to identify temperaments that do not subjectively fall into an extremely excitable category but still have an impact on handling, feedlot performance, and carcass characteristics. This study aimed to identify the relationship of chute side objective measures to physiological

markers as well as the correlations of the measures to exit velocity. Additionally, this study aimed to identify possible objective chute side measures to replace or augment exit velocity as a predictor of an animal temperament. Plasma lactate as measured by the Lactate Pro® meter was significantly related to exit velocity and when exit velocity was used as a classification, plasma lactate concentration was significantly different between the three classes indicating the potential ability to use plasma lactate as a way to sort animals into temperament classes similar to exit velocity. Furthermore, Akaike information criterion indicated plasma lactate in conjunction with body temperature was the strongest candidate for predicting exit velocity.

Steers and heifers react differently to handling stress as indicated by the significant differences in chute side measures, physiological measures, and exit velocity. The increased excitable behavior expressed in heifers cannot be easily explained by the data presented within this study. Variation in animal's physiological responses to stressors is impacted by differences in homeostatic "set points" which makes it difficult to model chute side measures capturing a physiological response to exit velocity. Nonetheless, the use of objective chute side measures can greatly improve our selection and removal of temperamental animals. In addition the use of chute side measures in combination to exit velocity will remove subjectivity from selection criteria.

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