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## RELATIONSHIPS BETWEEN BEHAVIORAL MEASURES AND PRODUCTIVITY IN GROWING BEEF CATTLE

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RELATIONSHIPS BETWEEN BEHAVIORAL MEASURES AND PRODUCTIVITY  
IN GROWING BEEF CATTLE

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THESIS

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A thesis submitted in partial fulfillment of the requirements for the  
degree of Master of Science in the  
College of Agriculture, Food and Environment  
at the University of Kentucky

By

Kelsey Amelia Bruno

Lexington, Kentucky

Director: Dr. Eric Vanzant, Associate Professor of Animal Science

Lexington, Kentucky

2015

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## ABSTRACT OF THESIS

### RELATIONSHIPS BETWEEN BEHAVIORAL MEASURES AND PRODUCTIVITY IN GROWING BEEF CATTLE

The relationships between behavioral measures, growth performance, and immunocompetence in receiving beef steers were explored in three experiments. Specifically, exit velocity (EV) and objective chute score (OCS) were examined as temperament measures. In experiment 1, no main effects or interactions with degradable intake protein (DIP) or interactions between temperament measures were observed ( $P \geq 0.11$ ); however, high OCS steers had greater intake and gain:feed ( $P < 0.10$ ) and slow EV steers had higher intake and gain ( $P < 0.10$ ) than their counterparts. In experiment 2, during week two of social observations, dominance hierarchy rankings were dependent on OCS and EV ( $P < 0.05$ ); slow EV steers also had increased antibody responses and gain ( $P < 0.10$ ) and high OCS steers had increased gain ( $P < 0.10$ ). In experiment 3, subjective chute scores and OCS were positively correlated ( $P < 0.01$ ) and both EV and OCS treatments changed over time ( $P < 0.10$ ); intake, vaccine titer response, and gain:feed responses to monensin were dependent on OCS treatment ( $P < 0.10$ ). The studies suggest that temperament, measured by EV and OCS, affects growth performance and health related measures and is related to social dominance behavior in receiving beef steers.

**KEYWORDS:** temperament, social behavior, monensin, growth performance, Beef cattle

Kelsey Bruno  
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March 30, 2015  
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Date

RELATIONSHIPS BETWEEN BEHAVIORAL MEASURES AND PRODUCTIVITY  
IN GROWING BEEF CATTLE

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## DEDICATION

I dedicate this thesis to my beloved grandparents, Anthony and Amelia Bruno

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I would like to extend my most sincere gratitude to those involved in the successful completion of my graduate program and thesis. First, I would like to thank Dr. Eric Vanzant, my major professor, for his never-ending support and guidance during my graduate studies and research. I greatly appreciate his time, efforts, and advice that has helped me to succeed as well as shaping my professional character. This would not be possible without him. To my committee members, Dr. Jeff Lehmkuhler and Dr. Kyle McLeod, thank you for your added advice, guidance, and invaluable assistance in the completion of my thesis.

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# CHAPTER 1

## INTRODUCTION

Although relationships between animal behavior and productivity have long been assumed by producers, until fairly recently, little scientific proof has related behavioral measures to physiological responses. Research interest in these relationships has increased over the past few decades. Researchers have identified various ways to measure temperament and social behavior in cattle, as well as how to further predict and increase productivity in the beef industry.

Cattle temperament has previously been defined as the fearfulness and reactivity to humans and novel or threatening environments (Fordyce et al., 1988; Grandin, 1993; Murphey et al., 1994; Burrow, 1997). Temperament can be measured through numerous restrained and non-restrained testing methods that, theoretically, measure different behavioral responses of cattle to stressors (Burrow, 1997). Stressors can be any internal or external stimuli or threats that disrupt homeostasis and stimulate a stress response in cattle; stressors can include many common management practices, such as weaning, regrouping, and transportation. Animals with a more “excitable temperament” can have more active stress responses to environmental stressors, whereas calmer animals can have a less severe response. The response is quantified as “temperament” and used to determine the level of fear, excitement, or other responses displayed by a particular animal.

Commonly, exit velocity (Burrow, 1997) and chute score (Grandin, 1993) are used to measure temperament. While exit velocity is an objective measurement of an animal’s response to human handling, chute score is a subjective score representing

animal movement when confined in a weighing chute during handling. Recently, some researchers have referred to temperament as an index, combining both scores for an average temperament index (Hulbert et al., 2011; Francisco et al., 2012). There are two main concerns with this approach. Firstly, because each measure uses different units, averaging the two dissimilar numbers weights the average in some arbitrary way, and both measurements can utilize different scales (e.g., one could use a 1-4 scoring scale as compared to the common 1-5 scale for chute score). Secondly, averaging the measures assumes they have similar relationships to the response variables, which has not been proven as a reliable assumption. Further investigation should be done to quantify relationships between chute scores and exit velocity. Due to the subjective nature of the chute score measurement, the results can also vary across scorers and over time; therefore, an objective chute score could prove to be a superior measuring methodology.

Additionally, while some studies use initial measures of exit velocity or chute score to assign temperament treatments, others use an average of scores collected over time; it is unclear if these different approaches affect how temperament relates to growth performance. Moreover, previous research has found that animals habituate to handling over time (Petherick et al., 2009; Cafe et al., 2011), which can affect the animal's response during temperament testing. The first handling experience may indicate the animal's unique response to human handling, more accurately conveying their "true temperament". Therefore, patterns of change in exit velocity and chute score over time need further clarification, in addition to whether or not the initial measure may serve as a superior representation of the true response to human handling.

In addition to cattle temperament, dominance and social behaviors also have the possibility of impacting productivity of beef cattle. Dominance can be viewed as an attribute of a relationship between two individuals, whenever an unevenness in the outcome of agonistic interactions is measured (Drews, 1993). Dominance behavior is a major component of social behavior of cattle, as groups of cattle will establish social hierarchies, which can reduce the level of aggression in the herd (Wiener, 2015). These interactions become more common as resources, such as space or food, become limited. Early work with dominance ranking and production traits found that intake was affected by ranking, but no differences in gain were detected (McPhee et al., 1964; Wagnon, 1965), although one study reported a correlation between social ranking and weight gain (Blockey et al., 1974). Confirmation as to whether an animal's role in the dominance hierarchy can affect production traits can be important for housing management strategies.

Finally, while temperament has previously been related to production traits, the specifics of how temperament relates to growth remain unclear. Previous researchers have suggested that differences in gain stem from difference in efficiency (Petherick et al., 2002) while others have suggested that intake is the causative factor (Cafe et al., 2011). It seems more likely that intake is ultimately affecting growth and that intake is most likely regulated by an inherent physiological mechanism. Particularly, the hepatic oxidation theory of intake regulation in ruminants provides an interesting understanding that might link temperament and intake. In addition, differences in growth rate between animals of different temperaments suggests the possibility that nutrient requirements differ.

Thus, the following studies will 1) introduce a novel measurement technique for beef cattle temperament, 2) assess the effects of temperament and diet on growth performance and health related measures, and 3) assess how temperament can affect social behavior and dominance relationships. The results will suggest new insights into the relationships between temperament and performance in growing beef steers to improve management strategies and animal health.

## **CHAPTER 2**

### **LITERATURE REVIEW**

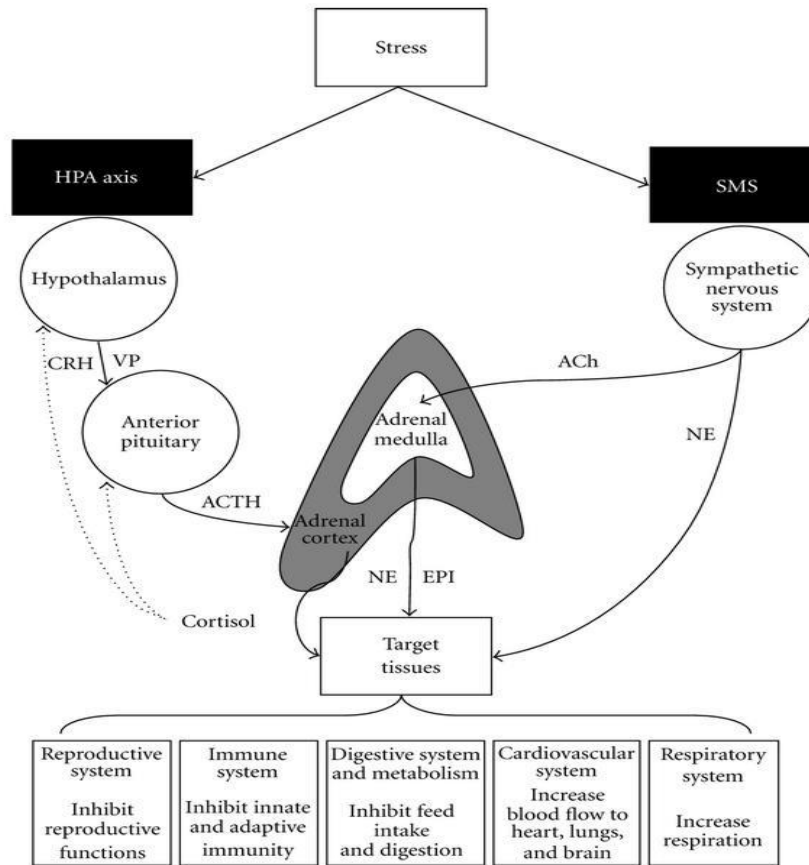
#### **Introduction**

Animal temperament is important in the livestock industry because it can impact productivity and production costs. In the past two decades, there has been a considerable increase in research interest in the relationships between productivity, temperament, and stress response. Numerous researchers have studied the animal response to stress and how it impacts economics, health, and performance. Temperament can be considered as a factor that reflects an individual animal's response to stress.

#### **Stress**

##### *Stress Response*

According to Burdick et al. (2011a), a stressor is any internal or external stimulus or threat that disrupts homeostasis. Stressors encountered by livestock can result in economic costs to producers and consumers, as well as increased risk of personal harm to handlers and animals. Common management practices such as weaning, transportation, and regrouping are typical stressors encountered by livestock. Animals with higher levels of stress hormones have been found to have decreased growth rates, as well as weaker immune responses to pathogens (Burdick et al., 2011a). In order to modify the influence of stress on cattle production, the mechanisms of biological stress responses must be understood. Figure 1 represents biological responses to stressful stimuli (Burdick et al., 2011a).



**Figure 2. 1. The biological pathways of the Hypothalamic-Pituitary-Adrenal axis and Sympathomedullary System in response to stress (Burdick et al., 2011a)**

The body is affected by a stressor with the activation of the hypothalamic-pituitary-adrenal axis (HPA axis) system and the sympathetic nervous system (Burdick et al., 2011a). In response to a stimulus, the brain activates neurons in the paraventricular nucleus (PVN) of the hypothalamus, which in turn stimulates synthesis and secretion of corticotrophin-releasing hormone (CRH) and vasopressin (VP) (Plotsky, 1991; Burdick et al., 2011a). These neurohormones are exposed to the anterior pituitary gland's corticotrophs that will activate the adrenal axis. The anterior pituitary corticotrophs synthesize and secrete ACTH into the body's circulation; ACTH stimulates production of glucocorticoids by the adrenal cortex (Webster Marketon, 2008).



Without a stress stimulus, CRH and VP are released at a low frequency (~2-3 secretory episodes per hour) with higher pulse amplitudes in the morning. Management techniques, such as lighting and feeding schedules, can alter the release of CRH and ACTH, as well as stress to an animal (Burdick et al., 2011a).

The primary glucocorticoid for cattle is cortisol (Burdick et al., 2011a). Glucocorticoids are distributed in the circulatory system via carrier proteins that prevent degradation and allow for quick availability. CRH stimulates release of corticotropin from the anterior pituitary and prompts the synthesis and secretion of cortisol by the adrenal cortex (Rhen, 2005). Albumin is the major carrier protein for cortisol, but cortisol can also be transported by binding to cortisol-binding globulin (transcortin) (Burdick, 2011). Approximately 90% of the secreted cortisol is bound to corticosteroid-binding globulins (Rhen, 2005). Roughly 1-10% of cortisol circulates in the system as a free steroid; the free steroid is biologically active and commonly converted to cortisone (Rhen, 2005). Tissues can manage available cortisol by increasing or decreasing available cortisol in cells using  $11\beta$  hydroxysteroid dehydrogenase (HSD11 $\beta$ ) enzymes, which convert cortisone to cortisol and vice versa (Rhen, 2005; Burdick et al., 2011a).

Glucocorticoids bind to either mineralocorticoid receptors (MR) or glucocorticoid receptors (GR). The MR are found in some limbic brain areas, such as the hippocampus, as well as the heart, kidney, and colon (Carrasco, 2003); the GR are distributed throughout the body (Carrasco, 2003; Smoak, 2008). After the binding of glucocorticoids to cytosolic GR, the receptor undergoes characteristic changes, causing exposure of the nuclear translocation signal. Within the nucleus, the GR will bind to glucocorticoid response elements (GREs), which can allow for direct regulation of gene expression

(Rhen, 2005). The following effects of regulation of gene expression are tissue-specific but will include effects on prostaglandins, pro/anti-inflammatory cytokines, and cell adhesion molecules (Burdick et al., 2011a).

Glucocorticoids elicit many biological responses, including carbohydrate and protein metabolism, regulation of stress response, and influence on overall immune function (Carroll et al., 2007). Glucocorticoids can increase blood glucose concentrations by stimulating the liver to convert fat and protein to gluconeogenesis intermediate metabolites, such as glycerol and amino acids, that will be converted to glucose for energy (Carroll et al., 2007). Glucocorticoids can also suppress the inflammatory and immune systems (Carroll et al., 2007). They also play an important role in gluconeogenesis, by increasing blood glucose concentrations by stimulating the liver to convert fat and protein to intermediate metabolites, such as pyruvate or glycerol, that will be ultimately converted to glucose (Carroll et al., 2007). They can increase synthesis and secretion of catecholamines, which control physiological processes such as heart rate, pupil dilation, vasoconstriction in the skin and gut, vasodilation in leg muscles, and increased glucose production by the liver. A known secondary response is the inhibition of immune and inflammation responses (Carroll et al., 2007). In livestock, increases in glucocorticoids are linked to reduced rates of reproduction, suboptimal growth, and suppression of immune function making the animal more susceptible to pathogens (Carroll et al., 2007).

The sympathetic nervous system is also activated by stressors, often before the stimulation of the HPA axis. When stimulated, neurons of the brain and peripheral organs secrete norepinephrine into circulation, which can increase blood pressure, heart rate, and

respiration rate. Moreover, brain nerve impulses communicate messages to the limbic system to release norepinephrine, serotonin, and acetylcholine, activating the paraventricular nucleus (PVN) (Black, 2002). Preganglionic sympathetic fibers of the adrenal medulla stimulate production and secretion of epinephrine and norepinephrine by acetylcholine. In cattle, the majority of these secretions are epinephrine (Burdick et al., 2011a).

The sympathetic nervous system controls the actions of many biological systems, including the gastrointestinal, cardiovascular, renal, and respiratory systems. After the activation of the stress response, increased levels of epinephrine in cattle can result in decreased neurovegetative activities, such as eating or sleeping (Burdick et al., 2011a).

Whether or not an animal identifies an incident as stressful depends in part on the past development and experiences of that animal. Responses may vary depending on the individual animal and the responses may make them vulnerable to some stressors or protect from others (Burdick et al., 2011a). Differences are dependent upon animal and animal history, but may be closely linked to immune function and production.

Stress responses in cattle may vary depending on duration of the stimulus. The body may be able to recover from cellular action of an acute response, but entire systems can be altered with a chronic stress response.

#### *Fear response and stress*

Animals can be stressed by either a psychological trigger (such as restraint, handling, or novelty) or a physical trigger (such as hunger, injury, or thermal extremes) (Grandin, 1997). Animals can also be stressed by social situations, known as social stress.

An individual animal's stress response is dependent upon its past experiences and a complex interaction of some genetic factors.

Fear is an important emotion throughout the animal kingdom that motivates animals to avoid predators and increase survival. The amygdala of the brain is the central neural system involved in fear behavior and conditioning fear (Grandin, 1997). Studies involving over 20 different animal species showed that electrical stimulation of the amygdala elicits fear response and in some species increases corticosterone (Grandin, 1997).

Because novelty is such a prevalent stressor, Grandin (1997) suggested that animals be gradually conditioned to handling to reduce stress. "Less severe" management procedures should be done first when cattle are first being introduced to scales and squeeze chutes (Grandin, 1997). However, the applicability of her suggestion in industry is limited by large herd numbers and labor availability.

### **Temperament**

Temperament has previously been defined as the response of cattle to humans or novel environments and particularly used within a handling setting (Burrow, 1997a; Grandin, 1997; Burdick et al., 2011a). However, classifying an animal's temperament based solely on one specific response is weak and a broader approach could be advantageous (Müller et al., 2006). Rather, the definition of temperament may be closer to the definition of personality, which can be defined as consistent individual behavior which differs from the behaviors exhibited by other individuals in comparable situations (Müller et al., 2006), and not a response to one specific stimulus, necessarily. Behavioral responses can range from docility to fear or nervousness, non-responsiveness, escape or

withdrawal, and aggressive behaviors (Burrow, 1997a). Poor temperament can have costly effects on the beef industry due to increasing production costs for management, compromised health, and danger in handling. Additionally, poor temperament can also hurt the profitability of the industry by decreasing productivity traits, such as growth, fertility, and meat quality (Burrow, 1997a).

Cattle producers desire cattle that are easy to handle during standard management procedures and transport. Producers that utilize such practices as artificial insemination, implantation, and dehorning may appreciate docile cattle that are easy to restrain and therefore have calm temperaments.

### *Methodology*

The definition of temperament in production settings is largely described by the method of measurement and which behavioral response it measures. Because there are numerous methods of temperament measurements, temperament itself can be defined in a number of ways by numerous behaviors. Therefore, temperament is operationally defined in many ways and each behavior does not necessarily relate to others.

There are many methods to measure temperament in beef cattle, and one measurement alone may not identify all the behaviors that producers aim to modify (Burrow, 1997a). Methodology is broken down into non-restrained testing where animals have ample room to move around during testing, restrained tests where animals are controlled, and ease of movement tests where animals are recorded using large scale movements over time. Some additional and specific tests are maternal temperament tests, dairy temperament tests, and dominance testing (Burrow, 1997a).

Some non-restrained tests can be labor intensive and time consuming, although that is not always the case. The flight speed test, which measures the time taken by an animal to move a set distance after exiting a weighing scale (Burrow, 1988), may be the most valuable for beef producers over the other tests because it is fast, objective, and simple to measure and implement on the farm. Many different distances have been used but the measurement is ultimately measured as time required to cover some set distance, or as velocity (meters/seconds). The test presumably measures the animal's fear response to handling, rather than fear of the actual human handler (Burrow, 1997a).

Restrained tests are generally quick, inexpensive, and easy to use and implement in farm settings. However, restrained tests may not represent the temperament of the animal well because some animals may have a "freeze" response, characterized by little to no movement, to a novel stimulus in an unrestrained environment but also react very aggressively in the chute or vice versa (Burrow, 1997a).

Non-restrained tests include a few different approaches that measure different behaviors. The approachability test (Murphy, 1980) and the flight distance test (Murphy, 1981) measure the distance an observer can approach before an animal reacts, whereas the docility test records the minimum distance an observer can approach as well as the time an animal will allow an observer to stroke them in an open field without moving (Le Neindre et al., 1995). The docility test would be inadequate because in most beef production situations the number of animals that an observer can approach would be zero. These tests require the observer to actively approach in order to stimulate and measure a fear response in the animal (Burrow, 1997a).

In contrast, the approach/avoidance test (Murphy, 1981), arena test (Fell and Shutt, 1989), and the behavioral test used by Boissy and Bouissou (1988) involve the observer taking a passive role and measures time taken for the animal to approach the observer, amount of time the animal interacts with the observer, and number of interactions within a defined test period (Burrow, 1997a). The behaviors being measured are mostly fear and exploratory responses. These testing styles may not be effective in testing beef cattle, as they are usually less interactive and handled less; these tests may be better suited for dairy cattle or other livestock species.

More open area tests include the flightiness test (Hughes and Black, 1976), goat-human contact test (Lyons, 1988), the open field test (Beilharz and Cox, 1982), and the pound test (Fordyce et al., 1982), which measure the reaction of the animal to a novel stimulus and record the amount and speed of movement in an open yard (Burrow, 1997a). The non-restraint testing methods may be useful with some livestock animals and production facilities, but are unrealistic practices for the beef industry as a whole due to time and labor commitments involved.

Restrained tests involve an animal's movement being restricted while an observer subjectively assesses the behavior (Fordyce et al., 1982; Grandin, 1993). The scoring scales may fluctuate depending on the location and animals. Behaviors that can be measured include amount of movement, vocalizations, eliminations, tail swishing, kicking, balking, audible respiration, and attempts to escape (Burrow, 1997a).

Tulloh (1961) found no relationship between a subjective temperament score and cattle movements while entering the scales, the crush, and the bail, concluding that cattle that are difficult to handle in the chute do not necessarily have a poor temperament.

Alternatively, Fordyce et al. (1982) measured temperament in restrained and non-restrained situations and reported that, generally, all tests were significantly and positively correlated. However, the repeatability estimates and correlations were highest for their flight distance (closest distance an observer could approach to the animal in an open yard) and pound test (number of times animals crossed a line in an open yard + speed of movement; 1-5 scale) compared with the restrained testing methods and indicated that these tests were more useful measures for “improving temperament”, which would lead to improved behavior while in the chute. Burrow et al., (1988) found that the flight speed test is negatively correlated with the flight distance test used by Fordyce et al. (1982), such that animals with low flight speed times would have greater flight distances and were generally reported as being difficult to handle by stockman. Burrow et al. (1988) suggested that tests of temperament in a non-restrained situation can likely identify problem animals in extensively managed beef herds.

Other researchers have found that chute score is not associated with other temperament tests and therefore not actually measuring temperament of animals (Burrow, 1997a). MacKay et al. (2013) found that flight speed and chute score were highly correlated and Cooke et al. (2009) found that chute score, exit velocity, and pen score were positively correlated to each other. Other researchers have combined temperament tests (chute score, pen score and/or exit velocity) for an average temperament score (Cooke et al., 2009; Hulbert et al., 2011; Francisco, 2012), but it is not clear if combining tests is an accurate way to measure temperament.

The ease of movement testing method measures the time it takes to move animals through a series of yards and open fields (Hinch and Lynch, 1987). This test was



originally created to assess differences in ease of handling between sexes and breeds. This test is also usually a group measurement rather than an individual measurement. The results can be hard to interpret because animals that move quickly through yards could be docile and therefore able to move efficiently or temperamental because the stress of moving makes them faster (Burrow, 1997a).

Dominance testing is not commonly used to measure temperament. Social dominance among animals has been defined as the relationship where an animal, through threat, force or pleasure, causes a subordinate animal to yield space (Beilharz et al., 1966; Kabuga et al., 1991; Burrow, 1997a). While dominance interactions have been used as a type of temperament testing, the dominance tests differ because they measure the behaviors of social interactions of animals, while a human is or is not present, rather than the before mentioned response of cattle to humans (Burrow, 1997a).

Both temperament scoring and dominance ranking are often done over a period of time rather than as a single incident; however, temperament testing has also been done as a single incident measurement (Burrow, 1997a).

In addition to subjective measurements, objective measurement techniques have been used for cattle temperament. A movement measuring device (MMD) was used to quantify movement in the chute by measuring counts of peaks of voltage changes from the device taken over a one minute time period (Stookey et al., 1994). Watts et al. (2001) found that MMD scores were correlated between observations and suggested it could represent an inherited personality trait. Exit time has been correlated with MMD peaks (Sebastian et al., 2011).

Moreover, the amount of force that an animal exerts on the head gate after being caught can represent an objective temperament score, where minimum, maximum, standard deviation, and absolute force were collected (Sebastian et al., 2011). Exit time was correlated with absolute strain force, whereas MMD was not (Sebastian et al., 2011). Sebastian et al. (2011) found that increasing subjective chute scores were correlated with higher strain forces, higher number of MMD peaks, and shorter exit times.

The relationships between exit time, MMD peaks, absolute strain forces and subjective scores found by Sebastian et al. (2011) showed that these measures were consistent across time and as reliable as subjective chute scores. Objective measures could be better candidates for assessing temperament. Objectivity of temperament testing may be compromised by an observer bias and this bias may be unavoidable (Lyons, 1989; Boivin et al., 1992).

#### *Factors affecting temperament*

Many factors influence the temperament response in cattle, including age, breed, sex, and past experience; age is generally confounded with the effects of previous experience (Burrow, 1997a). Favorable temperament can be thought of as docile and easy to handle animals, whereas unfavorable temperament would be excitable and hard to handle animals. Numerous studies have found that temperament becomes more favorable with age, handling experience, and body condition (Sato, 1981; Burrow, 1997a).

Sex is a factor that affects the temperament of beef cattle. Some studies have found that heifers are more temperamental than both steers (Beilharz et al., 1967; Voisinet et al., 1997; Cafe et al., 2011) and bulls, and that steers are more temperamental

than bulls (Shrode, 1971; Vanderwert et al., 1985). Compared to bulls, steers tend to have higher flight speeds and higher chute scores, which indicate more movement in the chute.

There is a clear effect on temperament from differing breeds as well. One study found that animals with  $\frac{1}{4}$  or  $\frac{1}{2}$  Brahman inheritance had “poorer temperaments” than British Breed crosses (Burrow, 1997a); Angus cattle also have slower flight speeds than Brahman cattle (Cafe, 2011). Grandin (1980) found that Brahman cattle had longer flight distances than British breed cattle. *Bos indicus* breeds and their crosses are more difficult to handle under extensive management than *Bos taurus* breeds (Burrow, 1997a). Additionally, there are some significant differences between breeds within the two species. Tulloh (1961) found that Hereford and Angus steers had lower chute scores than Shorthorn cattle. Using the same three breeds, Wagnon (1981) found a significant difference in dominance; stating that Angus cattle are most dominant and Hereford are least dominant.

Past experience is also an important influence on temperament when it is not confounded with age. Studies done with calves found significant differences due to increased handling in pre and post weaning calves and concluded that regular handling of post-weaning calves improved temperament scores (Burrow, 1997a). Cooke et al. (2009) also found that acclimated heifers had reduced chute scores compared to control heifers.

Temperament is a heritable trait. Numerous studies have been done with varying temperament tests, experimental settings, ages, breeds, and models; these studies have not been weighted for comparative purposes. However, the average, unweighted heritabilities of temperament related traits summarized in the non-restrained and restrained categories were 0.36 and 0.23 respectively (Burrow, 1997a; Schmutz et al.,

2001). Other studies have found heritability to be moderate (0.30-0.60) to high and therefore temperament is a selectable trait.

### *Temperament and Performance Traits*

Tulloh (1961) was one of the first to report that docile animals grew better than animals that were restless, nervous, wild, or aggressive. Cattle with a low temperament score have significantly higher daily gain averages than animals with a high temperament score (Voisinet et al., 1997; Burdick et al., 2011a). Temperament and ADG have generally been reported to have an inverse relationship; as chute scores and exit velocities have increased, ADG has typically decreased (Bates et al., 2014).

Temperamental cattle tend to have higher concentrations of non-esterified fatty acids (NEFA). Burdick Sanchez et al. (2012; 2014) found that temperamental bulls maintained greater circulating NEFA concentrations before and after a stress stimulus. This evidence supports the theory that temperamental beef cattle deposit lower amounts of fat than calm animals, indicating an influence of temperament on adiposity (Burdick Sanchez et al., 2012). They concluded that temperamental cattle utilize NEFAs for energy when glucose concentrations are low, therefore preventing the incorporation of fatty acids into triglycerides in fat cells (Burdick Sanchez et al., 2012).

Increasing flight speed in Brahman cattle was significantly related to decreased ADG, body weight, and dry matter intake (Cafe et al., 2011; Sanchez et al., 2013). Temperamental cattle, as measured by flight speed, tended to grow more slowly, produce smaller carcasses, deposit less fat, and have darker meat (Cafe et al., 2011).

Cause and effect relationships between temperament and performance characteristics are not well understood. Based on previous research, one could suggest

that animals with excitable temperaments have lower ADG and efficiency because of the greater inherent energy expenditure and stress response associated with alert or defensive behavior (Cafe et al., 2011). Additionally, temperamental cattle may have inherent metabolic and stress response differences, which may influence their response to an inflammatory stimulus, and explain physiological response differences (Burdick Sanchez et al., 2014). In contrast, other reports have associated lower ADG and efficiency with lower DMI related to feeding behavior, stating that temperamental animals spend less time eating, thus accounting for their reduced DMI and ADG more through behavioral than metabolic mechanisms (Cafe et al., 2011). MacKay et al. (2013) found that animals with high flight speeds may be more active overall and thus that performance differences may be unrelated to their fear response.

#### *Immunology and Temperament*

The degree of temperament and innate stress response in livestock can affect their immunological response to a stimulus as well. Animals with a more reactive or elevated stress response may have a lower immune response. Hulbert et al. (2011) found that temperamental cattle (measured by an average of exit velocity and pen score) may be more likely to be infected than calm cattle because of neutrophil function deficiencies. These cattle may also be more difficult to identify and treat because they do not display clinical signs of illness (Hulbert et al., 2011). These results are consistent with findings that temperamental animals have a decreased feedlot performance (Voisinet et al., 1997; King et al., 2006; Hulbert et al., 2011). Furthermore, an animal's social rank within the group can also impact stress response; submissive subordinate pigs were found to be more stressed by a 4-hour transport than dominant pigs (Grandin, 1997).

A reactive stress response can make the animal more vulnerable to common illnesses, such as bovine respiratory disease. Bovine respiratory disease (BRD) in newly received cattle is the most common and most significant health problem in the cattle industry (Duff, 2006; Snowden et al., 2006). Mortality, medical treatment, and labor are amongst the most costly effects of BRD on the beef industry; however, decreases in growth performance and carcass quality also contribute to the economic losses of BRD (Duff, 2006; Quimby et al., 2001). BRD is a complex of diseases characterized by many types of infection, each with its own causes, clinical signs, and economic implications (Snowden et al., 2006). Predisposing causes can include age, stress, environment, and immunological history (Snowden et al., 2006). Environmental causes are managed to the best ability in order to prevent BRD in the beef industry. Roeber et al. (2001) found that animals that had been treated for illness two or more times had 12% lower ADG throughout the initial implant period (67 d) of their study. If animals of unfavorable temperaments are more vulnerable to disease they can also be vulnerable to less productivity.

Temperamental and stressed calves may also have a lower response to vaccinations, which can in turn make them vulnerable to illness. More temperamental cattle have been found to have a lower response to vaccinations compared to calm calves (Oliphint, 2006). Brahman bull calves were divided into two groups (calm or temperamental) based on exit velocity and pen scores. Calves received clostridial vaccines at the beginning of the study and received booster vaccines 42 days later. Both groups initially showed antibody responses by d 6 and peaks by d 13. Following the booster, calm and temperamental bulls had peak responses on days 54 and 49, although

from d 49 to the end of the 11-week study antibody responses decreased 3-fold for the temperamental calves, whereas calm cattle response had no significant decrease (Oliphint, 2006). By the end of the study calm calves had a 1.6-fold greater antibody responses and greater ADG compared to the temperamental cattle (Oliphint, 2006).

Temperamental animals are more costly to raise due to lower production efficiency. Temperament can be changed over time with additional handling. Curley et al. (2006) found that exit velocity was influenced by time and tended to decrease from day 0 to day 120 in Brahman bulls. This is predictable because their response to a stimulus will improve with increased exposure. However, the study by Curley et al. (2006) also indicated that there was no difference in exit velocity from day 60 to day 120. Based on this response, the authors suggested that there may be a limit to human-animal interaction acclimation for Brahman bulls. However, this fails to account for the fact that there must be some minimum baseline for exit velocity.

Animals that have higher levels of stress hormones (e.g. cortisol) have been found to have decreased growth rates and average daily gains, as well as weaker immune responses to pathogens (Burdick et al., 2011a). Hulbert et al. (2011) found temperamental animals (measured by average of chute score and exit velocity) had higher levels of circulating cortisol at two days after transport. Curley et al. (2006) found that pen score and exit velocity were both positively correlated with cortisol concentration; Grandin (1997) found that calves that had greater human contact earlier in life had lower levels of cortisol. Yet, while previous human experience is thought to be a major contributor to temperament of animals, repeated handling may not reduce reactivity of the more temperamental cattle (Grandin, 1997).

Furthermore, because temperamental cattle utilize free fatty acids for energy they are in a constant state of lipolysis of adipose tissue to fuel their bodily functions. Based on that, temperamental cattle do not deposit fat at the same rate as calm animals and producers should take that into consideration when formulating diets for beef cattle and ultimately feed temperamental cattle differently. Therefore management practices that treat each animal equally may decrease production and be more costly to the producer.

## **Temperament and Diet**

### *Hepatic Oxidation Theory*

One area that has received little attention is the relationship between diet and temperament. One theory of intake regulation that provides a testable hypothesis relating to diet interactions with temperament is the hepatic oxidation theory.

The hepatic oxidation theory (HOT) has been proposed to explain the idea that feed intake is controlled by a signal from the liver to the brain that is stimulated by oxidation of numerous fuels (Allen et al., 2009). The idea that the liver is involved in the control of food intake is not a new idea. Russek (1963) introduced the idea when he found that glucoreceptors in the liver of dogs influence feeding behavior. Subsequent studies with rats and other non-ruminant laboratory species aided in the development of the theory which Allen et al. (2009) applied to ruminants.

In a variety of lab animals it was found that infusing glucose decreases food intake (Forbes, 1995). Inhibition of glycolysis and FA oxidation with methylpalmoxirate and 2-deoxyglucose (Friedman and Tordoff, 1986), or inhibition of glycolysis and lipolysis by 2-deoxyglucose and nicotinic acid (Friedman et al., 1986), increased feed intake in rats (Allen et al., 2009). Other fuels metabolized in the liver, including pyruvate,



lactate, and glycerol have shown hypophagia in rats, which was eliminated by hepatic vagotomy (Allen et al., 2009). Stimulation of feed intake by blocking FA oxidation with methylpalmoxirate has also been associated with reduced hepatic energy status as measured by liver ATP concentration, ATP-to-ADP ratio, and phosphorylation potential (Allen et al., 2009). Research with metabolic inhibition revealed a cause-and-effect relationship between hepatic energy and feeding behavior; the effect is likely in the liver because latency for the eating response was less for portal compared with jugular infusion, hepatic vagotomy blocked the eating response, and significant radioactivity was found in the liver but not the brain after administration of radioactive 2,5-anhydro-D-mannitol (Allen et al., 2009).

Unlike the adult non-ruminant, ruminant livers do not utilize glucose as an energy source; ruminant livers mainly utilize short-chain FA (SCFA), lactate, amino acids (AA), and glycerol as oxidized fuels. Propionate is the main precursor for glucose in ruminants. Propionate can be converted to glucose, but it can also be utilized by oxidation in the TCA cycle as well as stimulate oxidation of acetyl CoA derived from other fuels (Allen, 2000, 2009). Ruminal digestion kinetics will determine the extent and site of nutrient digestion, which can affect the type and pattern of fuels absorbed, therefore impacting temporal patterns of fuel oxidation by the liver and feeding behavior (Allen et al., 2009). The retention of digesta within the rumen can supply a more consistent supply of starch, LCFA, and protein to the small intestine, which can delay their absorption. However, VFA are rapidly produced and absorbed and are likely responsible for stimulating satiety during meals (Allen et al., 2009). However, the intake of feedlot cattle is primary

regulated by metabolic signals, rather than ruminal distension, and diet fermentability can clearly influence intake (Allen et al., 2009).

The liver is likely involved in regulation of feed intake by propionate because hypophagic effects of portal infusions of propionate were eliminated by splanchnic blockade with anesthetic, bilateral splanchnotomy, and hepatic vagotomy, as well as with total liver denervation in sheep (Allen et al., 2009). Infusion of propionate has been shown to decrease feed intake, while acetate did not produce the same results (Elliot et al., 1985). This is consistent with HOT because propionate can be oxidized and stimulate oxidation in the liver, whereas acetate oxidation is negligible (Allen et al., 2009).

Increasing propionate linearly decreased ME intake compared with acetate in lactating dairy cows; as the proportion of propionate increased, the reduction in ME intake from the diet exceeded that supplied from the infusate (Allen et al., 2009). Therefore, depressed feed intake by propionate cannot be explained simply as additional energy supplied as propionate per se. Thus, it appears that animals do not consume feed to meet their energy requirements, but rather have fuel-specific mechanisms to regulate feed behavior (Allen et al., 2009).

When starch digestion is shifted from the rumen to the small intestine, propionate production decreases and lactate absorption increases from glucose metabolism in the gut (Reynolds et al., 2003). The shift often results in a higher feed intake. Starch digested in the small intestine likely stimulates hepatic oxidation less than ruminally fermented starch, especially within the timeframe of meals, because of the greater lag of starch passing the rumen, and because extraction of lactate from the blood by the liver is much less than that of propionate (Allen et al., 2009). Because of differences in metabolism,

HOT predicts that more energy can be absorbed in the form of lactate than propionate (Allen et al., 2009).

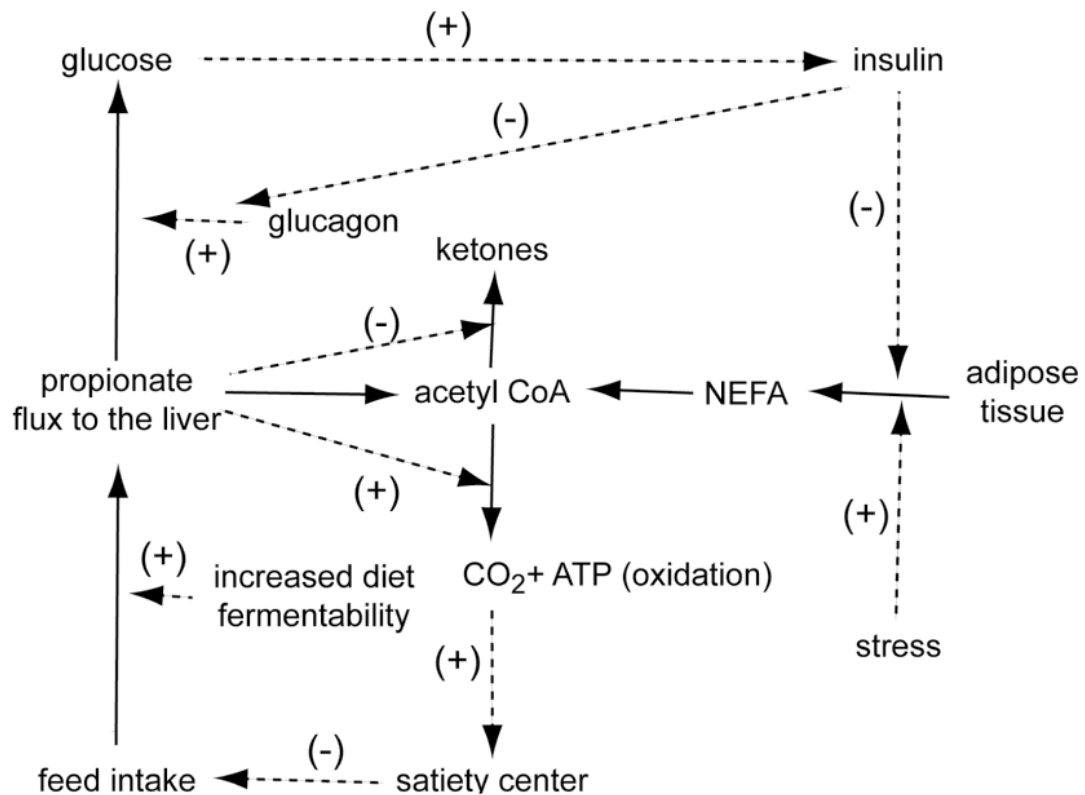
It is common for calves entering the feedlot to exhibit depressed feed intake for up to three weeks (Allen et al., 2009). Common management practices, such as loading, transport, and regrouping result in elevated plasma concentrations of cortisol and epinephrine, which are stress hormones. Cortisol, epinephrine, and norepinephrine are pleiotropic hormones, but each acts as a lipolytic agent (Allen et al., 2009). The stress response in receiving cattle often results in lipolysis and elevated plasma non-esterified fatty acids (NEFA) concentrations. The decrease in DMI by receiving calves may be related to the stress-induced increase of NEFA; the elevated plasma NEFA provides a new oxidative substrate for the liver, generating additional ATP and suppressing feed behavior (Allen et al., 2009).

It has been proposed that ruminants have receptors for propionate in the veins draining the splanchnic bed; G protein-coupled receptors activated by propionate have also been identified (GPR41 and GPR43) (Brown et al., 2003). These can also be activated by acetate, but GPR41 tends to respond ~10-35% more for propionate than acetate, depending on the concentration (Brown et al., 2003). However, peripheral concentrations of propionate are less than acetate and therefore this seems an unlikely mechanism for propionate to induce hypophagia (Allen et al., 2009).

Hypophagic effects of propionate infusions have been seen without an increase in insulin levels (Allen et al., 2009). Insulin might have indirect effects on intake in increasing oxidation of gluconeogenic precursors in the liver by speeding clearance of fuels from the blood and decreasing lipolysis and NEFA supply to the liver (Allen, 2005,

2009). This could explain inconsistent intake reports from peripheral insulin administration reported in previous literature. Additionally, there seems to be no effect on leptin concentrations from propionate in cattle.

The model and mechanisms of the HOT theory can be seen in Figure 2.



**Figure 2. 2. Model of feed regulation according to the hepatic oxidation theory (Allen et al., 2009). Solid lines indicate carbon flow and dashed lines show stimulation/inhibition of flow.**

The liver uptake of propionate can either be used for gluconeogenesis, utilizing ATP, or oxidized in the TCA cycle through acetyl CoA. Acetyl CoA from  $\beta$ -oxidation of fatty acids is oxidized in the TCA cycle or exported as ketones. Decreases in insulin release, increases in insulin resistance, and stress can increase lipolysis, therefore increasing the concentration acetyl CoA by  $\beta$ -oxidation of NEFAs. During meals,

propionate stimulates oxidation of acetyl CoA to CO<sub>2</sub> and generates ATP (Allen et al., 2009).

Hepatic oxidation increases throughout meals, increasing hepatocyte energy status and decreasing discharge rates of hepatic vagal afferents, which then cause satiety. After meals the oxidation decreases, hepatocyte energy status decreases, and the firing rate of the hepatic vagus increases, in turn causing hunger (Allen et al., 2009). The hepatic vagal afferents eventually go to the hypothalamus.

Propionate is rapidly taken up by the liver and converted to TCA cycle intermediates. There is an increased flux of carbon from propionate through pyruvate kinase, allowing oxidation based on the fate of pyruvate. When energy is low, pyruvate is oxidized in the TCA cycle; when energy is high, acetyl CoA activates pyruvate carboxylase, converting pyruvate to oxaloacetate. Hepatic oxidation of NEFA is low during meals due to increased insulin release inhibiting lipolysis in adipose tissue and uptake of NEFA by the liver and because propionate also inhibits  $\beta$ -oxidation of FA by decreasing FA transport into mitochondria and by decreasing activity of fatty acyl CoA dehydrogenase (Allen et al., 2009).

Fatty acids are oxidized in the mitochondria by  $\beta$ -oxidation to acetyl CoA and can be further broken down to CO<sub>2</sub> in the TCA cycle or exported as ketones. A possible mechanism that blocks oxidation of acetyl CoA is the buildup of NADH from  $\beta$ -oxidation of NEFA (Allen et al., 2009).

Propionate flux to the liver during meals stimulates oxidation of acetyl CoA in the TCA cycle, providing enough CO<sub>2</sub> to stimulate oxidative phosphorylation and ATP generation (Allen et al., 2009). The elevated plasma NEFA concentration of stressed

animals offers another oxidative substrate for the liver; increased lipolysis of stressed animals increases concentrations of acetyl CoA through  $\beta$ -oxidation of NEFAs (Allen et al., 2009). When stressed animals that already have high concentrations of circulating NEFAs are supplemented with monensin, HOT would predict a substantial decrease in intake, because propionate can serve to replenish depleted TCA cycle intermediates.

The hepatic oxidation of fuels has a big influence on intake in ruminants and non-ruminants. Formulating diets to prevent decreases in DMI for receiving cattle is difficult and involves understanding multifaceted alternative and redundant mechanisms that integrate the brain and liver. According to HOT, stressed animals can be more susceptible to diet induced DMI depression, such as that caused by increased propionate concentrations. The anaplerotic ability of propionate gives it the potential to have intake depressing effects in the presence of FA available for oxidation. The HOT offers a potential unifying mechanism to explain relationships between temperament and intake.

*Previous studies: Energy, microbes and monensin*

Previous studies with stressed receiving calves and diet have focused on alternate dietary considerations to alter DMI for receiving and stressed cattle. One study focused on varying levels of monensin (0, 11, or 33 mg/kg) on high-energy diets of receiving calves. When monensin was supplemented at moderate concentrations DMI increased for the first 28 days on feed, while the high dose tended to decrease DMI (Allen et al., 2009). The propionate production with the lower inclusion rate of monensin could have benefited DMI by promoting insulin secretion and suppressing lipolysis; however, the greater inclusion rate may have provided enough oxidative substrate to increase hepatic ATP concentration and suppress feeding behavior (Allen et al., 2009).

Diets ranging from 80-100% forage have been shown to decrease DMI in receiving calves (Lofgreen et al. 1975; Lofgreen et al. 1985). Previous studies with stressed receiving cattle found that DMI increased when animals were fed diets with higher levels of ME (Fluharty, 1996; Lofgreen et al. 1975; Lofgreen et al. 1981). Fluharty and Loerch (1996) found increased DMI during the first week on feed when animals were fed a ration containing 85% concentrate compared to 70% concentrate ration. In contrast, Berry et al. (2004) found that animals fed a low energy diet (55% concentrate) had increased DMI compared to animals fed a diet with higher concentrate (65%) during the first 42 days on feed.

Of the studies that found energy density to alter DMI during the receiving phase, those with higher morbidity rates ( $\geq 50\%$ ) found that the reduced energy density improved DMI (Lofgreen et al., 1981; Fluharty et al., 1994; Berry et al., 2004). However, studies where there were low morbidity rates ( $\leq 43\%$ ) found that rations with greater energy density results in increased DMI (Fluharty and Loerch, 1996). Because morbidity is commonly used as an indicator of stressed animals, these studies suggest that a more conservative energy density ration may be better suited for stressed receiving calves to maintain DMI.

There could be a relationship between rumen microbe populations and temperament in beef cattle. Bercik et al. (2011) showed that intestinal microbiota in mice can influence the central nervous system, in the absence of discernible changes in local or circulating cytokines or specific gut neurotransmitter levels, and intestinal microbes play a critical role in behavior and central neurotrophin expression. For example, work with germ-free mice indicated that intestinal microbiota can influence postnatal development

of the hypothalamic-pituitary response to stress (Sudo et al., 2004). Others have found induction of anxiety-like behavior after introduction of a pathogenic bacteria into the gut in mice, suggesting that intestinal microbes affect behavior (Lyte et al., 1998). Naglaa and Ghada (2014) found that administering probiotic Actisaf<sup>®</sup> SC 47 (*Saccharomyces cerevisiae* NCYC Sc 47) with ewes for one month improved temperament (measured with SCS), serum cortisol, serum lactic acid concentration, and serum glucose concentration.

Moreover, Bercik et al. (2011) found that there was a relationship between the amygdala and antimicrobial use. The amygdala is associated with mood, memory, and fear and conditioning behavior (Grandin, 1997; Bercik et al., 2011) and overactivation of the amygdala has been associated with anxiety (Drevets, 2000). Brain-derived neurotrophic factor (BDNF) and its receptor, tyrosine kinase receptor B (TrkB), have been implicated as mediators of hippocampal-dependent learning and memory (Rattiner et al., 2004a). A study with mice found increased BDNF expression in the amygdala during learning (Rattiner et al., 2004b); additionally, they found that BDNF signaling through TrkB receptors in the amygdala is required for the acquisition of conditioned fear (Rattiner et al., 2004a). When mice were treated with an antimicrobial, their BDNF levels decreased and observed exploratory behaviors increased (Bercik et al., 2011). If altering microbe populations can affect the biochemistry of the amygdala, ultimately impacting behavior or fear, gut microbes may have a direct relationship with temperament in cattle. Such relationships could account for behavioral differences between cattle and varying responses to a variety of stressful situations. There is limited work focusing on how altering intestinal microbe populations can alter behavior and the majority of available



literature features human or mouse models. Because microbes play an essential role in nutrition and metabolism in ruminants, cattle would be a suitable model to use for studying relationships between gut microbes and behavior. Thus, future research should focus on relationships between rumen microbe populations and temperament, and how this relationship can affect growth and efficiency.

### *Monensin*

Monensin is a dietary ionophore utilized in production settings and was approved for use in the 1970's. Monensin selectively inhibits gram-positive bacteria, which in turn promotes metabolic efficiency by improving nitrogen and energy metabolism, as well as lowering risk for acidosis and bloat (Duffield et al., 2012). Acidosis and bloat are both illnesses that can decrease production in cattle and are harmful to production as a whole, as well as the animals.

Monensin also alters the VFA concentration within the rumen, increasing propionate and decreasing the amount of acetate and butyrate (Duffield et al., 2012). Numerous studies have found increases in propionate when monensin was fed (Perry et al., 1976; Van Maanen et al., 1978; Prange et al., 1978). The increase in propionate increases the supply of energy to the animal.

Monensin grew in popularity when it was found to increase average daily gain (ADG) and feed efficiency (FE), while decreasing intake and methane production (Goodrich et al., 1984). Although monensin does increase the efficiency of the animal, it does not always affect the rate of gain compared with control animals (Raun et al., 1976; Perry et al., 1978).

Because of the negative implications of ionophores on intake, the concentrations supplemented for receiving cattle are usually kept fairly low. The impact of ionophores on intake for new cattle can be managed by altering the dietary concentration.

Additionally, the impact on intake may also be mediated by adapting the animals gradually to increasing levels, rather than sudden addition to the diet (Thomas, 2006).

Stressed receiving cattle on a diet containing monensin should have lower DMI due to the increase in propionate from the monensin. Some studies with dairy cattle have shown that monensin can decrease blood concentrations of NEFA (Duffield et al., 2007). The increased ruminal propionate supply from the monensin likely leads to reductions in mobilized body fat and supply of fat to the liver.

### **Animal Behavior**

Ethology is the scientific study of animal behavior and has become more accepted in the scientific community over the past 50 years. Previously, animal behavior studies have been done subjectively and were often prone to error. However, with clear definitions of behaviors to measure and a quantitative measurement technique, animal behavior can be used to help design management practices that will foster increased productivity and efficiency of livestock production.

Knowledge of the etiology of behavior and psychophysiological processes allows for a more accurate interpretation of observable behaviors (Hurnik, 1994). Ethology may contribute to development of better production practices, more closely related to the animals' psychological and physical needs (Hurnik, 1994). Therefore, ethology is an important consideration for ethical principals and management techniques for veterinary science and animal agriculture.

### *Defining Dominance in Animal Science*

Dominance has many definitions among animal behaviorists. In 1922, Schjelderup-Ebbe (1975) introduced the idea of the pecking order in animal dominance; Wilson further described different dominance relationships among animals. Historically, dominance has been used without a clear definition or the definition has been redefined to fit the findings of the study (Drews, 1993). Many scientists also have not explicitly specified which definitions they advocate and which they criticize (Drews, 1993). Dominance is used in animal behavior to describe a particular kind or set of observable interactions (Drews, 1993).

Some of the terms used in measuring dominance are defined and used here as follows:

*Agonistic behavior*: competitive interaction between two or more animals, with or without vocalization or body contact, characterized by certain structures specifically developed for this purpose (Fox, 1968).

*Winner*: the contestant that expresses consummatory behavior according to its initial goal. Winner/loser refers to the outcome of one single contest. (Baenninger, 1981; Hand, 1986; Drews, 1993)

*Submissive*: submissiveness is an active response to an aggressive action by another individual in which yielding or surrendering are displays given by the loser. Submissive behavior refers to single contests (Hanby, 1976; Hand, 1986; Drews 1993)

*Subordination*: to lose consistently certain conflict encounters. Subordination refers to a pattern based on several contests (Hand, 1986; Drews, 1993)

*Aggression*: Actual attacks, threats of attack or encroachments; signals which suggest that attack may occur can be called aggressive (Hand, 1986; Drews, 1993)

Of the many definitions of dominance, a few apply to the study of behavior of beef cattle. Drews (1993) mentions two definitions that consider dominance to be a trait of the individual: 1) “dominance is aggressiveness” where a dominance hierarchy is the set of sustained aggressive-submissive relations among a group of animals which coexist within one territory (Wilson, 1975) and 2) “Dominance is a trait that conveys rank” where a dominant individual routinely has encounters with significantly greater than chance probability against a variety of conspecific opponents (Baenninger, 1981).

Additional example definitions mentioned by Drews (1993) consider dominance to be an attribute of encounters and relationships rather than of the individual. These include: 1) “winner is dominant, loser is subordinate” where dominance status is a synonym for winner and loser is used to describe the outcome of any single encounter, 2) “dominance is a lack of aggressiveness” where dominance refers to the “peaceful” nature of encounters between animals, in which escalated fights do not take place and conflicts are resolved using non –agonistic behaviors using control of resources and minimum energy expenditure through the use of displays to control behavior (Vesey, 1981), 3) “consistent winner of agonistic contests” where A consistently wins agonistic encounters against B then A is dominant and B subordinate, 4) “consistent winner in a given context” where dominance is a descriptive term for individuals that consistently win dyadic encounters in a given context (Lee, 1983), and 5) “peck-order” where if A pecks at B and B never or seldom reciprocates then A is dominant to B and B is subordinate to A (Schjelderup-Ebbe, 1922).

However, the definition that seems to best describe cattle is listed as “modified peck-order” where dominance is an attribute of a relationship between two individuals, whenever an asymmetry in the outcome of agonistic interactions is measured (Drews, 1993). Some of the previous stated definitions are outdated; additionally, while others have a place in animal behavior studies, the latter appears to be the best definition for studying cattle behavior and social interactions.

A dominance relationship between individuals implies that the same individuals meet and resolve conflicts on several occasions in a clearly unidirectional pattern (Drews, 1993). In this respect, past conflicts may influence the outcome of future conflicts. Furthermore, individual recognition is not necessary to produce a behavioral pattern under the dominance definitions. An individual may assess another at the onset of any conflict to determine competitive ability. This relates to the “dominance is a lack of aggressiveness” explanation.

Scott (1958) emphasized that learning was important in aggressive behavior because success in fighting reinforces the aggressive behavior; therefore prior experience is important and may influence the final outcome of success or defeat (Fox, 1968). Time of birth may also be a driving factor in aggression or dominance, as animals born earlier in the season are physically more mature than animals born later (Fox, 1968).

#### *Agonistic Behaviors and Hierarchies*

After cattle engage in agonistic behaviors and determine who is dominant over whom, a hierarchy forms in the herd. Hierarchies can be simple (linear) or complex. Small groups (6-10 animals) of domestic animals of similar sex and size more frequently have linear relationships. In this setup, each individual is dominant to all listed below

them (Craig, 1986). Hierarchies become more complex when pecking triangles are present. Many studies have been done with chickens and the complexity of pecking orders. Social hierarchies are important to allow the coexistence of animals in social communities, or more commonly recognized in the case of cattle, confined areas.

The time requirement to form a social hierarchy in cattle is not well defined, especially in steers. Previous studies have found that once it is established the rank will persist for months. Studies with large herds have found instability in social dominance status, while other studies found that hierarchies form in a “relatively short time” (Oberosler et al., 1980). The time requirement was better addressed by Kondo et al. (1990), who indicated that “the time necessary for the process of social conditioning will determine the rate of social hierarchy stabilization within a group”. Generally, there are some reversals for individuals dominating others who are similar or slightly higher in rank, but these rates are usually low, seen at 8% in domestic goats and 5.5% in American bison and Dama gazelles (Barroso et al., 2000).

Previous studies have found that female cattle hierarchies tend to persist while male social status may change more frequently (Craig, 1986). Adolescent animals usually take longer to create a hierarchy when they are kept together rather than being mixed with strangers (Craig, 1986). Cattle should develop a social hierarchy by 3-6 months (Schein, 1955). Studies with poultry found similar results, showing that individuals have greater success in encounters with strangers when they are in familiar surroundings (Colias, 1943; Craig, 1986). It is also important to note that while alliances may be important in some species, they are not important within farm animals.

When environments change for animals, such as confining or mixing groups, dominance relationships change and agonistic interactions tend to increase. Periods of social activity usually occur during “change in phase” periods and in the morning hours (Hinch et al., 1981). When cattle are kept in smaller confinement and are frequently regrouped aggression increases, partly due to competition for feed and lying space (Galindo, 2000). Some animals will be more successful at gaining access to feed and space theoretically because of dominance, hence an advantage in productivity will likely result for those animals. Mench et al. (1990) conducted a study with beef cows where cattle were kept in small groups of ten animals with two animals being introduced from a different source (denoted as alien cattle). They found that when small numbers of cattle from different sources are mixed the aliens are at a social disadvantage, and speculated that under some conditions social stress on aliens and(or) subordinates may be additive over time.

It is generally accepted that the agonistic interactions of animals increase as resources are restricted; however, even when resources are abundant there is a baseline of these behaviors to maintain hierarchy status. Barroso et al. (2000) found that goats on pasture with no competition for resources spent more time displaying “passive” social interactions (75.5% of time), whereas goats in a stable setting with resource competition spent significantly more time acting aggressively or threatening. Even in a pasture setting with no feed competition there is some aggressive behavior. These behaviors will also increase as space is restricted. This introduces the idea of individual space, in which animals will act aggressively to defend their space (Craig, 1986). The individual space area can change between resting periods.

### *Dominance and Feeding Behavior*

As a consequence of social status, the most dominant animals will be rewarded with priority access to resources as well as possibly receiving more of the resource, specifically when resources are restricted. Intermediate ranking animals may receive equal amount of reward, but low status animals will be severely penalized and lose access to resources (Craig, 1986). As resources become more restricted, the lower status animals can become largely excluded (Craig, 1986). Therefore, social hierarchy can affect production characteristics, such as DMI and ADG.

However, other research suggests it is unclear whether or not feeding behavior is directly affected by social rank in herd animals. Galingo and Broom (2000) found no significant relationship between time spent feeding and time standing in dairy cattle, where time standing was used to indicate rank. Galindo and Broom (2000) found that lower-ranking dairy cattle were significantly more likely to spend more time standing than higher-ranking animals. Additionally, the lower-ranking animals were also found to be significantly more likely to become lame due to sole lesions because of the increased time standing, using pair-wise comparisons. However, Barroso et al. (2000) found a significant relationship between feed intake and social rank in goats. The direct effect of social rank on feeding behavior may depend on species and study specifics, such as environment, feed availability, number of animals, etc. Research with feeding behavior and dominance rank within beef cattle is limited.

Banks et al. (1979) performed a study measuring feeding frequency/duration and dominance hierarchy in poultry. Birds were evaluated subsequent to deprivation of food and water for 18 hours after previously having had ample supply of food and water when



feeders could only accommodate one animal at a time. They found a significant relationship between social rank and frequency and duration of feeding. However, the relationship was not found at the water sources, whichever bird arrived first drank, regardless of social rank. While this study only found food to facilitate aggression, previous works found hens displacing subordinates from preferred nesting sites as well, another example of aggression related to individual space (McBride et al., 1969).

Van Tienhoven (1983) found that only the lowest-ranking hen in a poultry flock of six had a depressed feeding activity, body weight, and egg production. Schake and Riggs (1972) did a similar study; they found that the group ranked as the lowest one-third in terms of social status was depressed in all items of performance, while those in the middle were depressed much less in body weight compared with the top-ranking group (Craig, 1986). Similar work with sheep found that dominance value was significantly positively correlated to supplement intake while on pasture (supplement access was restricted), liveweight, and chest girth (Lobato and Beilharz, 1979).

A handful of studies have examined the relationship between dominance ranking and production with beef cattle. Original studies were done by McPhee et al. (1964), who fed a drought ration *ad libitum* to steers in a feedlot, and Wagon (1965), who fed supplements to a mixed-age group of cows from troughs in the paddock. In both studies the animals of high social ranking spent more time feeding and were disturbed less during feeding than animals of low social ranking, but in neither study was there a significant difference in weight gain/loss between the social groups. Blockey et al. (1974) found a significant partial correlation between social ranking and weight gain during a period of supplemental feeding where intake was restricted.

Dominance order is correlated with body weight, chest girth, and age in cattle, sheep, and swine (Schein, 1955; Beilharz et al., 1967; Lobato and Beilharz, 1979; Hasegawa et al., 1997), although weight and age are not causative (Schein, 1955). Additionally, Barroso et al. (2000) found that age and body size strongly determined rank status of goats. This study also found that the presence of horns greatly affects rank status in goats by efficiently facilitating success in competitive interactions.

Physical injury may impact production of livestock animals. The increase in stress due to aggressive social interactions may lower the ability of the immune system to fight pathogens and increase incidence of illness (Barroso et al., 2000). A decrease in the immune system can greatly decrease production. Contrary to most hypotheses, the middle-ranking animals can sometimes be the most successful in production, as seen with goats (Barroso et al., 2000). These animals may suffer less stress in social situation by not being the bottom rank and also do not have to use energy for aggression to stay in the top ranking. This has also been seen in swine, as the higher-ranking sows spent more time defending the pile of food than actually feeding (Csermely et al., 1990).

### **Animal Monitoring Technology**

Historically, two approaches have been used for measuring animal motion and activity. The Lagrangian approach monitors a specific organism and records locations that it passes through, whereas Eulerian approaches monitor a specific location and record all organisms that pass through it (Kays et al., 2011). Eulerian approaches are commonly used to limit stress to the animal, but the data collection is usually less detailed and restricts the questions being asked. The Lagrangian approach can be used with livestock because they are handled more often and a monitoring device can be

placed on the organism easily. This is also better suited for answering questions about the individual organism.

The use of technology to monitor movement, behavior and activity of animals is relatively new in the field of animal science. Technology was first introduced in the 1960's and used with wildlife; later, the technology began to be used with domestic livestock as well. Radio telemetry was the first tracking technology to be used in 1962 with mallards (Lord et al., 1962). Within the last half-century there have been many new technologies developed to investigate different questions involving behavior with livestock.

Radio telemetry is still used with research for many different species. Radio telemetry uses radio frequency signals to collect data from sensors. Data collection by sensor signals can be limited by battery life, distance from the receiver, signal strength, and vegetation profile. However, because this is the most cost effective technique it is the most common technique (Kays et al., 2011). Although this is still commonly used, this type of monitoring is better for small species.

Another system, referred to as ARGOS, was created by the French. This system has been used extensively with marine and terrestrial mammals to record activity in a broad range of temporal measures (Hays et al., 2007). This system sends signals to polar orbiting satellites that orbit the earth at 850 km. the satellites store the data on-board and send it back to earth in real time. There are over 40 antennas worldwide that collect data; individuals collect data using their regional antenna (<http://www.argos-system.org/web/en/67-how-it-works.php>). Earlier studies only recorded data for days or weeks and more current studies have shown successful data collection for up to one year

(Hays et al., 2007). While this technology has improved, there can be problems with failure to collect data for many possible reasons, mainly signal loss (Hays et al., 2007).

Global positioning systems (GPS) and radio-frequency identification (RFID) have been used to monitor animal activity and health status with livestock animals with some success (Turner et al., 2000). One study used RFID to monitor time at the feed bunk with feedlot steers; healthy steers were found to spend 30% more time at the bunk than sick animals (Sowell et al., 1998). GPS has been used to monitor foraging behavior and selection in grazing cattle (Hessle et al., 2008). These systems can accumulate a large amount of data and require a large sensor network.

In the past, behavioral studies with domestic livestock have depended upon direct human observation, which can be limited by interference of human presence, or video recording, which can be limited by subjectivity and time (Bewley et al., 2009). More recently, animal monitoring technologies have been used to study various behaviors (feeding, social, estrus, etc.) for livestock. These technologies are more commonly used in dairy cattle. Animal activity monitoring tags have been developed and validated (McGowan et al., 2007; Bewley et al., 2009). Of significant importance are the technologies that measure activity (lying, standing, etc.), feeding behavior, and temperature. These types of measurement allows for a more objective measurement as well as the opportunity to measure the activity of more animals. Table 1 lists examples and details of animal monitoring technologies used in animal science (commonly dairy science).

**Table 2. 1. Common animal monitoring technologies used in agricultural research (Bewley, J.M. 2015. Personal communication)**

<b>Technology</b>	<b>Company</b>	<b>Description</b>	<b>Location</b>	<b>Parameters</b>
IceQube™	IceRobotics Ltd., Edinburgh, Scotland	A leg mounted technology that monitors lying and standing behavior, and activity. The information used is used to detect health status, lameness, and estrus behavior.	Left rear leg	Lying time, steps, and locomotion
Pedometer Plus™	afimilk®, S.A.E. AFIKIM, Kibbutz Afikim, Israel	A leg pedometer that calculates deviations of activity, standing and lying time, rest time	Right rear leg	Lying time and steps
SmartBow	MKW electronics GmbH, Jutogasse, Austria	A cow location and movement monitor that can be used for locating animals within a barn	Right ear	Position and movement
Track-a-Cow™	ENGS, Hampshire, UK	A technology monitoring cow activity and lying and standing behavior as predictors of health and estrus. Also monitors feeding behavior in order to detect digestive health	Right front leg	Lying time and time at the feed bunk
Cow Manager SensOor	Agis, Harmelen, the Netherlands	An ear tag monitoring cattle head movement, feeding behavior, rumination, and temperature as indicators of general health, digestive health and estrus	Left ear	Rumination time, feeding time, ear skin temperature, and activity
Fever Tags®	FeverTags, Amarillo, TX	An ear tag that utilizes temperature fluctuations to identify animals with higher than usual temperatures	Right ear	Temperature
IceTag™	IceRobotics Ltd., Edinburgh, Scotland	Uses accelerometers to measure lying, standing, grazing behavior and stepping behavior	Neck	Lying, standing, stepping, and grazing behavior

CowScout™	Gea Farm Technologies GmbH, Bönen, Germany	Leg mounted technology that monitors activity in order to predict health and estrus related behavior	Left front leg	Leg activity
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Previous work has shown that there is a relationship between temperament and production in beef cattle. The relationship between social behavior and productivity has not been widely studied, as well as the correlation between differing measurements of behavior. Inherent temperament and social behaviors may be an indicator for stress vulnerability or inherent metabolic mechanisms. The hepatic oxidation theory has been used to explain decreased feed intake in cattle due to these differences in metabolic pathways. Therefore, understanding different metabolic actions of animals with inherent variations in temperament and social behaviors is useful to obtain optimal production with beef cattle.

## CHAPTER 3

### **INTERACTIONS BETWEEN TEMPERAMENT AND DEGRADABLE INTAKE PROTEIN SUPPLY ON GROWTH PERFORMANCE OF RECEIVING CATTLE**

#### **Introduction**

Temperament in livestock has been defined as an animal's reactivity to human handling or to novel or threatening environments (Murphey et al., 1994; Burrow, 1997) and has previously been found to affect productivity of beef cattle. Cattle with excitable temperaments tend to have decreased growth rates and depressed immune responses (Voisinet et al., 1997; Burdick et al., 2011a). In addition to decreasing cattle productivity, overall profitability can be decreased due to increased production costs (Burrow, 1997).

Generally, each temperament test has been devised with specific objectives in mind. In some cases the objective has been to improve human safety during handling in yards or chutes (Grandin, 1993) or under conditions of close human-animal proximity (Boivin et al., 1992). In other cases the objective has been to relate behaviors to productivity, such as weight gain or feed intake (Voisinet et al., 1997; Petherick et al., 2002; Cafe et al., 2011). As a result, each test tends to measure behavioral aspects specific to the objective of the experiment.

The most common techniques used to measure temperament in cattle are exit velocity and subjective chute score because they are easy and quick for producers and found to be related to production (Grandin, 1993; Burrow, 1997). Some investigators have combined these two measures into a single average temperament score (Hulbert et al., 2011; Francisco et al., 2012), although evidence suggests that these measures do not necessarily serve as proxies of the same underlying behavioral trait (Kilgour et al., 2006). The subjective nature of the chute score measure makes it difficult to replicate and

validate. Thus, an objective measure that quantifies activity in the chute could potentially offer advantages over the commonly used subjective scoring approaches.

Currently, the specifics of how temperament relates to animal growth are unclear. There is some evidence suggesting that temperamental cattle are less efficient than calmer cattle (Petherick et al., 2002), whereas other studies suggest that decreased intake can play a major role in decreased gains (Cafe et al., 2011). Additionally, differences in growth rate among animals with different temperament scores suggest the possibility that nutrient requirements may differ. Moreover, animals of different temperaments may have different gut microbial populations, as recent studies with mice have demonstrated strong relationships between the gut microbial population and behavior (Bercik et al., 2011). Consequently, microbial protein requirements of rumen microbes may vary in animals with different temperaments.

Thus, the objectives of this study were to evaluate an objective chute score measurement and exit velocity as independent measures of temperament and to determine if temperament interacts with degradable intake protein to affect growth, intake, and morbidity of newly received feeder cattle.

## **Materials and Methods**

All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

*Animals and treatments.* One hundred ninety two mixed breed beef steers ( $243 \pm 1.32$  kg) in 48 pens (4 steers/pen) were used in a randomized complete block design with a 2 x 2 x 3 factorial treatment structure, using initial weight as the blocking factor. Steers were purchased from central Kentucky livestock auctions by an order buyer and housed



at the C. Oran Little Beef Research Unit in Woodford County, KY. Steers were comprised of several mixed breeds dominated by British influence. Within 48 h of arrival, cattle were weighed, ear tagged for individual identification, and temperament measures were obtained for treatment assignment.

Three temperament measures were obtained on each animal: 1) subjective chute score (Grandin, 1993), 2) objective chute score and 3) exit velocity (Burrow et al., 1989). Subjective chute score was completed by observing behavior in the chute (Silencer hydraulic squeeze chute, Moly manufacturing, Lorraine, KS, mounted on load cells) for 10 s after the head was caught, with no squeeze applied, and was represented as the average score across 4 observers. The 1-5 scoring scale used was that of Grandin (1993):

- 1: calm, no movement
- 2: restless shifting
- 3: squirming, occasional shaking of device (squeeze chute or scale)
- 4: continuous vigorous movement and shaking of device
- 5: rearing, twisting, or violently struggling.

Objective chute scores (OCS) were recorded during the first 10 seconds after the head was caught in the weighing chute (Silencer hydraulic squeeze chute, Moly manufacturing, Lorraine, KS, suspended from load cells). The scale head (Lynx, Mettler Toledo LLC, Columbus, Ohio) was programmed to export weights at 5 Hz via RS232 to a laptop computer (Acer Aspire V5, Acer America, San Jose, CA). During this period, animals were restrained in the head catch but otherwise undisturbed and OCS was determined as the coefficient of variation of the 50 recorded weight values across this 10 s interval. At the end of this ten-second period, squeeze was applied. Each animal's

weight was calculated as the average of weights measured at 200 ms intervals during the most stable two-second interval subsequent to squeeze being applied.

Upon being released from the chute, flight time was measured over 1.68 m using an infrared sensor (FarmTek Inc., North Wylie, TX) and converted to exit velocity (m/s).

Routine processing (day 0 except where indicated) included: 1) ear tag placement for unique animal ID number (at arrival), 2) viral and bacterial vaccinations (Bovi-Shield Gold 5, Zoetis, Florham Park, NJ; Once PMH, Merck Animal Health, Summit, NJ; Somubac, Zoetis; Ultrachoice 7, Zoetis), and 3) an injection of de-wormer (Dectomax, Zoetis). Cattle were reimmunized on d 14 with Ultrachoice® 7 and Somubac®.

Animals were assigned to treatments within weight strata. Treatments included two levels of exit velocity (fast or slow), two levels of OCS (high or low), and three levels of dietary protein supply (nominally described as 75%, 105%, and 120% of NRC DIP requirements) arranged in a 2 x 2 x 3 factorial. Temperament treatments were established based on the exit velocity and OCS measured on each animal during its first exposure to the handling facility. Steers were assigned to pens such that each pen had four animals of like temperament treatment. The experimental unit was pen, consisting of 48 pens total and 4 pens per treatment.

Because the two treatment factors were inherent characteristics of the animals (as contrasted with exogenously applied treatments), the ability to divide the animals into these treatment groups depended on the independence of and the relationships of the distributions of the treatment variables. Thus, prior to developing an allotment strategy, the distributions of the two factors were evaluated using SAS JMP (SAS, Inc., Cary, NC) and the selected allotment strategy (division into OCS groups preceding division into EV

groups) was the one which provided the least overlap among treatments. A comparison of the distributions of the treatment variables is provided in Figures 3.1 and 3.2. Of eleven distribution types compared within SAS JMP, the Johnson SI distribution provided the best fits (based on Akaike's Information Criterion) for both OCS and EV. Because animals were first divided into high and low OCS groups, there was essentially no overlap in the distribution of OCS for either of the high and low OCS treatment groups (Fig. 3.2). Additionally, across both EV groups, there was substantial similarity in the OCS distributions of each of the OCS groups. Thus, the allotment strategy provided for a clear delineation between high and low OCS treatments. Alternatively, EV allotments were determined subsequent to the establishment of the two OCS groups. Thus, EV delineations were not as distinct as those for OCS (Fig. 3.1). However, within each level of OCS (high and low), there was essentially no overlap between the EV distributions of the fast and slow groups. A high degree of overlap existed between the "slow" EV, high OCS group and the "fast" EV, low OCS group. More importantly, the EV distributions differed somewhat for the high and low OCS groups. This situation would be expected to increase the likelihood of detecting interactions between EV and OCS and would need to be considered in any interpretation of interaction effects. However, the distributions depicted in 3.2 indicate sufficient delineation in EV between the "fast" and "slow" groups as a whole to suggest confidence in main effects attributed to this factor. Overall, this approach increased the power of our design for detecting differences between OCS treatments while compromising on the ability to detect EV effects. However, the degree of confounding would have been greater (greater overlap between distributions) had the alternate strategy been chosen.

Steers were randomly assigned to pen and dietary treatment within weight blocks. Steers were housed in 2.44 by 14.63 m pens within a three-sided, concrete floored barn. Each pen had 2.44 linear meters of bunk along the fence line (0.61 m bunk space per animal). Pens were scraped clean and bedded with sawdust routinely.

*Dietary Treatments.* Each pen was fed a corn silage-based total mixed ration (TMR; Table 3.1) with one of three different DIP levels (denoted in table 3.1 as DIP levels 1, 2, or 3) once daily at approximately 0700 h. Diets were prepared and adjusted daily to provide ad-libitum intake with minimal amounts of feed refusals. To this end, feed bunks were observed twice daily at 0700 and 1500 h when the remaining amount of feed in the bunk of each pen was estimated. Ingredient dry matters were determined once weekly by drying samples for 24 hours in a forced air oven (100°C, Model 1690, VWR Scientific Products, Corneilius, OR, USA) and rations were adjusted accordingly. Steers had free access to water; adjacent pens shared a water source.

The DIP treatments were established to represent 75%, 105%, and 120% of requirements, calculated according to NRC (2001), using a DIP requirement equivalent to 11% of total digestible nutrients (TDN). Intakes were estimated at 2.8% of body weight, and diets were formulated to be isocaloric and to meet metabolizable protein requirements for 1.36 kg·d<sup>-1</sup> ADG. Using DIP requirements of 13% of TDN, these treatments would have provided 62%, 89%, and 102% of animal requirements. Protein degradability values for forages were determined at a commercial laboratory (Dairy One, Ithaca, NY) using a *Streptomyces griseus* protease assay according to Coblenz et al. (1999). Degradability values for other protein containing feedstuffs were determined as described by Kenney et al. (2015).

Once weekly, feed refusals were collected, weighed, recorded, and combined within treatments. Treatment composites were sub-sampled and dry matter was determined (duplicate 250-500 g samples dried at 100 C for 24 h or until constant weight) and recorded. Any feed refusals on the floor outside of the bunk were weighed, but not included in the orts sample for DM determination.

Animals were not withheld from feed or water prior to weighing, although weights were obtained prior to feeding. Animal weights were recorded on d 1, 2, 16, 30, 57, and 59. An average of the d 1 and d 2 weights was used as the initial weight. Average weight from d 57 and d 59 was used as the final body weight. Exit velocity and OCS were collected on d 1, 2, 16, 30, 57, and 59. Subjective chute scores (SCS) were collected on days 1 and 59.

*Medication protocol.* Steers were examined daily and treated for sickness if required. In order for animals to be diagnosed as being “sick”, and thus qualifying for treatment, the animals must have displayed clinical signs (e.g., lethargy, emaciation, coughing, runny nose) and had a rectal temperature exceeding 39.7°C. The treatment regimen for respiratory disease consisted of 1) a single subcutaneous injection of Draxxin (2.5 mg/kg BW; Zoetis), 2) a single subcutaneous injection of Nuflor (40 mg/kg BW; Merck animal health), and 3) a single subcutaneous injection of Baytril (7.5 mg/kg BW; Bayer HealthCare Animal Health division, Shawnee Mission, KS). One animal was treated with PenJect (6ml/100kg BW; Butler Schein Animal Health, Dublin, OH) for an injured leg. Animals were rechecked on d 1, 2, and 5 subsequent to treatment. Second and third treatments were only warranted if animals failed to respond to the initial treatment per label instructions. Two animals were treated more than three times, one for

a leg injury and one for coccidiosis. Nine animals in this study were removed for various health reasons. Diagnosis of illness of animal was based on clinical signs and at the discretion of the UK Beef Research Unit manager. No diagnostic testing was done.

*Growth performance.* Performance measures were collected for three periods: d 1 to 30, 30 to 59, and 1 to 59. Dry matter intake (DMI) was calculated for each pen by subtracting theorts from the total amount of feed offered during each period. Average daily gain (ADG) was calculated for each animal as the total body weight gain per period divided by the total number of days per period. Gain to feed ratio (G:F) was calculated as body weight gain divided by dry matter intake.

*Morbidity.* The prevalence of bovine respiratory disease was used as the measure of morbidity. Only animals displaying clinical signs of bovine respiratory disease complex were included in calculations for morbidity. Animals were grouped into two classes - those displaying clinical signs but not treated (rectal temperatures less than 39.7 C) and those displaying clinical signs that were treated (rectal temperature of 39.7 C or greater). Farm technicians considered steers to have a respiratory infection based on incidence of lethargy, unusual breathing, and quantity and character of nasal discharge.

*Statistical Analysis.* A distribution analysis was conducted for each variable in JMP 10 (SAS, Inc., Carey, NC, USA). No outlier data points were found using a criterion of greater than 1.5 times the interquartile range below the first quartile, or above the third quartile.

Data were analyzed using pen as the experimental unit. All performance data (ADG, DMI, G:F) were analyzed using GLM procedure of SAS (SAS, Inc., Carey, NC, USA). Initially, data were analyzed using a model that included main effects of EV, OCS,

DIP, all interactions among these three, and block. However, there were no main effects of DIP, nor any interactions with DIP for any of the response variables. Thus, for analysis of temperament treatments, DIP was removed from the model. The model statement for analysis of morbidity contained only the effects of temperament treatment using CATMOD procedures of SAS (SAS, Inc., Carey, NC, USA). Main effects and interactions were considered significant at  $P < 0.10$ .

## Results

Main effects of DIP are presented in Table 3.2. Level of DIP in the diet did not influence ( $P \geq 0.23$ ) any of the response variables in this study. Likewise, no interactions between EV and OCS were detected ( $P \geq 0.11$ ) for the variables analyzed and reported here.

Average daily gain was greater in slow as compared with fast EV animals for d 1 to 30 ( $P < 0.01$ ) and d 1 to 59 ( $P = 0.02$ ; Table 3.3). Likewise, DMI, both in absolute terms and as a percentage of BW, was greater in slow EV animals for all periods ( $P \leq 0.09$ ). There were no significant effects of EV on G:F ( $P \geq 0.14$ ).

There was no effect of OCS on ADG ( $P \geq 0.13$ ). High OCS animals had a higher absolute DMI than low OCS animals for all periods ( $P \leq 0.06$ ). As a percent of BW, high OCS animals had higher DMI than low OCS during d 30 to 59 ( $P = 0.08$ ) and d 1 to 59 ( $P = 0.09$ ). Low OCS animals had a higher G:F than high OCS animals during d 30 to 59 ( $P = 0.05$ ) along with a similar trend for d 1 to 59 ( $P = 0.11$ ).

There were no significant effects of EV on morbidity ( $P \geq 0.26$ ; Table 3.4). Compared with low OCS animals, more high OCS animals were pulled from pens for

displaying clinical signs of illness, without corresponding increase in rectal temperatures ( $P = 0.06$ ).

## **Discussion**

There were two reasons DIP was chosen as the dietary treatment to be analyzed with temperament in this study. The first was to evaluate whether differences exist in nutrient requirements between temperaments, as measured by EV and OCS. Previous research has found that animals with different EV have different growth rates (Burrow, 1997), where slow EV animals typically have higher ADG and DMI than fast EV animals (Cafe et al., 2011; Bates et al., 2014). Thus, we hypothesized that those differences between temperaments might correspond with differences in response to DIP provision. The second was that differences in rumen microbe populations could be linked to animal behavior. Naglaa and Ghada (2014) found that administering *Saccharomyces cerevisiae* probiotic to ewes for one month improved temperament (measured with subjective chute score; Grandin, 1993), showing that behavior of ruminants was somewhat related to gut microbes. Differences in gut microbes could insinuate differences in microbial protein requirements. However, no significant effects of DIP were detected in this study. There are two potential sources of variation accounting for the lack of significant DIP effects in this study. First, there are numerous challenges associated with obtaining accurate prediction of DIP requirements as a percentage of dietary TDN. Our intent was to include sufficient variation among DIP treatments to permit detection of treatment differences despite the presence of known errors in accuracy of estimating requirements. However, it is possible that our estimates of DIP requirements were sufficiently high relative to actual requirements and that all treatments had sufficient DIP to meet microbial needs.



Secondly, there also exist substantial errors in estimating ruminal protein degradability of feedstuffs. Protein degradability is a function of both inherent feed characteristics and exogenous factors that are related to the diet and animal (e.g. passage rate). Thus, it is possible that errors associated with estimation of diet degradability in this experiment could have contributed to the lack of differences among treatments. Regardless, the lack of any significant effects of DIP indicates that our DIP treatment values were not an adequate test of our hypothesis. Thus we cannot draw any conclusions about the relationship between DIP and temperament.

Exit velocity is a measure of temperament that has been suggested to be indicative of physiological stress related to animals' encounters with humans (Curley et al., 2006). The main stress hormone measured in cattle to indicate stress response is cortisol, which has been positively correlated to EV (Curley et al., 2006). Although no glucocorticoids were measured in this study, Carroll et al. (2007) suggested that increased glucocorticoids in livestock are linked to suboptimal growth. Differences in performance between EV treatments is possibly due to the elevated stress response, which is associated with increased stress hormone concentrations and decreases in activities such as eating and sleeping (Burdick et al., 2011a).

Differences in ADG between fast and slow EV were confined to the first period, even though slow EV animals had a higher intake across both periods. This may indicate an attenuation of temperament effects on ADG across time. However, it is more likely the difference in significance between the two periods is a consequence of end-point errors. More important are the effects across the entire study period, indicating that slow EV

animals have a higher ADG than fast EV animals, which is consistent with previous research (Petherick et al., 2002; Cafe et al., 2011; Bates et al., 2014).

The differences in DMI, as an absolute value and as a percentage of BW, between fast and slow EV during both periods was consistent with previous studies where slow EV animals had higher DMI than fast EV animals (Cafe et al., 2011; MacKay et al., 2013). Previously, behavioral reasons were suggested as the cause for differences in intakes between animals with different EV. However, alterations in behavior are possibly the result, rather than the cause, of differences in intake. The stress response has been well documented. Animals with a more reactive stress response have increased concentrations of neurohormones and glucocorticoids, such as epinephrine and circulating cortisol, released by stimulation of the HPA axis and the sympathetic nervous system (Burdick et al., 2011a). Stimulation of these stress-related hormones can inhibit feed intake and digestion (Burdick et al., 2011a); therefore, it seems likely that intake is directly affected by these endocrine actions, and that reductions in feeding time are a consequence of a reduced intake. Relationships among simple measures of temperament, like EV, social or feeding behaviors, intake and gain are worthy of further study.

The cause and effect relationship between temperament and growth performance is not yet clear. Some researchers have speculated that differences in the stress response of excitable animals imply that these animals are less efficient in maintaining and gaining weight (Petherick et al., 2003), although there were no differences in G:F between EV treatments in the present study. Alternatively, Cafe et al. (2011) found that temperament was related to DMI and time spent eating, with lesser effects on efficiency of feed utilization. It seems likely that depressions in time spent feeding are a consequence,

rather than a driver, of the lower intakes experienced by high EV animals that are caused by activation of the HPA axis (Burdick et al., 2011a). However, MacKay et al., (2013) suggested that differences in intake between temperament levels are due to differences in activity and home pen behavior such that animals with a fast exit velocity might have higher levels of extraneous activity and spend less time eating. Results of the present study add additional verification that differences in gain between EV treatments stem from differences in intake. Future research aimed at better understanding the mechanistic relationships between behavior and intake could help establish management strategies to fully capitalize on temperament-related effects.

The tendency for higher gains during the first 28 d with high OCS animals and the positive relationship between OCS and DMI contrast with previous studies, which have used subjective chute score (SCS) and have generally reported negative correlations between chute score and growth (Hoppe et al., 2010, Cafe et al., 2011; Sebastian et al., 2011). However, Graham et al. (2001) found a positive relationship between SCS and ADG in two out of four sire-groups (with no effect of SCS on ADG in the other two). Studies evaluating the relationship between SCS and intake are quite limited. Cafe et al. (2011) found a negative relationship between DMI and SCS, whereas MacKay et al. (2013) found no relationship between these measures. In the present study, initial SCS and OCS were weakly, but positively correlated (Figure 3.3), which is not surprising as these measures were taken simultaneously. Thus, the positive relationship between OCS and DMI cannot be attributed to some unique attribute of OCS as compared with SCS. More likely is that chute scores in general are a reflection of prior experience, as well as inherent variation in response to stress. Specifically, it is possible that, in the present

study, where chute scores were obtained at initial exposure to the facilities, animals with a higher stress response displayed freezing, as opposed to aggressive behavior in response to being restrained in the squeeze chute.

The lack of a significant difference in ADG between OCS treatments was likely due to the differences in efficiency during the study period. Low OCS animals were more efficient than high OCS animals during the last 28 days and tended to be during the entire study period. The lack of a significant relationship between OCS and ADG seen in this study is consistent with previous findings with SCS (Francisco et al., 2012; Mackey et al., 2013), but inconsistent where others found that cattle with a high SCS had lower ADG (Voisinet et al., 1997; Cafe et al., 2011; Bates et al., 2014). Because the relationship between chute score and productivity is inconsistent, further confirmation is needed as to how ADG is related to chute scoring measures.

Objective chute score did not significantly affect incidence of respiratory disease in cattle, but it did affect number of cattle that were pulled from their pens for displaying clinical signs of illness without the corresponding increase in rectal temperature, where high OCS animals were pulled more often than low OCS animals. Results suggest that high OCS animals may show more symptoms of illness than low OCS animals. Previous relationships between temperament and external display of illness have been seen. For example, Hulbert et al. (2011) suggested that temperamental animals are more difficult to treat for illness because they do not show clinical signs, but that study used an average of EV and SCS to define temperament scores. Thus, it is unclear how these measures might have related individually with observation of sickness.

## **Conclusion**

Results of this study give additional confirmation of a relationship between exit velocity and growth performance. Additionally, we have demonstrated the use of a novel objective chute score, which overcomes concerns of the subjectivity generally associated with chute scores. This objective score was positively, but weakly correlated with subjective scores, indicating that the behavior measured by this approach is not identical to that determined when using SCS. Furthermore, in this study, animals with high OCS had characteristics often associated with less temperamental animals (higher intakes, greater display of sickness, and tendency for higher gains during the first 30 d of the study). Although exit velocity relationships appear to be generally consistent across the literature (higher exit velocities associated with lower ADG), it appears that the relationship between chute scores and production characteristics may be more complex than is often assumed. At a minimum, researchers and practitioners should take care to use exit velocities and chute scores as independent measures, and not presume that they measure similar characteristics.

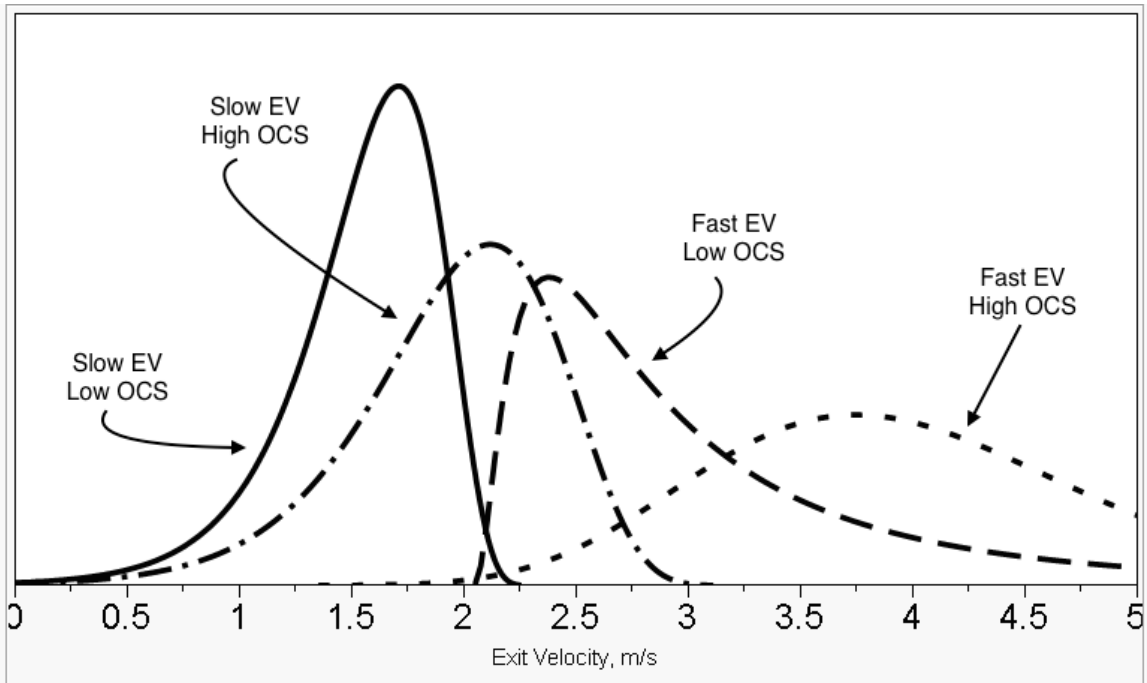


Figure 3.1. Fitted Johnson SI distribution curves for exit velocity (EV) for each treatment in the 2 x 2 factorial. These distributions were constructed from all animals ( $n = 192$ ) that were assigned to the various treatments. These curves provide a graphical description of the degree of overlap in distribution of EV among treatments.

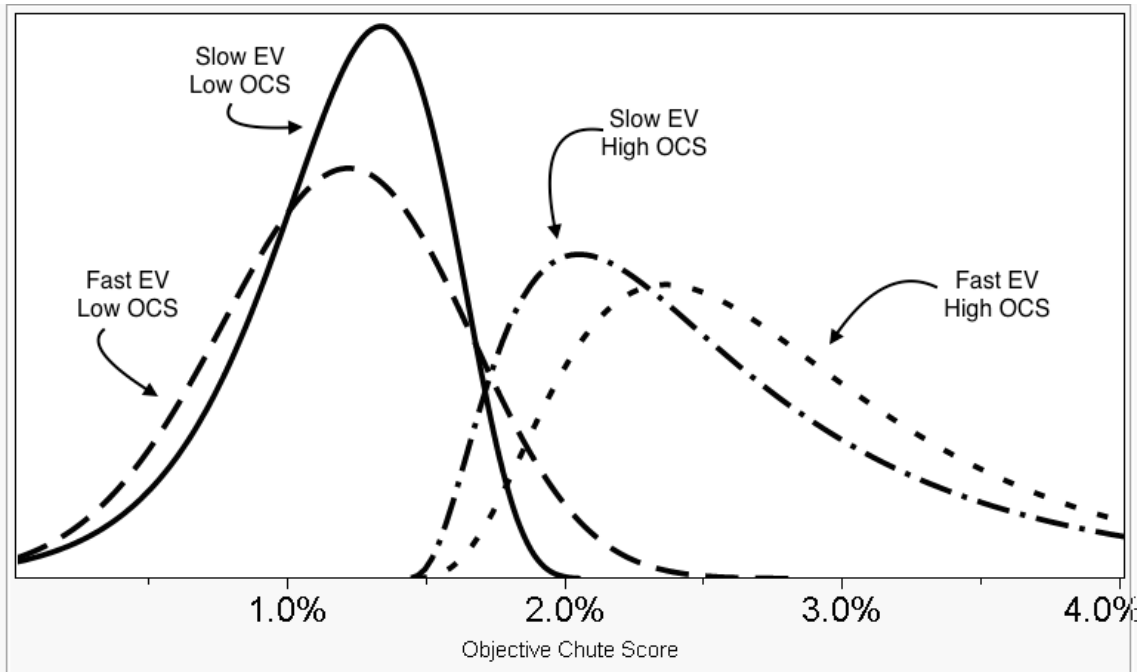


Figure 3.2. Fitted Johnson SI distribution curves for objective chute score (OCS) for each treatment in the 2 x 2 factorial. These distributions were constructed from all animals (n = 192) that were assigned to the various treatments. These curves provide a graphical description of the degree of overlap in distribution of OCS among treatments.

Table 3.1. Ingredient composition of experimental diets

	% of diet DM		
	DIP level <sup>a</sup> 1	DIP level 2	DIP level 3
Grass Hay	20.00	20.00	20.00
Switchgrass	20.00	20.00	20.00
Cracked corn	29.90	29.90	29.90
High moisture corn	12.82	12.82	12.82
AminoPlus® <sup>b</sup>	14.30	4.84	5.53
Soybean meal	0.00	9.38	8.15
Urea	0.00	0.08	0.62
Limestone	1.30	1.30	1.30
Potassium Chloride	0.50	0.50	0.50
Trace Mineral salt <sup>c</sup>	0.75	0.75	0.75
Vitamin ADE Premix <sup>d</sup>	0.05	0.05	0.05
Choice white grease	0.38	0.38	0.38

<sup>a</sup>Levels refer to the amount of DIP in the diet, nominally described as 75% (level 1), 105% (level 2), and 120% (level 3) of NRC DIP requirements

<sup>b</sup>AminoPlus® is a product of Ag processing Inc. (AGP, Omaha, NE)

<sup>c</sup>Trace mineralized salt provided 92.9% salt, 68 ppm Co, 1838 ppm Cu, 120 ppm I, 9290 ppm Mn, 19 ppm Se, and 5520 ppm Zn.

<sup>d</sup>Vitamin premix supplied 1820 IU/kg Vitamin A, 363 IU/kg Vitamin D, and 227 IU/kg Vitamin E.



Table 3.2. Effect of DIP treatments on ADG, DMI, and G:F conversion (n = 16)

	DIP Level <sup>a</sup>			SEM	P-value
	1	2	3		
ADG, kg/d					
d 0 to 28	2.24	2.27	2.13	0.100	0.57
d 28 to 56	3.39	3.37	3.41	0.112	0.97
d 0 to 56	2.81	2.82	2.77	0.070	0.85
DMI, kg/d					
d 0 to 28	14.7	14.8	14.0	0.37	0.23
d 28 to 56	17.3	17.5	17.0	0.33	0.55
d 0 to 56	16.0	16.2	15.5	0.31	0.28
G:F					
d 0 to 28	0.153	0.154	0.153	0.0079	0.99
d 28 to 56	0.195	0.193	0.202	0.0051	0.45
d 0 to 56	0.176	0.175	0.180	0.0042	0.69

<sup>a</sup> Levels refer to the amount of DIP in the diet, nominally described as 75% (level 1), 105% (level 2), and 120% (level 3) of NRC DIP requirements

Table 3.3. Effect of temperament treatments on ADG, DMI, and G:F conversion (n = 181)

	EV <sup>1</sup>		OCS <sup>2</sup>		SEM <sup>4</sup>	P-value		
	Slow	Fast	Low	High		EV x OCS	EV	OCS
Exit Velocity <sup>3</sup> , m/s	1.74	3.56	-	-	0.081	-	-	-
OCS <sup>3</sup> , %	-	-	1.17	2.79	0.001	-	-	-
Initial Wt, kg	245	241	240	245	1.0	-	-	-
ADG, kg/d								
d 1 to 30	1.09	0.91	0.97	1.04	0.035	0.89	<0.01	0.13
d 30 to 59	1.54	1.54	1.53	1.54	0.039	0.34	0.91	0.86
d 1 to 59	1.31	1.23	1.25	1.29	0.025	0.40	0.02	0.23
DMI, kg/d								
d 1 to 30	7.0	6.5	6.6	6.9	0.12	0.14	0.01	0.06
d 30 to 59	8.2	7.6	7.7	8.2	0.12	0.30	<0.01	0.01
d 1 to 59	7.6	7.1	7.1	7.5	0.11	0.15	<0.01	0.01
Intake % BW								
d 1 to 30	2.68	2.58	2.60	2.66	0.043	0.11	0.09	0.34
d 30 to 59	2.78	2.65	2.67	2.76	0.034	0.27	0.01	0.08
d 1 to 59	2.70	2.58	2.60	2.68	0.032	0.11	0.01	0.09
G:F								
d 1 to 30	0.153	0.143	0.148	0.148	0.046	0.64	0.14	1.00
d 30 to 59	0.193	0.201	0.203	0.191	0.041	0.95	0.16	0.05
d 1 to 59	0.175	0.175	0.178	0.171	0.028	0.61	0.99	0.11

<sup>1</sup>EV (exit velocity) = time taken for steers to travel 1.68m upon exiting the chute; assignment to: slow: and “fast” treatments described in text

<sup>2</sup>OCS (objective chute score) = the coefficient of variation of 50 recorded weight values across a 10 s interval while the animal is restrained by the head in a chute; assignment to “low” or “high” treatments described in text

<sup>3</sup> Average initial (d 0) measures of OCS and EV of animal by treatment

<sup>4</sup>SEM = standard error of the mean (n = 8).

Table 3.4. Effect of temperament treatments on morbidity

	Slow EV		Fast EV		P-value		
	Low OCS	High OCS	Low OCS	High OCS	EV x OCS	EV	OCS
Respiratory <sup>1</sup>	6	7	11	8	0.47	0.25	0.70
Pulls, not treated <sup>2</sup>	1	4	0	3	0.56	0.49	0.06
Weight Loss <sup>3</sup>	1	5	0	0	0.72	0.12	0.13
Other <sup>4</sup>	4	1	0	1	0.28	0.14	0.39

<sup>1</sup> Number of animals displaying respiratory illness as described in the materials and methods

<sup>2</sup> Number of animals that were pulled from their pens for displaying clinical signs of illness without the corresponding increase in rectal temperature, and thus were not treated

<sup>3</sup> Number of animals that were observed to be losing weight but were not displaying clinical signs of illness

<sup>4</sup> Number of animals that were treated for illness other than respiratory (leg injury, coccidiosis, etc.)

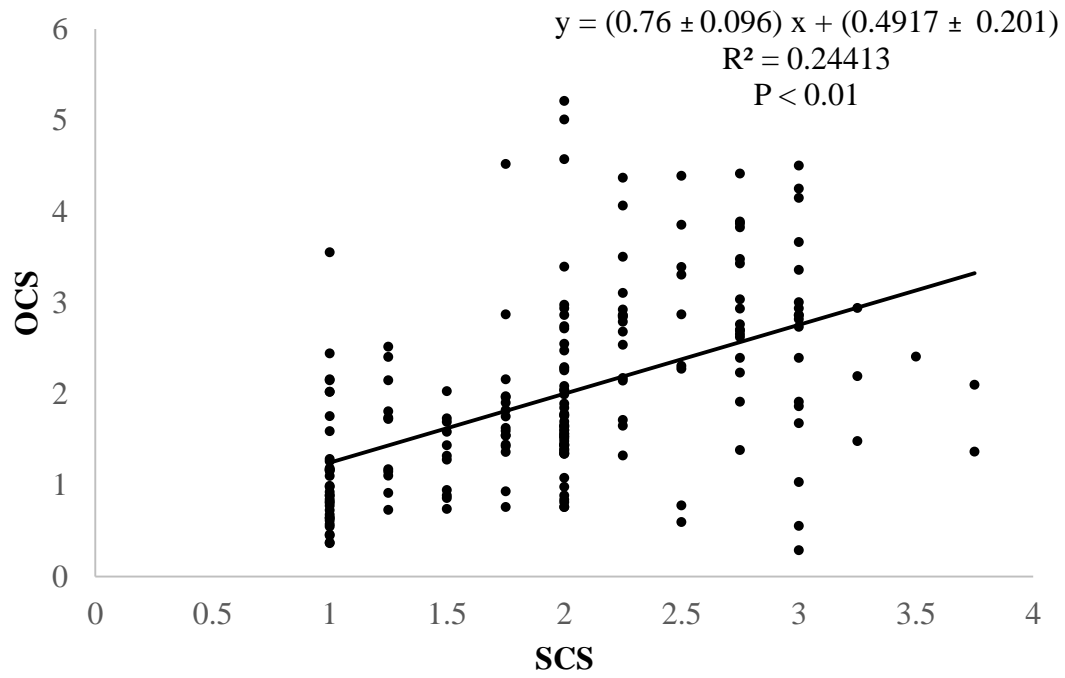


Figure 3.3. Linear regression analysis of initial objective chute score (OCS) and subjective chute score (SCS) at d 0 (n = 192)

## CHAPTER 4

### RELATIONSHIP BETWEEN QUANTITATIVE MEASURES OF TEMPERAMENT AND OBSERVATIONAL BEHAVIORS IN RECEIVING CATTLE

#### Introduction

Temperament has been defined previously as fearfulness and reactivity to humans and to novel or threatening environments (Grandin, 1993; Burrow, 1997). Cattle with favorable temperaments often have greater growth and efficiency (Burrow, 1997). One possibility is that temperament may relate to growth and efficiency at least in part through relationships with social behavior. However, it is unclear how common measures of temperament relate to social dominance behavior in cattle. A major component of social behavior in cattle is social dominance behavior, as groups of cattle will establish social hierarchies, which can reduce the level of aggression in the herd (Wiener, 2015). Dominance can be viewed as an attribute of a relationship between two individuals, whenever there is an unevenness in the outcome of agonistic interactions (Drews, 1993). However, it is unclear whether an animal's place in the dominance hierarchy directly affects productivity traits.

Much of the recent research with cattle temperament has used a combination of chute exit velocity (an objective measure; Burrow, 1997) and chute scores (a subjective measure; Grandin 1993). Recently, temperament has been represented as an index which is calculated as an average of the two scores (Hulbert et al., 2011; Francisco, 2012). There are at least two concerns with this approach. First, the averaging of dissimilar numbers weights the measures in some arbitrary fashion. The units for both measures are arbitrary (e.g. one could choose to measure exit velocity in ft/s rather than m/s, thereby

increasing the weighting of this term; one could use a 1-4 chute scoring scale as compared with a 1-5 scale, similarly adopting arbitrary weighting). Secondly, the use of a single index presupposes that the two measures have similar relationships with response variables of interest. Further, it seems that the mathematical combination of these scores derives from and reinforces the idea that both measures are related to the same underlying behavioral component. There does not appear to be any substantiation of this assumption in the research literature. Finally, although the widely adopted chute scoring system is relatively straight forward, it remains a subjective score. The possibility exists that an objective measure of activity in the chute could prove superior to a subjective scoring system.

Thus the primary objective of this experiment was to evaluate relationships between two measures of “temperament” (exit velocity and objective chute score), social dominance behaviors, and growth in steers during the receiving period.

## **Materials and Methods**

All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

*Animals and treatments.* Thirty-two mixed breed beef steers ( $254 \pm 5$  kilograms) in 8 pens (4 steers/pen) were used in a randomized complete block design experiment, with pen as the blocking factor. Animals were selected from a group of approximately 200, the remainder of which were used in a companion study. Animals used in this experiment represented the third lightest of six weight groups, increasing uniformity of weight. Steers were purchased by an order buyer from central Kentucky livestock auctions and were housed at the C. Oran Little Beef Research Unit in Woodford County,

KY. Animals were comprised of several mixed breeds dominated by British influence. Within 24 h of arrival, cattle were weighed, ear tagged for individual identification, and temperament measures were obtained for treatment assignment.

Three temperament measures were obtained on each animal: 1) subjective chute score (Grandin, 1993), 2) objective chute score and 3) exit velocity (Burrow et al., 1989). Subjective chute score was completed by observing behavior in a squeeze chute (Silencer hydraulic squeeze chute, Moly Manufacturing, Lorraine, KS, suspended from load cells) for 10 s after the head was caught, without squeeze applied, and was represented as the average score across 4-5 observers. The 1-5 scoring scale used was that of Grandin (1993):

- 1: calm, no movement
- 2: restless shifting
- 3: squirming, occasional shaking of device (squeeze chute or scale)
- 4: continuous vigorous movement and shaking of device
- 5: rearing, twisting, or violently struggling.

Objective chute scores (OCS) were recorded during the same time interval as subjective chute scores. The scale head (Lynx, Mettler Toledo LLC, Columbus, Ohio) was programmed to export weights at 5 Hz via RS232 to a laptop computer (Acer Aspire V5, Acer America, San Jose, CA). During this period, animals were left undisturbed and OCS was determined as the coefficient of variation of the 50 weight values recorded across this 10 s interval. At the end of this ten-second period, squeeze was applied. The animals' weight was calculated as the average of weights measured at 200 ms intervals during the most stable two-second interval subsequent to squeeze being applied.

Upon being released from the chute, flight time was measured over 1.68 m using an infrared sensor (FarmTek Inc., North Wylie, TX) and converted to exit velocity (m/s).

The experiment began (d 1) 8 days after the initial measures of temperament were obtained. Routine processing (d 1 except where indicated) included: 1) ear tag placement for unique animal ID number (at arrival), 2) viral and bacterial vaccinations (Bovi-Shield Gold 5, Zoetis, Florham Park, NJ; Once PMH, Merck Animal Health, Summit, NJ; Somubac, Zoetis; Ultrachoice 7, Zoetis), and 3) an anthelmintic injection (Dectomax, Zoetis). Cattle were reimmunized on d14 with Ultrachoice® 7 and Somubac®. Additionally, to determine the ability of steers to mount an immunological response to an antigen to which they were naïve, steers were vaccinated with a leptospirosis vaccine (L5 SQ, Merck Animal Health) on day 1 and administered a booster on day 14. Earlier experiments in our laboratory (unpublished) have found only about 0.5% of steers to have a measureable serum leptospirosis titer prior to vaccination.

Treatments were established based on the exit velocity and OCS measured on each animal during its first exposure to the handling facility. Treatments included two levels of exit velocity (fast or slow) and two levels of OCS (high or low) arranged in a 2 x 2 factorial structure. Steers were randomly assigned to pens such that each pen had one animal representing each treatment. The experimental unit was animal and there were 8 animals per treatment.

Because the two treatment factors were inherent characteristics of the animals (as contrasted with exogenously applied treatments), the ability to divide the animals into these treatment groups depended on the independence of and the relationships of the distributions of the treatment variables. Thus, prior to developing an allotment strategy,



the distributions of the two factors were evaluated using SAS JMP (SAS, Inc., Cary, NC) and the selected allotment strategy (division into OCS groups preceding division into EV groups) was the one which provided the least overlap among treatments. Means and standard errors for the treatment factors are presented in Table 4.3. However, a comparison of the distributions of the treatment variables is more informative. Such comparisons are provided in Chapter 5 as Figures 5.1 and 5.2.

Steers were housed in 2.44 by 14.63 m pens within a three-sided, concrete-floored barn. Each pen had a 2.44 linear meters of bunk line (0.61 m bunk space per animal). Pens were scraped clean and bedded with sawdust routinely.

*Diets.* Animals were fed a corn silage-based total mixed ration (TMR; Table 4.1) at 2.25% of BW (DM) once daily at approximately 0700 h. Diets were prepared daily and adjusted weekly to increase by 0.45 kg DM/pen/week. In order to encourage competition for feed resources for the behavioral measurements, in addition to feeding below *ad libitum* intake levels, feed was delivered in a single pile in the middle of the feed bunk. Ingredient dry matters were determined weekly by drying samples for 24 hours in a forced air oven (100°C, Model 1690, VWR Scientific Products, Cornelius, OR, USA) and rations were adjusted accordingly. Steers had free access to water with adjacent pens sharing a water source.

Once weekly, bunks were checked for feed refusals. However, throughout the study period there were no feed refusals.

Three steers in each pen were given colored ear tags and collars to differentiate individuals within each pen for behavioral measurements. Additionally, steers were fitted with electronic ear tags (Smith, 2012) that contained accelerometers to continuously

measure animal activity. Data from electronic tags were transmitted via RF to a receiver attached via USB to a laptop computer and stored in a Microsoft Access database.

All cattle were monitored by one of 4 video cameras: 3 fixed network and 1 pan/tilt/zoom (PTZ) network camera (Axis Communications; Chelmsford, MA). These were used to collect video recordings for behavioral observations. Video footage was stored on a local server (HP Proliant DL160 G6, Hewlett-Packard, Palo Alto, CA). Video was recorded 24 hours/day for the duration of the study period, with some unexpected video outage (31 h on days 37-39 and 69 hours on days 50-53). These outages occurred subsequent to time periods selected for collection of behavioral data. Videos were accessed for viewing using Axis software (Axis Camera software, Chelmsford, MA).

Animals were not withheld from feed or water prior to weighing, although weights were obtained prior to feeding. Animal weights were recorded on d -7, 0, 14, 28, 55, and 56. A weighted average of d-7 and d 0 weight was used as the initial weight. This weighted average was based on the idea that with consecutive day weights taken 24 h apart, the estimate of weight (the average of these two weights) is an estimate of weight at a time point 12 h prior to the final weight measurement. In this case, the “consecutive” weights were 7 d apart. To account for weight gain or loss during the intervening period, the d -7 and d 0 weights were used in a 2-point regression, in which initial weight was the predicted weight 12 h prior to the time of the final weight measurement as with the common approach above. This approach maintained the ‘gut fill smoothing effect’ of including weights from consecutive days, while compensating for weight change across the 7 d interval between weights. Average weight from two consecutive dates (d 55 and 56) was used as the final body weight. Exit velocity and OCS were collected on d -7, 0,

14, 28, 55, and 56. Subjective chute scores were collected on d -7 and 56. On d 28 (14d after L5 booster), approximately 10 mL of blood was collected from each animal's jugular vein for analysis of leptospirosis titers and serum NEFA concentrations. Whole blood was collected into vacutainer (red-top) tubes (Becton, Dickson and Co., Franklin Lakes, NJ, USA) and kept on ice for ~1h prior to centrifugation at 3500 x g for fifteen minutes. Serum was stored at -20°C until analysis.

*Medication protocol.* Steers were examined daily and treated for sickness if required. In order for animals to be diagnosed as being “sick”, and thus qualifying for treatment, the animals must have displayed clinical signs (e.g., lethargy, emaciation, coughing, runny nose) and had a rectal temperature exceeding 39.7°C. Animals in this experiment were treated with a single subcutaneous injection of Draxxin (2.5 mg/kg BW; Zoetis) when they were found to be sick. Cattle were rechecked on d 1, 2, and 5 subsequent to treatment, but none required additional treatment. Diagnosis of illness of animals was based on clinical signs at the discretion of the UK Beef Research Unit manager. No diagnostic testing was performed.

*Growth performance.* Performance measures were collected for three periods: 0 to 28, 28 to 56, and 0 to 56. Average daily gain (ADG) was calculated as the total body weight gain per period divided by the total number of days per period.

*Video Analysis.* Steers were recorded 24 hours a day, seven days a week for the duration of the study starting on d 0. Footage covering the 2 h time period beginning with feed delivery each day from d 1 to d 13 was used to quantify social interactions and feeding behavior. This time interval was chosen because feeding time is when cattle are most socially active and earlier work suggested that 2-hour intervals should be adequate

to identify dominance hierarchies (Kondo and Hurnik, 1990). Additionally, preliminary observations of the cattle on this study indicated that activity levels dropped off substantially after feed delivery. One observer scored animals for the duration of observations.

Animals were scored based on dyad interactions. Each time animals interacted, one animal was scored as the “dominant” animal while the other was scored as the “subordinate” animal. These counts were totaled for each animal each day resulting in a matrix of dominance scores with daily wins and losses listed for each possible pair. Additionally, all wins and losses were totaled by day for each animal and labeled as average daily interactions.

An animal was considered the winner in a given conflict with observation of one or more of the behaviors listed in Table 4.2 (adapted from MacKay, 2013). Additionally, the winner had to be able to change the behavior of the recipient steer (displace from feeder, move standing position, etc.).

Daily dominance scores were then used to calculate each of two dominance indices. The first index, average dominance index (ADI), was calculated according to Hemelrijk, et al. (2005). The dominance index reflects the number of times an individual has “beaten” a certain opponent as a proportion of the total number of interactions in which the pair was involved with each other, therefore  $w_{ij} = x_{ij} / (x_{ij} + x_{ji})$ , where  $x_{ij}$  refers to the number of times individual i was dominant over j and vice versa. The  $w_{ij}$  is the calculated dominance score for an individual compared with another individual. The average dominance index of the individual is the average of all its dominance indices with all its interaction partners, thus  $ADI = 1/N \sum_j w_{ij}$ .

The second dominance index evaluated was the David's score (DS) described in Hemelrijk et al. (2005), which is a weighted version of the ADI. Whereas ADI simply reflects numbers of wins and losses for an individual, with DS, each win or loss is weighted by the strength of the opponent, which is derived from that animal's win/loss ratio. Example calculations for each index can be seen in the Appendix (Table A1.1 and A1.2).

*Non-esterified fatty acids (NEFA).* An enzymatic colorimetric method assay was used to quantitatively determine NEFA concentrations in serum. A NEFA-HR(2) test kit (Wako Diagnostics) was used which relies on the acylation of coenzyme A (CoA) by fatty acids in the presence of added acyl-CoA synthetase (ACS). The produced acyl-CoA is oxidized by added acyl-CoA oxidase (ACOD) with the generation of hydrogen peroxide. With the presence of peroxidase, hydrogen peroxide permits oxidative condensation of 3-methyl-N-ethyl-N-( $\beta$ -hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple colored product. The concentration of the product was determined colorimetrically at 540 nm (Konelab 20XTi, Thermo Scientific).

*Leptospira Titers.* A microscopic agglutination test (MAT) was used to detect *Leptospira* serovar hardjo antibodies in serum (Steinman, 2007). The sample test was a set of serial dilutions, ranging from 1:100 to 1:6400. After 120 minutes of incubation, samples were read microscopically for agglutination. Titers were log normally distributed. Thus, the natural log of each animal's titer was used in statistical analysis.

*Statistical Analysis.* A distribution analysis was conducted for each variable in JMP 10 (SAS, Inc., Carey, NC, USA). Anything more than 1.5x the interquartile range below the first quartile or above the third quartile was flagged as a potential outlier. Two

individuals were determined to be outliers with low ADG, one of which had been treated for respiratory disease. These two animals were removed from analysis for all response variables. However, these animals' contributions to other animals' dominance scores were not excluded because sickness was not observed until after the observational period. When interactions were significant, means were separated using protected ( $P < 0.10$ ) Fisher's LSD.

Data were analyzed using individual steer as the experimental unit. Average daily gain, leptospirosis titers, and serum NEFA concentrations were analyzed using GLM procedure of SAS (SAS, Inc., Carey, NC, USA). The model statement included main effects of EV and OCS and their interaction and pen was included as a block. Dominance indices were analyzed separately for week 1 (7 d) and week 2 (6 d) using the mixed procedure of SAS allowing for repeated measures analysis; the model statement included EV, OCS, day, and their interactions, pen was included as a random effect.

Daily average activity measures were analyzed separately in four phases using the mixed procedure of SAS allowing for repeated measures analysis; the model statement included EV, OCS, day, and their interactions, pen was used as a random effect. Phases 1 (7 d) and 2 (6 d) matched up with "week 1" and "week 2" used in analysis of each dominance index. Phase 4 had missing data for all 12 days for two animals, 2 days for one animal, and 3 days for one animal. Thus, phases 3 (10 d) and 4 (12 d) were separated to account for missing data in one phase and not the other. Correlation analysis between ADG and activity counts was conducted using REG procedure of SAS. Activity counts during phase 3 was regressed against ADG from day 0 to 56. Denominator degrees of

freedom were estimated using the Kenward Roger approach and the autoregressive (1) covariance structure was used.

Main effects and interactions were considered significant at  $P < 0.10$ .

## Results

There were no EV by OCS interactions ( $P \geq 0.23$ ) for ADG, titers to leptospirosis antigen, or serum NEFA. Thus only main effects are presented in Table 4.3. High OCS animals had higher ( $P < 0.01$ ) daily gains than low OCS animals for days 0 to 28, 28 to 56 and 0 to 56. Gains across the 56-day study period were 0.25 kg greater for high, as compared with low OCS animals. Additionally, slow EV animals had higher ADG than fast EV animals for days 28 to 56 ( $P = 0.06$ ) and 0 to 56 ( $P = 0.02$ ). Slow EV animals had a higher antibody response to leptospirosis vaccine than fast EV animals ( $P = 0.05$ ).

There were no significant effects on serum NEFA concentrations ( $P \geq 0.13$ ).

Although they represent different ways of calculating dominance scores and have different scales, treatment effects on both ADI and DS were similar. The only difference in detected treatment effects was that during the first week of observations there was an OCS\*EV\*day interaction for ADI ( $P = 0.04$ ) and a trend for DS ( $P = 0.12$ ). However, in the second week of observations there was no three-way interaction for either ( $P \geq 0.39$ ). No other treatments by day interactions were detected ( $P \geq 0.19$ ).

During both week 1 and week 2 both ADI and DS had an OCS\*EV interaction ( $P \leq 0.01$ ). There were no consistent relationships established until the second week of the study, when “low, fast” and “high, slow” animals had larger dominance indices compared to their “low, slow” and “high, fast” counterparts (Figure 4.1).

There were no significant interactions between EV and OCS on daily activity counts for any of the four phases ( $P \geq 0.14$ ; Table 4.4). Activity counts were lowest on days that animals received vaccines (d 0 and d 14), and steadily increased across about a 10 d period subsequent to vaccination (Fig. 4.2). During phase 1 there was a significant interaction between OCS and day ( $P = 0.05$ ), although there were no differences between OCS treatments on any individual day ( $P \geq 0.36$ ). There were no significant effects during phase 2 ( $P \geq 0.32$ ). During phase 3 there were main effects of both EV ( $P = 0.10$ ) and OCS ( $P = 0.05$ ) where fast EV animals and low OCS animals were less active than their counterparts (Figure 4.2). Similar trends with EV ( $P = 0.16$ ) and OCS ( $P = 0.15$ ) were seen during phase 4. Average daily activity counts during phase 3 were significantly correlated to total ADG ( $R^2 = 0.17$ ;  $P = 0.02$ ; data not shown).

## **Discussion**

The difference in gain between fast and slow EV was expected based on results from previous studies (Burrow and Dillon, 1997; Voisinet et al., 1997; Müller et al., 2006; Burdick et al., 2011a; Bates et al., 2014). The cause and effect relationship between temperament measures and performance is not well defined. However, Cafe et al. (2011) suggested two potential causes for decreased ADG in steers with a fast EV. The first is that these animals may have a decreased DMI as a consequence of a higher stress response, as seen previously in temperament literature (Cafe et al., 2011; MacKay et al., 2013). Animals can have a depressed DMI due to increased lipolysis and NEFA oxidation for energetic fuel (Allen et al., 2009). Although we did not detect a significant difference in circulating NEFA concentrations in the present study, previous studies have found that fast EV animals tend to have higher NEFA concentrations (Sanchez et al.,



2012; Burdick Sanchez et al., 2014). The second speculation was that animals with a fast EV might have greater energy expenditure due to an increase in vigilant behavior, resulting in poorer feed efficiency (Cafe et al. 2011), as seen previously (Petherick et al., 2002). Cafe et al. (2011) reported no relationships between EV or chute score with feed efficiency, suggesting that differences in intake may play a larger role than efficiency effects. Because of the experimental design, individual intake was not measured in the present study. However, in the companion study (Chapter 5), we also found evidence of lower intake in high EV steers. One potential explanation for lower intakes for high EV animals would be that measures of EV could be related to social interactions among animals, as discussed below.

Fast EV animals are, in theory, animals with a heightened stress response. Activation of stress response mechanisms can suppress adaptive immunological responses (Carroll and Forsberg, 2007). The depression in antibody titers in the fast EV group in the present study is in agreement with the general effects of chronic stress on immunological function. Additionally, these data agree with Oliphint (2006) in that cattle with high exit velocity had lower responses to vaccines (Oliphint, 2006).

Differences in gain between OCS treatments were notable for at least two reasons. First, the magnitude of response to OCS was almost twice the magnitude of response to EV in this study. Secondly, the direct relationship between chute score and ADG is in contrast to most reports, in which chute scores have been inversely related to animal growth (Voisinet et al., 1997; Cafe et al., 2011; Sebastian et al., 2011; Bates et al., 2014). One possibility is that our OCS could be measuring something different than the commonly used subjective chute scores. However, across all of the animals in this, and

the companion study (Chapter 5), OCS and subjective chute score (SCS) were positively correlated ( $R^2 = 0.38$ ;  $P < 0.01$ ). This correlation would be expected between these two measures, which were obtained simultaneously on each animal. Thus, it is unclear why chute scores in the present study were related to growth in the opposite direction of most published reports. However, these results are corroborated by data from a preliminary study at our facility with 192 steers in a 56-day growing study with a similar treatment structure (Chapter 3). In that study, high OCS cattle had greater DMI and tended to have greater gains than low OCS cattle, particularly in the first 30 days. Additionally, Graham (2001) reported a positive relationship between SCS and ADG in two of four sire groups studied (with no relationship found in the other two). This points to the need for a clearer understanding of the mechanisms that link temperament to growth. Thus, one of our key objectives was to determine whether social behaviors could help explain these relationships. Regardless of the mechanism, however, these results indicate that chute scores should not be confounded with measures of exit velocity when assessing “temperament”. These measures appear to be quantifying different behavioral components that can have different relationships with production characteristics and should be evaluated independently.

One possible explanation for the relationship between OCS and ADG could be that OCS is related to aggressiveness: high OCS animals may be more aggressive than low OCS animals and thus have increased access to resources because of their place in a dominance hierarchy. However, there were not consistent relationships between OCS and dominance in this study. In fact, the relationships were influenced by EV, such that high OCS animals that also had high EV tended to be subordinate. Thus, it appears that to

understand the relationships between OCS and ADG is not as simple as considering OCS as a measure of aggressiveness or social dominance. Additional research will be necessary to elucidate the nature of this relationship.

This study is one of the first that we are aware of to relate an objective chute score (OCS) with growth in beef cattle. Other studies have used objective measures to quantify temperament (Stookey et al., 1994; Sebastian et al., 2011), but none have used the same method as presented here. Stookey et al. (1994) created a movement-measuring device (MMD) that objectively quantified movement in the chute by measuring counts of peaks of voltage changes from their device taken over a one minute time period. Sebastian et al. (2011) used strain gauges to measure the amount of force exerted by the animals on the head gate while in the chute due to struggle. Because OCS had the opposite relationship to gain compared with exit velocity (high OCS animals having a higher ADG), it seems clear that it measures a different behavioral trait than does EV. Since OCS and EV were related to ADG, yet were not directly related to dominance score, results suggest that these are independent measures that do not define behavior of cattle in social situations. Additionally, because relationships between these two temperament measures and performance were found to be inverse, these results also suggest that different temperament measurements should not be combined for an average measurement without substantiated justification because it is not clear what specific behaviors are being measured and how they may combine to influence production parameters.

The effects of EV in this study are consistent with those of Chapter 3, where significant differences were seen in ADG between EV treatment levels. However, in the previous study, no significant effects of OCS on ADG were detected. Likewise, in the

companion study to this work (Chapter 5), no main effects of OCS or EV were detected for ADG. Differences between study results could be linked to housing differences. Chapters 3 and 5 had experimental designs where animals were grouped with animals of like temperament treatments, whereas animals in this study were grouped such that one animal from each treatment was represented within each pen. The pen allotment used in this study could allow for temperament treatments to have a more significant effect. If effects of temperament are at least partially driven by social interactions with other animals, being grouped with animals of different temperament treatments could help drive significant differences in average daily gains. However, the relationships between temperament measures and dominance ranking that were uncovered were not in alignment with the relationships between temperament measures and ADG. Thus, it is not clear whether grouping animals of like treatment together directly influences the impact of temperament on animal growth.

Treatment effects on activity counts during phase 3 and trends during phase 4 were inversely related to ADG, where slow EV animals and high OCS animals gained more than their counterparts. Thus, our results suggest that the more temperamental animals will have lower ADG and increased home pen activity. Additionally, in the present study there was a correlation between the final ADG (d 0 to 56) and daily activity counts during phase 3 ( $R^2 = 0.17$ ;  $P = 0.02$ ) where animals with a higher activity count gained less than those with less activity. Cafe et al. (2011) stated that differences in production could be due to more vigilant behavior of stressed cattle, which would be supported by our results where stressed cattle were more active overall. These results are consistent with previous findings (MacKay et al., 2013) where a relationship was seen

between average daily step counts and exit velocity (AKA flight speed), but inconsistent in that no correlation existed between chute score and daily step counts in that study. However, the chute score they used was a SCS, whereas this study used an OCS. It should be noted that the previous findings were found when activity-monitoring devices were placed on the leg of the animal as opposed to the ear as in our study. There are some inherent differences of activity monitoring because of placement; accelerometers mounted on the ear would be expected to have more variation than those mounted on the leg, while a monitor on the leg will primarily pick up on leg movements, such as walking, an ear-mounted monitor can detect anytime the ear moves, such as headshakes or licking from another animal. Regardless, results of this study add evidence that there are differences in home pen activity between EV and OCS treatments, where more temperamental animals will have increased activity. Increases in activity across the first two phases coincided with administration of vaccines on d 0 and d 14. Although it is well recognized that vaccine administration can decrease intake and general activity of cattle in the short term, to our knowledge, this is the first report to document the time course of the effects on activity. Here, activity counts required about 10 days to reach plateau following administration of booster vaccines on d 14.

The methodology used was chosen to encourage interactions to better measure dominance behaviors and hierarchies; morning hours, regrouping periods, and time in confined spaces are usually prime opportunity to observe social behaviors and dominance interactions (Hinch et al., 1981; Galindo, 2000). Feeding time was chosen as the optimal time for observation based on previous studies that found the highest frequency of displacements and interactions around feeding (DeVries et al., 2004; Val-Laillet et al.,

2008). Craig (1986) found that as resources are restricted, lower status animals can become excluded, while intermediate and top ranking animals continue to receive ample resources. Distributing feed in the center of the feed bunk and feeding at restricted levels were used to restrict resources and encourage dominance behaviors. Following the initial regrouping (d 0, when animals were allotted to pens by treatment), animals were not moved from their pens for 13 days when they were moved for weighing and temperament assessment.

Previous researchers have explained that a function of dominance among social animals is to reduce aggression within the group (Syme et al., 1979). It has been suggested that the primary role of aggressive behavior, has been to ensure adequate supply of resources to high status animals when competing with their own kind (Craig, 1981). Animals use aggressive interactions with others in the group to establish their position in the hierarchy and once the position is established it can be maintained for the most part (Canali et al., 1986). However, maintaining status can be more difficult for higher-ranking animals, as they spend more time maintaining this position than previously thought. Studies with goats, ibexes, and dairy cattle suggested that higher-ranking animals constantly reinforce their status using incessant aggression (Orgeur et al., 1990; Wierenga, 1990; Alados et al., 1994; Barroso et al., 2000). Thus, even in a stable group, the dominance hierarchy may not prevent aggression (Barroso et al., 2000).

The dominance hierarchy was expected to be a linear hierarchy because of the small number of animals per pen (Craig, 1986). Because the hierarchy was linear there was almost always a clear absolute dominant animal and an absolute subordinate animal, there were a few exceptions where there were two completely subordinate animals. The

time requirement for a dominance hierarchy to form in a herd of cattle is not well defined and has mainly been described as a ‘relatively short time’ (Oberosler et al., 1980) and likely depends on a number of factors including housing conditions and group size. Under the conditions of this experiment, during the first week there was a significant interaction between temperament treatments and day on ADI, which suggests that the dominance index was not yet stabilized. The lack of any significant interactions between temperament treatments and day during the second week indicates that the dominance hierarchies were stabilized within this time period. Under the conditions of this experiment beef steer dominance hierarchies appear to have been established within approximately one week of regrouping.

Other studies with ruminants found that there are some role reversals within the dominance hierarchy, generally with individuals dominating another of similar but slightly higher rank (Barroso et al., 2000); these results can vary with the species being observed. Dairy cattle have been found to have no one cow dominant over all others in the group (Beilharz et al., 1982; Val-Laillet et al., 2008), but these studies were done with larger groups of cows of varying ages. In a study by Barroso et al. (2000), dominant animals were sometimes displaced by a lower ranking animal; Beilharz et al. (1982) suggested that this reversal could be due to dominant cows at the feeder no longer being hungry and displaced by lower ranking, hungrier animals. Hunger could provide sufficient motivation to occasionally displace animals of a higher rank. Infrequent role reversals in this study can account for the occasional rise in average daily dominance score of lower ranked animals. Animals that were low ranking tended to “wait their turn”

during feeding, approaching the feed bunk after other animals had left, following establishment of rank.

No correlation between average dominance ranking and ADG was detected ( $R^2 = 0.05$ ;  $P = 0.22$ ). This result was not expected because of previous findings that higher-ranking animals have increased access to resources (Craig, 1986), that feeding behavior is significantly related to social rank (McPhee et al., 1964; Barroso et al., 2000), and a previous correlation between social ranking and weight gain (Blockey et al., 1974). However, early work with dominance ranking and production found that intake was affected by ranking but differences in gain were not detected (McPhee et al., 1964; Wagnon, 1965). Diets and amounts of feed offered (restricted vs. *ad libitum*) varied between studies, which could explain some variation in findings. There was also no correlation between dominance index and titer antibody response ( $R^2 = -0.007$ ). The findings of this study are not suggestive of any clear relationship between dominance and performance in beef cattle grouped in small pens. Research on the relationship between dominance, temperament, and growth performance are scarce in cattle. However, it does appear that there is some relationship between dominance index and temperament measures, although the relationship appears to be complex when temperament is described in terms of independent exit velocity and chute score measures.

## **Conclusions**

These results complement conclusions from previous literature, which have found slow exit velocity animals to have an increased average daily gains and a higher immunological response to vaccines compared to fast exit velocity animals. Additionally, animals with a high objective chute score, measured with our technique, had higher



average daily gains than animals with a low objective chute score, which is inconsistent with most previous works evaluating chute score and productivity. Previous research with chute score and productivity has utilized the traditional subjective chute score and found an inverse relationship compared to our results. However, our measure of objective chute score was positively correlated with the traditional measure of subjective chute score. Thus, these results highlight the need for further research of the relationship and mechanisms relating temperament measures and growth performance in beef cattle.

Daily activity counts were also related to temperament measures, but there is little available data to compare these results. Thus, future research could focus on activity measures of beef cattle for possible further understanding of temperament and growth. Moreover, there is an active dominance hierarchy in beef steers that required approximately one week to stabilize after regrouping but had no correlation to ADG or immune response to vaccines.

Temperament is thought to represent a general measurement of an animal's excitability and response to humans or a stress stimulus (Burrow, 1997); many short-term testing methods have been used to quantify and assign a numerical value to temperament in cattle. However, "temperament" as a general trait is complex with interactions of various behavioral responses, not limited solely to fear or aggression. Furthermore, relationships between measureable factors (like exit velocity and chute score) and behavioral characteristics (such as fear or aggression) are, and will remain, imperfect. These considerations, combined with the evidence from this study that exit velocity and chute scores have independent influences on production parameters indicate that more rapid advances will be made in application of these concepts by focusing on explicit

measures as opposed to the vague concept of “temperament”. Specifically, one method cannot be used exclusively to define temperament or relate temperament to performance. Instead, specific measures should be related to performance; for example, exit velocity is often directly related to productivity in beef cattle. Objective chute score can be used as another individual temperament measurement to predict productivity. The lack of a correlation between dominance and ADG in this study indicates that dominance is not a measure that can be used to predict productivity.

Table 4. 1. Ingredient composition of experimental diet

Feedstuff	% of diet DM
Corn Silage	46.17
Cracked corn	18.00
Soybean meal	11.00
Wheat Straw	22.00
Dicalcium Phosphate	0.08
Limestone	1.23
Potassium Chloride	0.50
Trace Mineral salt <sup>a</sup>	0.75
Urea	0.20
Vitamin ADE Premix <sup>b</sup>	0.05
Deccox	0.03

<sup>a</sup>Trace mineralized salt provided 92.9% salt, 68 ppm Co, 1838 ppm Cu, 120 ppm I, 9290 ppm Mn, 19 ppm Se, and 5520 ppm Zn.

<sup>b</sup>Vitamin premix supplied 1820 IU/kg Vitamin A, 363 IU/kg Vitamin D, and 227 IU/kg Vitamin E.

Table 4. 2. Behaviors and social interaction examples that define dominance indices

Dominant Social Behaviors	Category	Definition
Butting	Contact	An animal uses head to head, head to neck, or head to flank contact to displace another animal from the feed area
Pushing	Contact	An animal forcefully enters the feed area and displaces another animal from the feed area
Threatening	No contact	An animal takes up a threatening posture by presenting the head in the direction of the recipient animal, no contact occurs
Defending	Contact	An animal uses physical contact to push the recipient animal to the back wall of the pen and keeps threatening posture to ensure animal stays there
Mounting	Contact	An animal mounts another recipient animal and forces the recipient animal away from the feed area

Table 4. 3. Effect of temperament on ADG, immunological response, and serum NEFA concentrations

	Low OCS <sup>1</sup>	High OCS	Slow EV <sup>2</sup>	Fast EV	SEM <sup>3</sup>	P-Value		
						EV*OCS	OCS	EV
Initial EV, m/s	-	-	2.10	3.66	0.310	-	-	-
Initial OCS, %	1.14	2.72	-	-	0.218	-	-	-
Initial weight, kg	254	253	254	253	5.3	-	-	-
ADG, kg/d								
Days 0 to 28	0.67	0.94	0.86	0.75	0.094	0.36	< 0.01	0.27
Days 28 to 56	0.99	1.23	1.18	1.04	0.071	0.66	< 0.01	0.05
Days 0 to 56	0.83	1.09	1.02	0.90	0.058	0.23	< 0.01	0.02
Leptospirosis Titers, ln	6.29	6.21	6.58	5.93	0.311	0.23	0.81	0.05
NEFA, mEq/L	0.65	0.58	0.60	0.63	0.046	0.66	0.13	0.48

<sup>1</sup>OCS (objective chute score) = the coefficient of variation of 50 recorded weight values across a 10 s interval while the animal is restrained by the head in a chute.

<sup>2</sup>EV (exit velocity) = time taken for steers to travel 1.68 m upon exiting the chute

<sup>3</sup>SEM = standard error of the mean (n = 16).

Table 4. 4. Effect of temperament on ADI, DS, and daily activity counts

		Low OCS <sup>1</sup>		High OCS		P-Values			
		Slow EV <sup>2</sup>	Fast EV	Slow EV	Fast EV	SEM <sup>6</sup>	EV*OCS	OCS	EV
ADI <sup>3</sup>	Week 1	0.45	0.57	0.58	0.39	0.061	0.02	0.67	0.57
	Week 2	0.37	0.59	0.62	0.40	0.067	< 0.01	0.69	0.93
DS <sup>4</sup>	Week 1	-0.41	0.87	1.02	-1.31	0.677	0.01	0.58	0.44
	Week 2	-1.31	1.06	1.31	0.80	0.779	< 0.01	0.63	0.86
Daily Activity Counts <sup>5</sup>	Phase 1	1411	1188	1032	1520	235.6	0.14	0.92	0.58
	Phase 2	2178	2296	1842	2537	402.4	0.48	0.91	0.32
	Phase 3	2372	2936	1830	2271	297.2	0.83	0.05	0.10
	Phase 4	3132	3690	2353	3129	468.1	0.81	0.15	0.16

<sup>1</sup>OCS (objective chute score) = the coefficient of variation of the 50 recorded weight values across a 10 s interval while the animal is restrained by the head in the a chute.

<sup>2</sup>EV (exit velocity) = time taken for steers to travel 1.68 m upon exiting the chute

<sup>3</sup>ADI (Average Dominance Index) = calculated as the average of all dominance indices with all interaction partners

<sup>4</sup>DS (David's score) = a weighted version of the ADI

<sup>5</sup>Daily activity counts = average activity counts collected from electric accelerometer ear tags (Smith, 2012)

<sup>6</sup>SEM = standard error of the mean (n = 8)

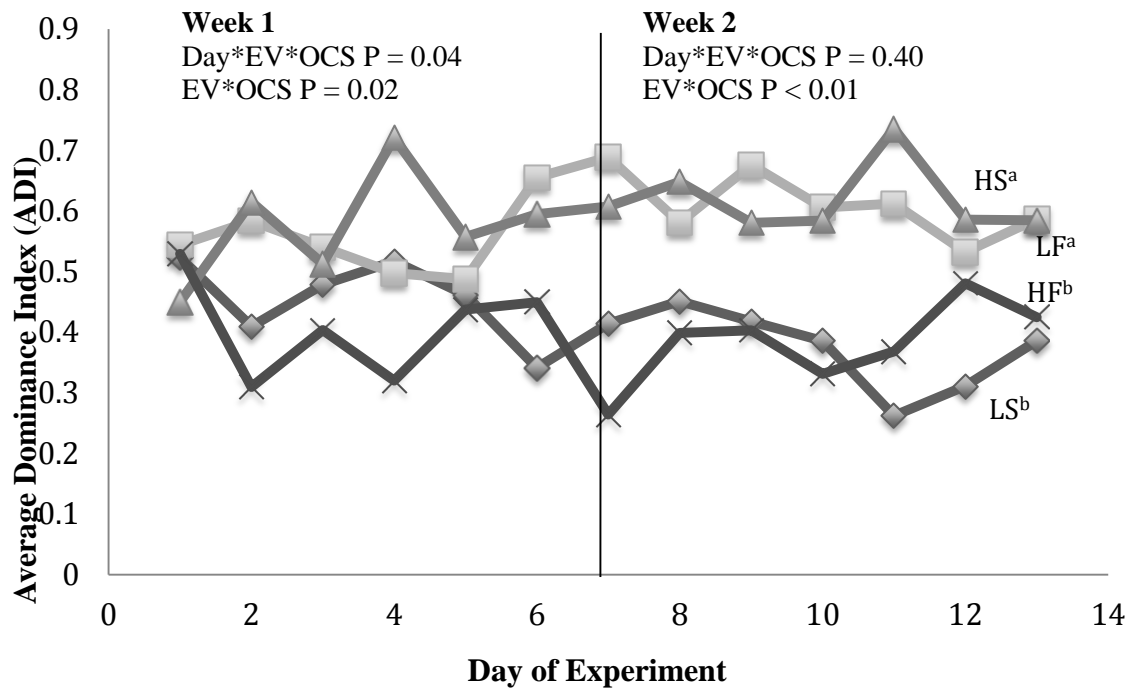


Figure 4. 1. Average dominance index (ADI) by day for each treatment type. During week 2, treatments with difference superscripts differ ( $P < 0.05$ ). LS= low OCS slow EV, LF=low OCS fast EV, HS=high OCS slow EV, HF=high OCS fast EV.

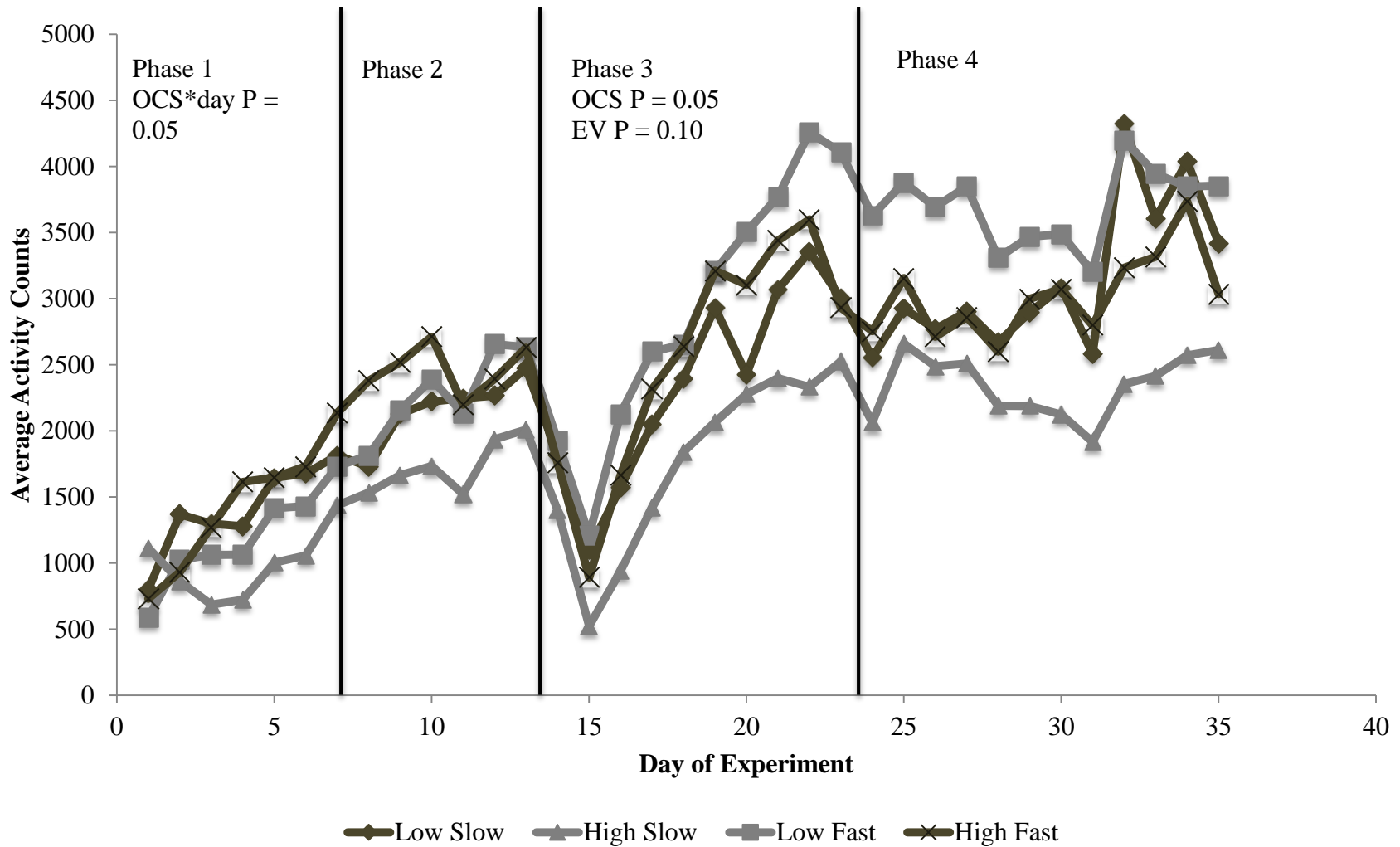


Figure 4. 2. Average activity counts by day for the first 35 days based on objective chute score (low or high) and exit velocity (slow or fast) treatment (n = 32). Phase 1 = d 1 to d 7, phase 2 = d 8 to d 13, phase 3 = d 14 to d 23, and phase 4 = d 24 to d 35.



## CHAPTER 5

### A NOVEL OBJECTIVE CHUTE SCORE INTERACTS WITH MONENSIN TO AFFECT GROWTH OF RECEIVING CATTLE

#### Introduction

Livestock temperament has been defined as the reactivity to handling by humans and to novel or threatening environments (Murphey et al., 1994; Burrow, 1997) and has been known to affect productivity of cattle, as well as overall profitability by increasing production costs (Burrow, 1997). Cattle with unfavorable temperaments tend to have decreased growth rate, immune response, and carcass quality (Voisinet et al., 1997; Oliphint, 2006; Carroll et al., 2007; Cafe et al., 2011).

Commonly, chute exit velocity and subjective chute score have been used to measure temperament (Grandin, 1993; Burrow, 1997; Curley et al., 2006) and these scores are often combined into a single measure of temperament (Cooke et al., 2009; Hulbert et al., 2011). Although chute score is commonly used, it is a subjective measure and can be difficult to replicate and validate. An objective measure of activity in the chute could potentially be a better temperament scoring system.

At present, it is unclear how temperament is related to animal growth. Some evidence suggests that temperamental cattle are less efficient than their less temperamental counterparts (Petherick et al., 2002), while other studies suggest that depressed intake is a major causative factor (Cafe et al., 2011). The hepatic oxidation theory (HOT) of intake regulation in ruminants (Allen et al., 2009) provides an interesting framework for understanding the relationship between intake and temperament. Of particular interest is the suggestion that increased propionate in stressed

cattle increases oxidation of fatty acids and decreases feed intake to a greater extent than would be seen in non-stressed animals (Allen et al., 2009). Thus, we hypothesized that provision of monensin, through its well-documented propionate-enhancing effect, would depress intake to a greater extent in cattle with temperaments that pre-disposed them toward lower ADG.

Thus, the objectives of this study were to evaluate an objective chute score measurement along with exit velocity as independent measures of temperament and to identify whether dietary ionophore supplementation interacts with temperament to influence gain, intake, health, and immunocompetence of newly received feeder cattle.

## **Materials and Methods**

All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

*Animals and treatments.* One-hundred sixty mixed breed beef steers ( $262 \pm 21$  kilograms) in 40 pens (4 steers/pen) were used in a randomized complete block design experiment with a 2 x 2 x 2 factorial treatment structure, using initial weight as the blocking factor. Steers were purchased from central Kentucky livestock auctions by an order buyer and were housed at the C. Oran Little Beef Research Unit in Woodford County, KY. Steers were comprised of several mixed breeds dominated by British influence. Within 24 h of arrival, cattle were weighed, ear tagged for individual identification, and temperament measures were obtained for treatment assignment.

Three temperament measures were obtained on each animal: 1) subjective chute score (Grandin, 1993), 2) objective chute score and 3) exit velocity (Burrow et al., 1989). Subjective chute score was completed by observing behavior in the chute (Silencer

hydraulic squeeze chute, Moly manufacturing, Lorraine, KS, mounted on load cells) for 10 s after the head was caught, without squeeze applied, and was represented as the average score across 4-5 observers. The 1-5 scoring scale used was that of Grandin (1993):

- 1: calm, no movement
- 2: restless shifting
- 3: squirming, occasional shaking of device (squeeze chute or scale)
- 4: continuous vigorous movement and shaking of device
- 5: rearing, twisting, or violently struggling.

Objective chute scores (OCS) were recorded during the same time interval as subjective chute scores. The scale head (Lynx, Mettler Toledo LLC, Columbus, Ohio) was programmed to export weights at 5 Hz via RS232 to a laptop computer (Acer Aspire V5, Acer America, San Jose, CA). During this period, animals were restrained in the head catch but otherwise undisturbed and OCS was determined as the coefficient of variation of the 50 recorded weight values across this 10 s interval. At the end of this ten-second period, squeeze was applied. Each animal's weight was calculated as the average of weights measured at 200 ms intervals during the most stable two-second interval subsequent to squeeze being applied.

Upon being released from the chute, flight time was measured over 1.68 m using an infrared sensor (FarmTek Inc., North Wylie, TX) and converted to exit velocity (m/s).

The experiment began (d 1) 8 days after the initial measures of temperament were obtained. Routine processing (d 1 except where indicated) included: 1) ear tag placement for unique animal ID number (at arrival), 2) viral and bacterial vaccinations (Bovi-Shield

Gold 5, Zoetis, Florham Park, NJ; Once PMH, Merck Animal Health, Summit, NJ; Somubac, Zoetis; Ultrachoice 7, Zoetis), and 3) an injection of de-wormer (Dectomax, Zoetis). Cattle were reimmunized on d14 with Ultrachoice® 7 and Somubac®. Additionally, to determine the ability of steers to mount an immunological response to an antigen to which they were naïve, steers were vaccinated with a leptospirosis vaccine (L5 SQ, Merck Animal Health) on day 1 and administered a booster on day 14. Earlier experiments in our laboratory (unpublished) have found only about 0.5% of steers to have a measureable serum leptospirosis titer prior to vaccination.

Animals were assigned to treatments within weight strata. Treatments included two levels of monensin supplementation (0 or 40 g/T DM monensin), two levels of exit velocity (fast or slow) and two levels of OCS (high or low) arranged in a 2 x 2 x 2 factorial. Temperament treatments were established based on the exit velocity and OCS measured on each animal during its first exposure to the handling facility. Steers were assigned to pens such that each pen had four animals of like treatment. The experimental unit was pen, consisting of 40 pens total and 5 pens per treatment.

Because the two treatment factors were inherent characteristics of the animals (as contrasted with exogenously applied treatments), the ability to divide the animals into these treatment groups depended on the independence of and the relationships of the distributions of the treatment variables. Thus, prior to developing an allotment strategy, the distributions of the two factors were evaluated using SAS JMP (SAS, Inc., Cary, NC) and the selected allotment strategy (division into OCS groups preceding division into EV groups) was the one which provided the least overlap among treatments. Means and standard errors for the treatment factors are presented in Table 5.2. However, a

comparison of the distributions of the treatment variables is more informative. Such comparisons are provided in Figures 5.1 and 5.2. Because the animals were divided into treatment groups from a larger set of animals ( $n = 192$ ), and because meaningful distribution curves could not be constructed from the subset ( $n = 32$ ) used in the companion experiment (Chapter 4), these figures depict the distribution of the treatment variables for all animals used in two companion experiments. Of eleven distribution types compared within SAS JMP, the Johnson SI distribution provided the best fits (based on Akaike's Information Criterion) for both OCS and EV. Because animals were first divided into high and low OCS groups, there was essentially no overlap in the distribution of OCS for either of the high and low OCS treatment groups (Fig. 5.1). Additionally, within both EV groups, there was substantial similarity in the OCS distributions of each of the OCS groups. Thus, the allotment strategy provided for a clear delineation between high and low OCS treatments. Alternatively, EV allotments were determined subsequent to the establishment of the two OCS groups. Thus, EV delineations were not as distinct as those for OCS (Fig. 5.2). However, within each level of OCS (high and low), there was essentially no overlap between the EV distributions of the fast and slow groups. A high degree of overlap existed between the "slow" EV, high OCS group and the "fast" EV, low OCS group. More importantly, the EV distributions differed somewhat for the high and low OCS groups. This situation would be expected to increase the likelihood of detecting interactions between EV and OCS and would need to be considered in any interpretation of interaction effects. However, the distributions depicted in Figure 5.2 indicate sufficient delineation in EV between the "fast" and "slow" groups as a whole to suggest confidence in main effects attributed to this factor. Overall,

this approach increased the power of our design for detecting differences between OCS treatments while compromising on the ability to detect EV effects. However, the degree of confounding would have been greater (greater overlap between distributions) if the alternate strategy had been chosen.

Steers were randomly assigned to pen and treatment within weight blocks. Steers were housed in 2.44 by 14.63 m pens within a three-sided, concrete floored barn. Each pen had a 2.44 linear meter bunk along the fence line (0.61 m bunk space per animal). Pens were scraped clean and bedded with sawdust routinely.

*Diet.* Each pen was fed a corn silage-based total mixed rations (TMR; Table 5.1.) either with or without monensin once daily at approximately 0700 h. Diets were prepared and adjusted daily to provide ad-libitum intake with minimal amounts of feed refusals. To this end, feed bunks were observed twice daily at 0700 and 1500 h when the remaining amount of feed in the bunk of each pen was estimated. Ingredient dry matters were determined once weekly by drying samples for 24 hours in a forced air oven (100°C, Model 1690, VWR Scientific Products, Corneilius, OR, USA) and rations were adjusted accordingly. Steers had free access to water; adjacent pens shared a water source.

Once weekly, feed refusals were collected, weighed, recorded, and combined within monensin treatments and blocks. Treatment composites were sub-sampled and dry matter was determined (duplicate 250-500 g samples dried at 100 C for 24 h or until constant weight) and recorded. Any feed refusals on the floor outside of the bunk were weighed, but not included in the orts sample for DM determination.

Animals were not withheld from feed or water prior to weighing, although weights were obtained prior to feeding. Animal weights were recorded on d -7, 0, 14, 28,

55, and 56. A weighted average of the d -7 and d 0 weights was used as the initial weight. This weighted average was based on the idea that with consecutive day weights taken at 24 h apart (which are commonly used to minimize weight difference due to gut fill), the estimate of weight contains an equal weighting of each of the two days' weight measurements, which results in an estimate of each animal's weight at a time point 12 h prior to of the final weight measurement. In this case, the "consecutive" weights were 7 d apart. To account for weight gain or loss during the intervening period the d -7 and d 0 weights were used in a 2-point regression, in which starting weight was the predicted weight 12 h prior to the time of the final weight measurement (similar to the consecutive day approach). This approach maintained the 'gut fill smoothing effect' of including weights from consecutive days, while compensating for weight change across the 7-day interval between weights. Average weight from two consecutive dates (d 55 and 56) was used as the final body weight. Exit velocity and OCS were collected on d -7, 0, 14, 28, 55, and 56. Subjective chute scores were collected on d -7 and 56. On d 28 (14 d after L5 booster), approximately 10 mL of blood was collected from each animal's jugular vein for analysis of leptospirosis titers and serum NEFA concentrations. Whole blood was collected into vacutainer (red-top) tubes (Becton, Dickson and Co., Franklin Lakes, NJ, USA) and kept on ice for ~1h prior to centrifugation at 3500 x g for fifteen minutes. Serum was stored at -20°C until analysis.

*Medication protocol.* Steers were examined daily and treated for sickness if required. In order for animals to be diagnosed as being "sick", and thus qualifying for treatment, the animals must have displayed clinical signs (e.g., lethargy, emaciation, coughing, runny nose) and had a rectal temperature exceeding 39.7°C. The treatment

regimen consisted of a single subcutaneous injection of Draxxin (2.5mg/kg BW; Zoetis) for respiratory disease (indicated by breathing through mouth, by incidence and a scoring scale of lethargy, unusual breathing, nasal discharge, quantity of discharge, and character of discharge as well as a temperature of 39.7°C or over) or a single subcutaneous injection of Noromycin 300 LA (4mg/kg BW; Norbrook, Newry, Northern Ireland) for pinkeye (indicated by tearing or photophobia). One animal was treated with PenJect (6ml/100kg BW, Butler Schein Animal Health, Dublin, OH) for an injured leg. Animals were rechecked on d 1, 2, and 5 subsequent to treatment. Second and third treatments for respiratory disease were only warranted if animals failed to respond to the initial treatment. The order of medical treatment consisted of 1) a single subcutaneous injection of Draxxin (2.5 mg/kg BW) , 2) a single subcutaneous injection of Nuflor (40 mg/kg BW; Merck animal health), and 3) a single subcutaneous injection of Baytril (7.5 mg/kg BW; Bayer HealthCare Animal Health division, Shawnee Mission, KS). Only one animal was treated more than three times, for a leg injury. Two animals in this study were removed due to indications of respiratory disease and bloat; these animals were treated three times and failed to respond to treatment before being removed. Diagnosis of illness of animal was based on clinical signs and at the discretion of the UK Beef Research Unit manager. No diagnostic testing was done.

*Growth performance.* Performance measures were collected for three periods: 0 to 28, 28 to 56, and 0 to 56. Dry matter intake (DMI) was calculated for each pen by subtracting the accumulated feed from the total amount of feed offered, during that period. Average daily gain (ADG) was calculated for each animal as the total body weight gain per period divided by the total number of days per period. Gain to feed ratio



(G:F) was calculated as body weight gain divided by feed intake for each pen and calculated for each period.

*Non-esterified fatty acids (NEFA).* An enzymatic colorimetric method assay was used to quantitatively determine NEFA concentrations in serum. A NEFA-HR(2) test kit (Wako Diagnostics) was used which relies on the acylation of coenzyme A (CoA) by the fatty acids in the presence of added acyl-CoA synthetase (ACS). The produced acyl-CoA is oxidized by added Acyl-CoA oxidase (ACOD) with the generation of hydrogen peroxide. With the presence of peroxidase, hydrogen peroxide permits oxidative condensation of 3-methyl-N-ethyl-N-( $\beta$ -hydroxyethyl)-aniline (MEHA) with 4-aminoantipyrine to form a purple colored product. This product was measured colorimetrically at 540 nm.

*Leptospirosis Titers.* A microscopic agglutination test (MAT) was used to detect *Leptospirosis* serovar hardjo antibodies in animal serum (Steinman, 2007). The sample test was a set of serial dilutions, ranging from 1:100 to 1:6400. After 120 minutes of incubation, samples were read microscopically for agglutination. Titers were log normally distributed. Thus, the natural log of each animal's titer was used in statistical analysis.

*Morbidity.* The prevalence of bovine respiratory disease was used as the measure of morbidity. Animals were broken into groups of animals treated once or animals treated more than once. Most cases of respiratory disease occurred during the first two weeks after arrival (d -7 to d 6). Thus, most cases were not attributed to monensin treatment, which was initiated on d 1. However, because temperament treatments are inherent characteristics of individual animals, we were able to analyze relationships between

temperament treatments and morbidity for d -7 through d 0. Therefore, the model statement for morbidity only included EV, OCS, and their interactions.

*Statistical Analysis.* A distribution analysis was conducted for each variable in JMP 10 (SAS, Inc., Carey, NC, USA). Anything more than 1.5x the interquartile range below the first quartile or above the third quartile was flagged as a potential outlier. Three animals were removed from analysis because they were found to be outliers for ADG; two of the animals were treated at least once for respiratory illness and described as “lethargic”. The pens with these animals were removed and handled as missing data points for analysis of DMI and G:F, the pen averages with the individuals removed were used for ADG, leptospirosis titers, NEFA concentrations and morbidity analysis.

Data were analyzed using pen as the experimental unit. All performance data (ADG, DMI, G:F), leptospirosis titers, and serum NEFA concentrations were analyzed using GLM procedure of SAS (SAS, Inc., Carey, NC, USA). The model statement included main effects of EV, OCS, monensin, all interactions among these three, and block. The model statement for analysis of morbidity contained the effects of EV, OCS, and their interactions using CATMOD procedures of SAS (SAS, Inc., Carey, NC, USA).

Time effects on EV and OCS were analyzed using the mixed procedure of SAS allowing for repeated measures analysis. The model statement included EV, OCS, day, and their interactions. Denominator degrees of freedom were calculated using the Kenward Roger method. Day was specified as the repeated term, and a first-order autoregressive structure was used for the error variance/covariance matrix. Time effects were characterized using linear, quadratic, and cubic contrasts.

Regression analysis between objective and subjective chute scores, between initial EV and ADG, and between initial OCS and ADG were conducted using the regression platform in the Analysis ToolPak add-in of Microsoft Excel (version 15.0).

Inter-rater reliability of SCS was calculated using Krippendorff's alpha (Hayes and Krippendorff, 2007). This statistic was used as a reliability index because it can be used with any number of observers, accommodates missing data, and satisfies all of the important criteria for a good measure of reliability. Krippendorff's alpha values and confidence intervals were determined using a SAS macro available from:

<http://www.afhayes.com/spss-sas-and-mplus-macros-and-code.html>. The measurement type was specified as "ordinal" and a bootstrap sample size of 10,000 was used for confidence interval construction.

Main effects and interactions were considered significant at  $P < 0.10$ .

## **Results**

Using measures collected from all the animals in this study as well as the companion study (Chapter 4), OCS and SCS were positively related. Coincidentally, objective chute score had approximately a 1:1 relationship with SCS, meaning that a score on one scale would be roughly equivalent to a score on the other scale.

Objective chute score and EV means within treatment groups changed over time (Figures 5.4. and 5.5.). There was a significant day by OCS interaction ( $P = 0.01$ ) and day by EV interaction ( $P < 0.01$ ). The time effects of the low OCS treatment were characterized by a linear ( $P < 0.01$ ) and quadratic ( $P = 0.09$ ) effect; time effects of the high OCS treatment were characterized by a quadratic ( $P < 0.01$ ) effect. Generally, OCS decreased for all animals, except for an initial increase in low OCS animals from the first

to the second experience in the chute. The time effects of slow and fast EV treatments were characterized by linear ( $P \leq 0.02$ ) and quadratic ( $P < 0.05$ ) effects. Fast EV animals tended to have a consistent decrease in EV over time, while slow EV animals had little difference in EV from the first to the last experience in the chute.

There was a low but significant positive correlation between OCS and ADG ( $P = 0.10$ ; Figure 5.6.). A correlation between initial EV and ADG was not detected ( $P = 0.17$ ; Figure 5.7).

No three-way interactions between diet, EV and OCS ( $P \geq 0.17$ ) nor two-way interactions between EV and OCS ( $P \geq 0.24$ ) were detected for the variables analyzed and reported (Table 5.3).

There were no treatment effects for ADG during the first 28 days, although there was a trend ( $P = 0.12$ ) for low OCS steers to outgain high OCS steers during this period (Table 5.4). There was an OCS by monensin interaction ( $P \leq 0.07$ ) for ADG during d 28 to 56 and d 0 to 56. Viewed in light of OCS effects, high OCS steers gained more (0.11 kg/d) than low OCS steers ( $P < 0.10$ ) when receiving monensin, but not ( $P > 0.10$ ) when receiving the control diet. Intake, expressed either in absolute amounts or as a percentage of body weight, did not display a monensin by OCS interaction ( $P \geq 0.41$ ), and monensin depressed intake ( $P < 0.01$ ) in each 28 day period and across the 56 day study. In the absence of an interaction with OCS in the first 28 days ( $P = 0.24$ ), monensin increased G:F ( $P = 0.02$ ). Interactions between monensin and OCS on G:F were similar to those seen for ADG, with significant interactions detected during the second 28-day and overall 56-day periods. As with ADG, G:F was improved ( $P < 0.10$ ) by monensin in these two time periods for the high OCS group only, and high OCS animals had greater G:F

compared with low OCS animals ( $P < 0.10$ ) only when receiving monensin. Serum NEFA concentrations were lower ( $P = 0.01$ ) in steers receiving monensin, and were unaffected ( $P \geq 0.36$ ) by temperament treatments.

Significant effects of EV were confined to ADG during the second 28-day period of the study, and to measures of morbidity. Steers with fast EV had lower d 28 to 56 ADG, and more animals treated once for a respiratory disease ( $P = 0.10$ ) compared with slow EV steers.

Titer responses to leptospirosis vaccine were affected by a monensin by OCS interaction ( $P = 0.05$ ; Table 5.3.) and a monensin by EV interaction ( $P = 0.02$ ; Table 5.3.). Steers with a high OCS had a decreased titer response ( $P < 0.10$ ) with supplementation of monensin and fast EV animals also had a lower response ( $P < 0.10$ ) with monensin supplementation.

## **Discussion**

### *Objective vs. subjective chute scores*

Objectivity of temperament testing may be compromised by an observer bias (Boivin et al., 1992) and such bias may be unavoidable in behavior studies, but mechanical instruments used in behavior research may not give complete information about animal temperament (Lyons, 1989). Additionally, Bates et al. (2014) suggested that a higher repeatability of EV than subjective chute score (SCS) could be due to the objective nature of the EV testing method. Although we used OCS as the primary measure in this study (i.e. we assigned treatments based on OCS), we felt it was important to collect SCS data to allow for comparison with existing literature, and to help characterize our use of OCS. The methodology for measuring SCS in this study was

designed so that more than one observer scored each animal in order to be able to assess inter-rater reliability. Previous literature either did not specify the number of observers (Curley, 2006; Oliphint, 2006) or used only one observer (Voisinet et al., 1997; Cafe et al., 2011; Fransisco et al., 2012). In our study, 4-5 observers scored each steer during observation periods and final SCS used for comparison with OCS were an average of all observers. Inter-rater reliability of SCS varied considerably between observations conducted at the beginning (d -7) and end (d 56) of the study. On d -7, Krippendorff's alpha values for repeatability amongst observers was 0.58 (95% CI 0.53 to 0.64), whereas on d 56, Krippendorff's alpha was 0.67 (95% CI 0.62 to 0.73). The marginal values, coupled with the large variation in repeatability estimates obtained at different times, indicate that the subjective nature of SCS is potentially problematic from the standpoint of collecting reliable data. Additionally, average SCS scores were more highly correlated with OCS scores than any single observer's score. Hence, using more than one observer when collecting SCS data could be a more accurate method.

The measure of objective chute score presented in this study is a new technique to objectively measure temperament in beef cattle. A handful of other studies have used objective measures to quantify movement in the chute (Stookey et al., 1994; Sebastian et al., 2011), but none have used the same method presented here. Stookey et al., (1994) used a movement-measuring device (MMD) to quantify movement in the chute by measuring counts of peaks of voltage changes from their device taken over a one minute time period. Additionally, MMD measures, in addition to the amount of force (strain force) that an animal exerts on the head gate after being caught in the chute has been used as an objective measure, where minimum, maximum, standard deviation, and absolute

force were collected (Sebastian et al., 2011). In that study, MMD was positively correlated with EV, whereas strain force was negatively correlated with EV. In the present study, OCS was determined while the animals' heads were restrained. Additionally, our OCS measures accounted for the magnitude of force exerted over the measurement period, unlike Sebastian et al. (2011) MMD measures, which were obtained on unrestrained animals and accounted for number of movements, as opposed to force of movements. Our OCS measure probably relates more closely to the strain gauge measures of Sebastian et al. (2011) which accounted for force exerted while animals were in a head catch.

Results from our regression analysis indicated that OCS and SCS were positively correlated, and that OCS had approximately a 1:1 relationship with SCS. A positive correlation would be expected between OCS and SCS, which were obtained simultaneously on each animal. However, it is worth mentioning that OCS would not be expected to detect many differences that SCS observers may notice and can account for. For example, SCS can account for other behaviors aside from moving in the chute that can indicate stress, such as pinned ears and vocalizations. It can also account for environmental conditions that may influence the response, such as loud noises or effects of weather conditions. Lyons et al. (1989) suggested that mechanical devices are only capable of detecting the most simplistic aspects of behavior. Sole use of an OCS can miss these behaviors or circumstances and animals can be assigned unfitting scores. It is possible that combined OCS and SCS data could be utilized to better characterize animals behavior than either alone. However, we believe that, with the demonstration of

relationships between OCS and growth performance of cattle, the objective and quantitative characteristics of OCS render it a superior measure compared with SCS.

*Repeatability of temperament measures*

Previous studies have found that animals can calm down and habituate to handling over time (Burrow and Dillon, 1997; Kilgour et al., 2006; Petherick et al., 2009; Cafe et al., 2011) and that animals can become accustomed to repeated non-aversive procedures (Temple, 1997), thus altering temperament measures over time. Both OCS and EV changed over time within treatment groups. In all cases, time effects were quadratic in nature, though the shapes of the curves differed amongst treatments. The OCS scores of both low and high treatment groups decreased over time, except for an initial increase from d -7 to d 1 in the low OCS group (Figure 5.5). Generally, our findings of change in OCS over time are consistent with previous findings where SCS decreased over time (Kilgour et al., 2006; Cafe et al., 2011), although it should be noted that these comparisons were with subjective measures of chute activity. Fast EV group scores decreased over time, while slow EV group scores changed subtly with a minor increase during the first 28 days followed by a minor decrease over the last 28 days, as indicated by the quadratic nature of the line. Our results are consistent with others, who found that exit velocity decreases over time, particularly in high EV animals (Curley et al., 2006; Petherick et al., 2009; Cafe et al., 2011), suggesting the idea that animal temperament measures can decrease with increased handling. However, other researchers have found that exit velocity initially increased from the first handling experience to the second or third handling, and decreased thereafter (Petherick et al., 2002; Kilgour et al., 2006), suggesting that, initially, repeated restraint of cattle can lead to increased flight



speeds due to increased fearfulness, and subsequent reduction of EV could be due to higher liveweights (Petherick et al., 1998, 2002). Regardless of whether or not temperament measures increased or decreased, most important is that EV and SCS have been found to change over time. Additionally, significant differences in mean EV between EV treatment groups on days 55 and 56, suggests that, although animals can habituate to handling, differences in temperament were persistent through the 56 d of this experiment. Thus, it is possible that the physiological differences between temperament levels also persist over time, such that more temperamental animals will continue to have a decreased growth performance. Moreover, if EV, SCS, and OCS decrease over time, the “temperamental” animals may be harder to identify after they have been acclimated to facilities and handling procedures. For this reason, and for practical application of management strategies to account for differences in temperament, it would seem to make more sense to use temperament measures obtained during the initial exposure to a new environment.

Considerable variation exists in the literature in the time periods used to determine temperament ranks within SCS or EV. Some researchers have used the initial measures, whereas some have used an average of all measures across a study when separating animals into temperament ranks. However, as seen in this study and others, these measures can change over time as animals habituate to handling (Burrow and Dillon, 1997; Curley et al., 2006; Cafe et al., 2011). Additionally, general trends in EV and SCS scores over time vary in the literature, but regardless of whether measures increase or decrease, time effects are generally present (Curley et al., 2006; Cafe et al., 2011; Sebastian et al., 2011). We believe the increase in OCS between d -7 and d 1 is important

with regard to interpretation of this data. As described, OCS treatment groups were established based on this measure, and, importantly, as discussed below, the relationships between OCS and growth in this study are opposite those reported previously for relationships among SCS and growth. One possibility is that OCS measurement on first exposure to the working facilities detected a fear response that was expressed through “freezing” behavior, i.e. lower movement, as compared with subsequent measurements. We suggest that the initial measure of these tests may, in some cases, uniquely represent an animal’s response to its first handling experience which differ from an average score calculated over time. Therefore, initial measures of EV, OCS, and SCS could provide a more accurate representation of an animal’s unique behavioral response to human handling or a new environment, before they become habituated. At a minimum, initial and average measures should not be considered as equivalent measures.

Previous studies found significant positive correlations between exit velocity and SCS (Petherick et al., 2003; Cooke et al., 2009; MacKay et al., 2013). However, it is important to note that in this study there were no interactions and weak correlations ( $OCS = 1.01 \pm 0.13 + 0.45 \pm 0.04 \times EV$ ;  $R^2 = 0.09$ ;  $P < 0.01$ ; data not shown) between EV and OCS, suggesting that these measures evaluate different behavioral responses.

#### *Relationships between measures of temperament and measures related to production*

Interactive monensin x OCS effects on ADG are inconsistent with previous findings that animals with a lower chute score will have higher ADG (Voisinet et al., 1997; Bates et al., 2014). The results of this study suggest that the “poor temperament” animals may not have a high OCS when measured at arrival, but rather a low one. Poor temperament in cattle is often ascribed to animals that are “excitable” or act

“aggressively” in the chute, including pushing, kicking, or trying to escape from being restrained. These reactions are generally considered to represent their fear response to human handling, characterized by their movement in the chute. However, animals with a poor temperament can also display a “freezing” behavior (Burrow, 1997a; Cooke et al., 2009), where with restraint the animal will not fight back but rather keep still from fear. The low OCS animals could be representative of the animals that freeze in response to fear. The increased activity in the chute from high OCS animals could be due to a few reasons. One explanation could be that the high OCS may not represent a fear response to human handling, rather a response to confinement or social isolation (Petherick et al., 2002, 2003). Alternatively, another explanation could be that the high OCS reflects a situation-specific fear response, rather than the intrinsic fearfulness of an animal, which could be why high OCS animals did not have the decreased growth performance (Petherick et al., 2002). Finally, the high OCS, as a product of a restraint test, could represent general agitation of an individual animal (Kilgour et al., 2006) and not necessarily a reaction to human handling, explicitly.

Because the low OCS steers could be animals that are predisposed to a lower ADG by inherently having a more reactive stress response or a “poor temperament”, we hypothesized that the inclusion of monensin could further decrease ADG. The negative effects of monensin on growth of low OCS animals would appear at first glance to fit our hypothesis that animals with a more reactive stress response would be more negatively affected by monensin. However, our hypothesis was based on expected differences in intake. Our data indicate that, although monensin depressed intake, it has similar effects regardless of temperament group. Additionally, no interactions were observed between

EV treatments and monensin consumption, also in disagreement with our hypothesis. At present, we do not have an explanation for the observation that monensin improved efficiency in high OCS but not low OCS cattle. Nonetheless, this observation has practical implications in that it suggests that different dietary management strategies may be appropriate for cattle of different temperament classifications.

Müller et al. (2006) suggested that because there is a genetic component to EV, there might be individual differences in genetic disposition and physiology of animals that can contribute to differences in the relationship between ADG and EV. These inherent individual differences may account for varying effects of OCS on ADG as well. Petherick et al. (2002) indicated that environment and previous experiences could alter temperament response in these short-term tests. Therefore, some temperament tests are more likely to measure intrinsic fearfulness of the animals, whereas others reflect situation-specific fear resulting from environmental or previously experienced conditions. These explanations can account for some variation seen in temperament responses and varying relationships with growth performance.

Intakes over the 56-day study were decreased by 0.92 kg/d with the inclusion of monensin, which is consistent with previous research; monensin is commonly used to improve efficiency by increasing ADG, though it generally decreases intake (Goodrich et al., 1984). Monensin causes increases in ruminal propionate concentrations. The flux of propionate during meals stimulates oxidation of acetyl CoA, ultimately generating ATP and decreasing intake (Allen et al., 2009). However, the original hypothesis that inclusion of monensin would differentially affect intake of animals with different temperaments was not supported. Monensin did not have a greater effect on intake in “temperamental”

animals. Further research should be conducted to clarify the existence of a relationship between temperament and ionophore supplementation.

Slow EV animals tended to have a greater absolute DMI than fast EV animals during the second period of the study as well as the total 56-day study. Decreased intake for steers with a fast EV, compared to steers with a slow EV, has been seen previously (Cafe et al., 2011; MacKay et al., 2013; Burdick Sanchez et al., 2014). However, absence of effects on DMI as a percentage of body weight in the present study indicate that the differences in intake among EV treatments could be attributed to differences in body size. Regardless, the numerical trends in intake were consistent with previous work as well as data in Chapter 3. Additionally, exit velocity had a significant effect on ADG during the second period and a trend during the 56-day study period. Previous studies have mentioned that having a small range of EV or SCS, where cattle were more docile, may affect impact of temperament on performance (Graham et al., 2001); although, the range of EV and OCS within our data are similar to previous studies.

Differences in DMI and ADG between differing EV ranks have also been seen in previous research (Petherick et al., 2002, 2003; Cafe et al., 2011; Bates et al., 2014). Because there were trends between EV and DMI, but not G:F, we suspect that ADG is primarily altered by differences in intake rather than efficiency. Cafe et al (2011) suggested that because EV commonly affects ADG and DMI, but not efficiency, differences are tied to behavioral reasons rather than metabolic differences, such as the more vigilant behavior of stressed cattle influencing time at the bunk. The results of Chapter 4 indicate that there is some correlation between ADG and home pen activity when animals have an ear tag accelerometer. However, to argue that decreased feeding

time causes lower intake, as opposed to lower intake causing decreased feeding time is somewhat paradoxical. It seems more likely that intake is regulated by an inherent biological mechanism, and that feeding time is a consequence, rather than a driver, of lower intake. Activation of the stress response in more “temperamental” animals stimulates the activation of the HPA axis and secretion of stress-related neurohormones, such as CRH, epinephrine, and ACTH, which inhibit feed intake and digestion (Burdick et al., 2011a). Thus, we suspect that differences in intake, where they exist, between EV treatments are directly affected by endocrinological factors related to stress and temperament and that decreases in feeding time are a consequence of decreased appetite.

*Relationships between measures of temperament and health-related measures*

While the animals’ titer responses to the leptospirosis vaccine were significantly affected by interactions between monensin and OCS, there were no significant effects of OCS in either the control or monensin-supplemented groups. Likewise, OCS had no effect on this response in our companion study (Chapter 4). The results did indicate a decrease in antibody titer response in fast, but not slow EV cattle, with the inclusion of dietary monensin. This response is difficult to align with the growth response to treatments, which was essentially unchanged by monensin supplementation within the high OCS treatment. Additionally, the interaction effect on leptospirosis titers between monensin supplementation and EV was in agreement with growth responses to EV, at least within the monensin-supplemented group, in which fast EV steers had lower antibody titers than slow steers. However, it is unclear why this difference only existed within the monensin-supplemented group. Previous studies have also reported that cattle with a fast EV produce a lower titer response to vaccinations, compared to cattle with a

slow EV (Oliphint, 2006). Our results were consistent when animals were on the monensin diet, but not on the control diet (Figure 5.9). Because the OCS by monensin relationship was significant in the present study, but OCS was not significant in the companion study, the relationship between OCS, monensin and antibody titer response remains unclear. Results of this study give additional evidence that fast EV animals can have a decreased response to vaccines in comparison to slow EV animals and further work with temperament and vaccine response is necessary.

Twice as many fast EV animals were treated for respiratory disease as compared with slow EV animals in this study. Significant differences between EV treatments were not detected for animals that were treated more than once because there was one animal from each treatment; but the differences between EV treatments treated once was strong enough for a trend to persist when analyzed as a total (Table 5.5). The increased prevalence of illness in fast EV animals is consistent with previous findings that animals with an unfavorable temperament are more vulnerable to illness. Hulbert et al. (2011) found that animals with a high temperament score (measured by EV and pen score) had decreased levels of neutrophils compared to calm cattle, making them more likely to be infected. They also suggested that these cattle would be more difficult to treat because they exhibit fewer clinical signs (Hulbert et al., 2011). If true, this would suggest that the true difference in disease prevalence may have been greater than measured here, because animals were not evaluated for elevated temperature unless they displayed clinical signs. Moreover, stress may not only affect antibody production, but also the seroconversion from IgM to other isotypes (Feng et al., 1991). Delayed seroconversion during a pathogenic challenge can increase the likelihood of morbidity (Feng et al., 1991). Our

results are consistent with previous results of relationships between EV and morbidity and add additional evidence that more temperamental animals, measured with exit velocity, may have an increased vulnerability to illness.

## **Conclusions**

Using an objective measure of chute score could be a useful tool either alone or in conjunction with a subjective chute score to measure behavior in the chute and relate to growth performance. Measures obtained at first exposure to new facilities should be used when assigning temperament scores, as the initial measurement appears to provide a greater degree of resolution of differences and has been shown here to be related to growth and/or intake. Additionally, because performance and health related measures were affected differently by OCS and EV, we suggest that these measures are evaluating different underlying traits, and should therefore be maintained as separate measures when assessing temperament characteristics that are related to production traits. Thus, describing an animal as more or less “temperamental” can confuse quantitative understandings of the relationships between behavioral measures and production traits, rather individual behavioral measures should be related to production traits. Thus, in this study, both low OCS and fast EV appeared to be associated with lower ADG, at least under some management scenarios.



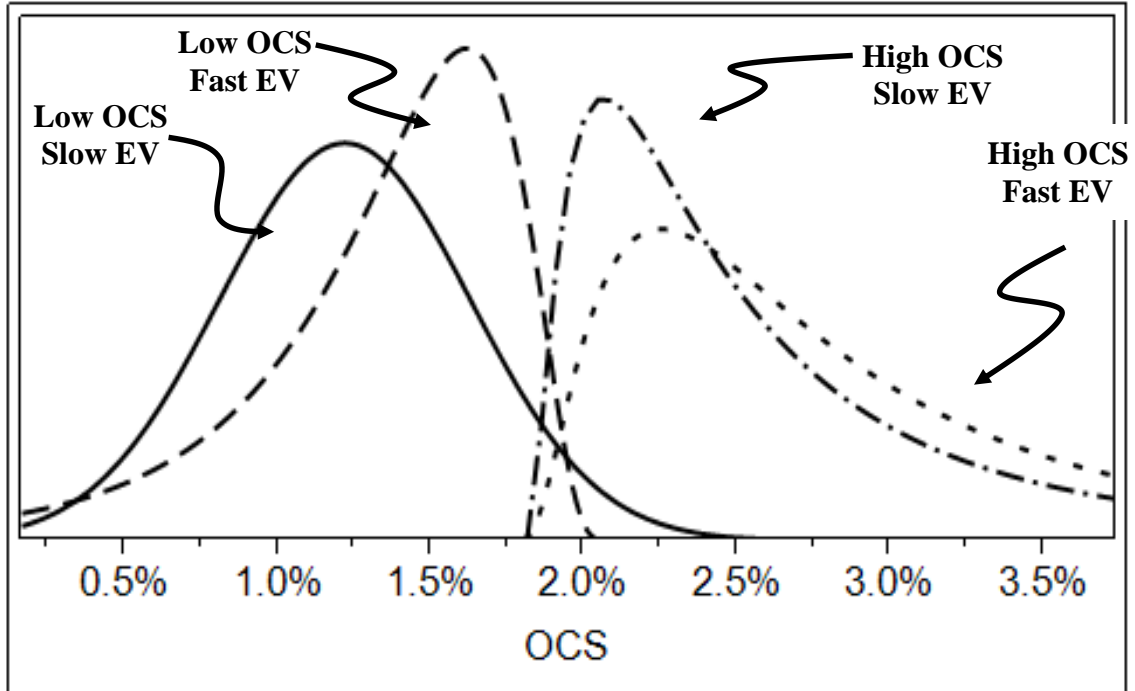


Figure 5. 1. Fitted Johnson SI distribution curves for objective chute score (OCS) for each temperament treatment in the 2 x 2 factorial. These distributions were constructed from all animals (n = 192) that were processed together and assigned to the various treatments. There were too few animals utilized in the companion experiment (Chapter 4; n = 32) from which to separately determine these distributions. These curves provide a graphical depiction of the degree of overlap in distribution of OCS among treatments.

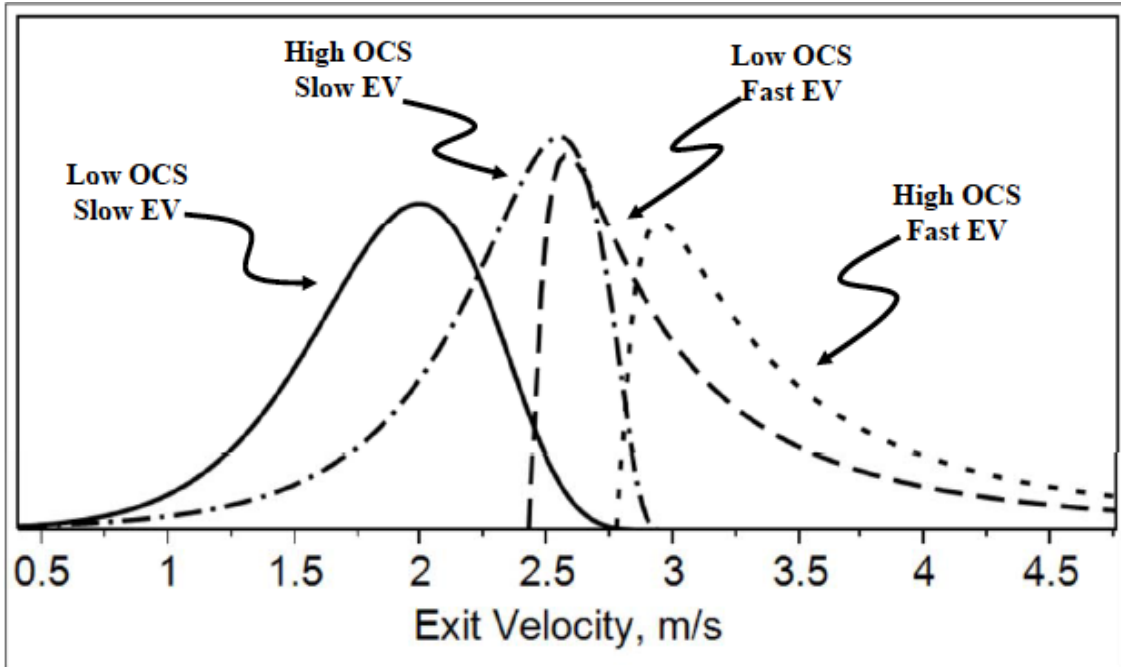


Figure 5. 2. Fitted Johnson SI distribution curves for exit velocity (EV) for each temperament treatment in the 2 x 2 factorial. These distributions were constructed from all animals (n = 192) that were processed together and assigned to the various treatments. There were too few animals utilized in the companion experiment (Chapter 4; n = 32) from which to separately determine these distributions. These curves provide a graphical depiction of the degree of overlap in distribution of EV among treatments.

Table 5. 1. Ingredient composition of experimental diets

Feedstuff	Control	Monensin
	% of diet as DM	
Corn Silage	46.17	46.14
Cracked corn	18.00	18.00
Soybean meal	11.00	11.00
Wheat Straw	22.00	22.00
Dicalcium Phosphate	0.08	0.08
Limestone	1.23	1.23
Potassium Chloride	0.50	0.50
Trace Mineral Premix <sup>a</sup>	0.75	0.75
Urea	0.20	0.20
Vitamin ADE Premix <sup>b</sup>	0.05	0.05
Deccox	0.03	0.03
Rumsensin - 90	0.00	0.021

<sup>a</sup>Trace mineralized salt provided 92.9% salt, 68 ppm Co, 1838 ppm Cu, 120 ppm I, 9290 ppm Mn, 19 ppm Se, and 5520 ppm Zn.

<sup>b</sup>Vitamin premix supplied 1820 IU/kg Vitamin A, 363 IU/kg Vitamin D, and 227 IU/kg Vitamin E.

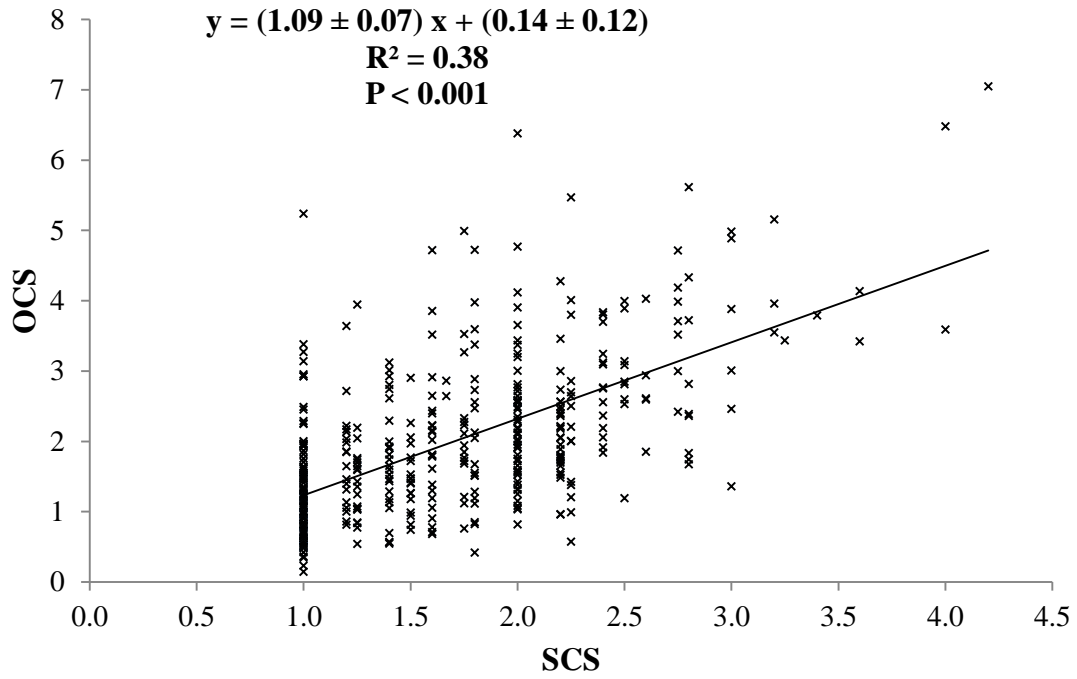


Figure 5. 3. Linear regression analysis of objective chute score (OCS) and subjective chute score (SCS) at d -7 and d 56 (n = 417)

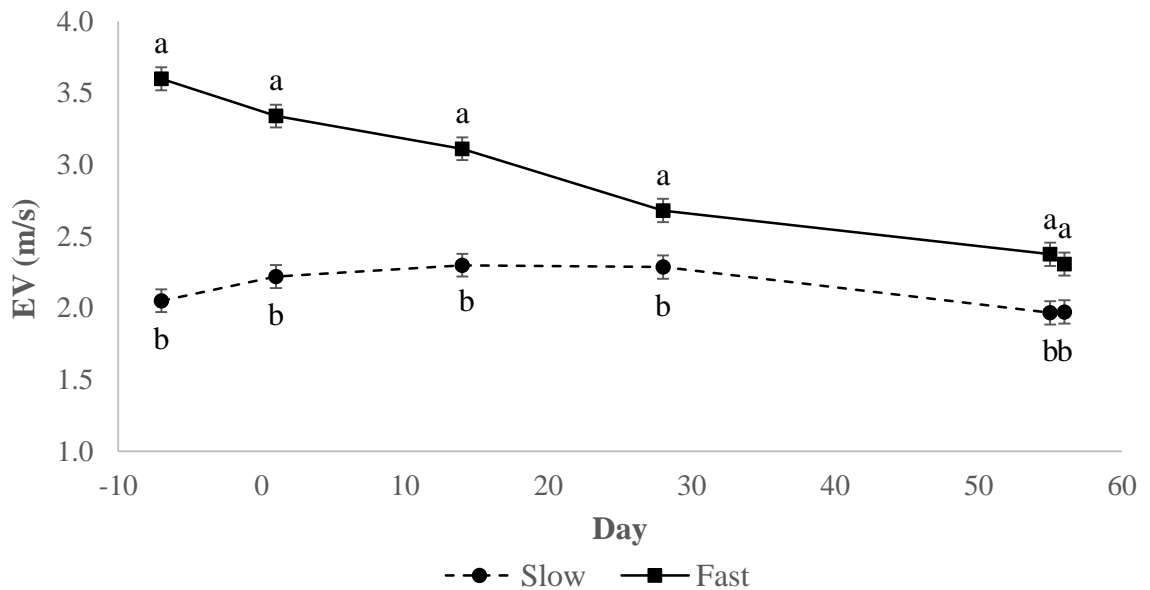


Figure 5. 4. Least square means ( $\pm$  SEM) of EV on each day of temperament assessment (d -7, 0, 14, 28, 55, and 56) by EV treatment (slow or fast). Treatment was based on initial measures of exit velocity (d -7). The initial average EV for the slow treatment was 2.01 and initial average EV for the fast treatment was 3.6 (n = 158). There was a significant EV by day interaction ( $P < 0.01$ ). Points with different letters on a given day are significantly different ( $P < 0.01$ ). The time effects of the slow EV treatment were characterized by a linear ( $P = 0.05$ ) and quadratic effect ( $P < 0.01$ ). The time effects of the fast EV treatment were characterized by a linear ( $P < 0.01$ ) and quadratic effect ( $P = 0.05$ ).

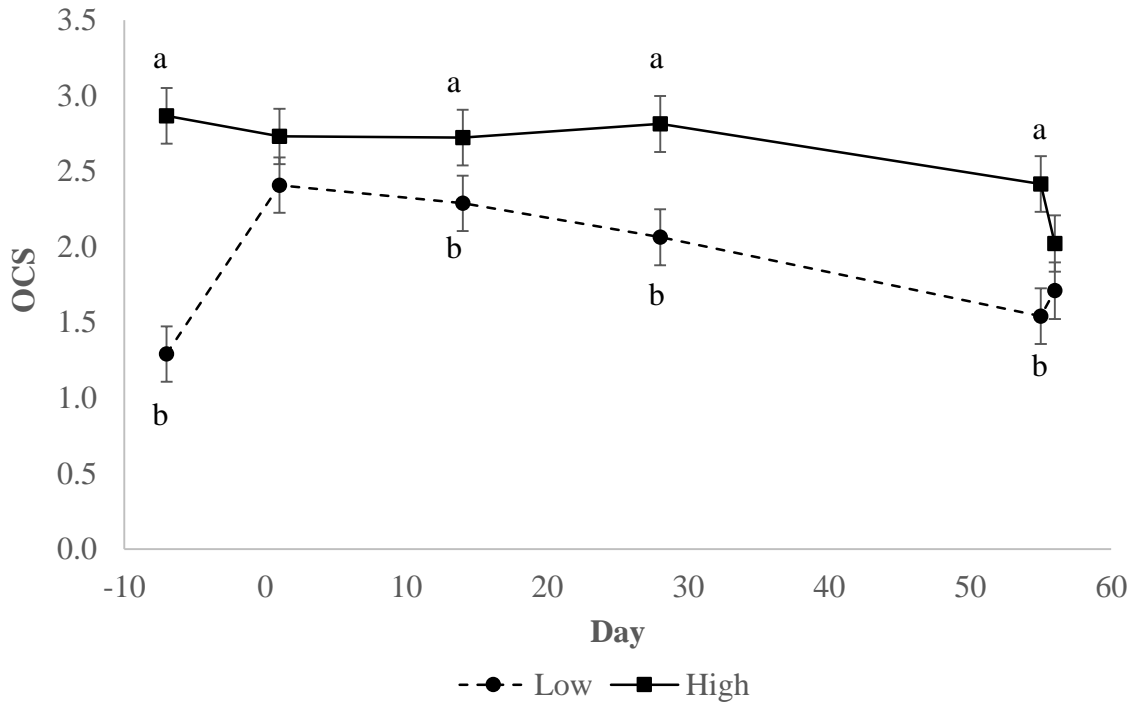


Figure 5. 5. Least square means ( $\pm$  SD) of OCS on each day of temperament assessment (d -7, 0, 14, 28, 55, and 56) for OCS treatment (low or high). Treatment was based on initial measures of OCS (d -7). The initial average OCS for low treatment was 1.2 and initial average OCS for high treatment was 2.9 ( $n = 158$ ). There was a significant OCS by day interaction ( $P < 0.01$ ). Points with different letters on a given day are significantly different ( $P < 0.10$ ). The time effects of the low OCS treatment were characterized by a linear ( $P < 0.01$ ) and quadratic effect ( $P = 0.10$ ). The time effects of the high OCS treatment were characterized by a quadratic effect ( $P < 0.01$ ).

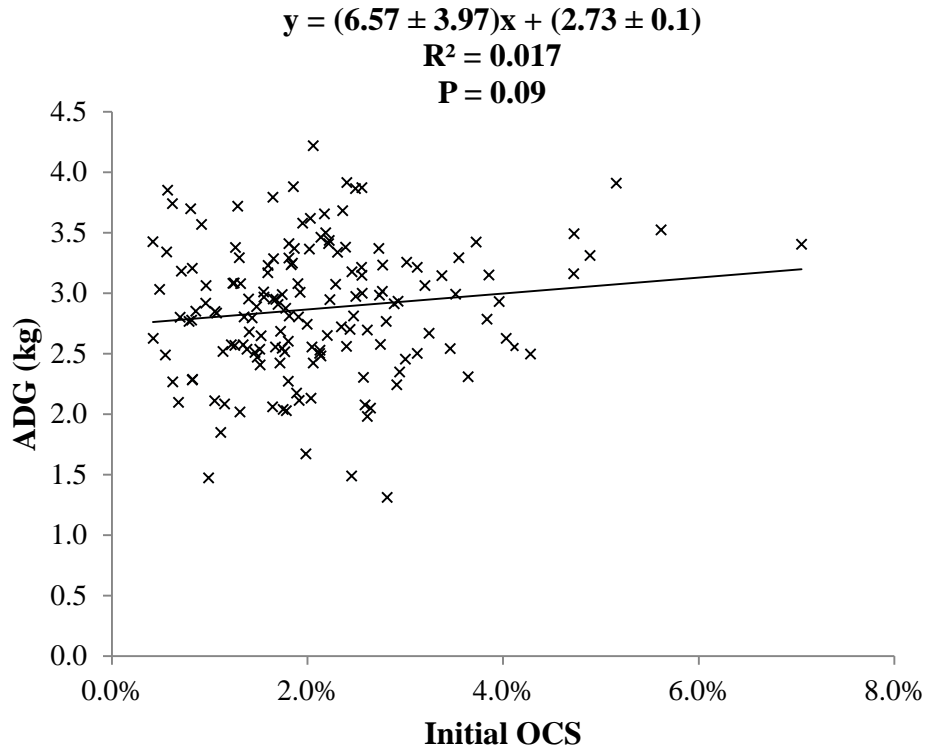


Figure 5. 6. Linear regression analysis of initial OCS (d -7) and final ADG for each animal (n = 158)

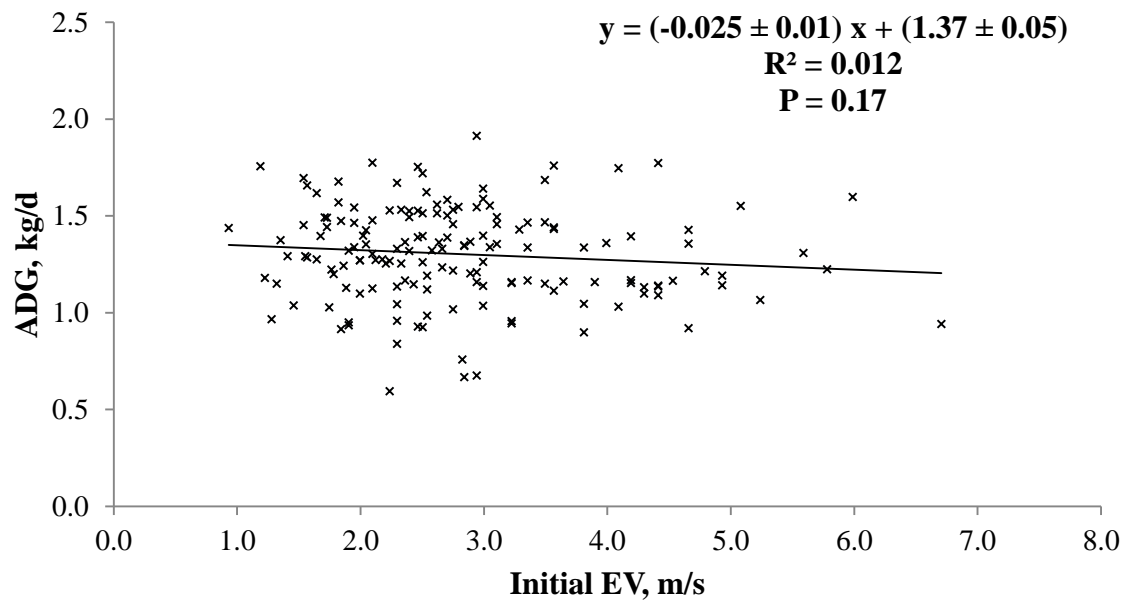


Figure 5. 7. Linear regression analysis of initial EV (d -7) and final ADG for each animal (n = 158)



Table 5. 2. Initial measures of temperament treatments and body weight

	Low OCS <sup>1</sup>		High OCS		SEM <sup>3</sup>
	Slow EV <sup>2</sup>	Fast EV	Slow EV	Fast EV	
Exit Velocity, m/s	1.86	3.36	2.24	3.83	0.1084
OCS, %	1.35	1.37	2.72	3.00	0.1318
Initial body weight, kg	264	262	263	259	3.518

<sup>1</sup>OCS (objective chute score) = the coefficient of variation of the 5 prerecorded weight values across the 10 s interval while the animal is restrained by the head in the a chute.

<sup>2</sup>EV (exit velocity) = time taken for steers to travel 1.68m upon exiting the chute

<sup>3</sup>SEM – standard error of the means

Table 5. 3. Statistical significance for interactions and main effects

	Probability of a greater F-value						
	M*EV*OCS <sup>1</sup>	M*EV	EV*OCS	M*OCS	M	EV	OCS
<b>ADG</b>							
d 0 to 28	0.40	0.82	0.80	0.26	0.22	0.72	0.12
d 28 to 56	0.65	0.90	0.48	0.07	0.01	0.04	0.33
d 0 to 56	0.36	0.80	0.55	0.06	0.01	0.15	0.08
<b>DMI</b>							
d 0 to 28	0.35	0.42	0.87	0.67	<0.0 1	0.15	0.28
d 28 to 56	0.15	0.77	0.35	0.88	<0.0 1	0.12	0.66
d 0 to 56	0.17	0.61	0.77	0.88	<0.0 1	0.11	0.39
<b>Intake, % BW</b>							
d 0 to 28	0.32	0.34	0.42	0.80	<0.0 1	0.28	0.12
d 28 to 56	0.11	0.67	0.31	0.41	<0.0 1	0.23	0.58
d 0 to 56	0.12	0.53	0.90	0.79	<0.0 1	0.24	0.18
<b>G:F</b>							
d 0 to 28	0.41	0.90	0.87	0.24	0.02	0.40	0.48
d 28 to 56	0.23	0.98	0.85	0.02	0.37	0.18	0.42
d 0 to 56	0.95	0.97	0.99	0.01	<0.0 1	0.97	0.13
Leptospir osis titers	0.36	0.02	0.15	0.05	0.64	0.55	0.84
<b>Morbidity, number of animals treated</b>							
Once	-	-	0.24	-	-	0.10	0.61
Twice	-	-	1.00	-	-	1.00	1.00
Total	-	-	0.29	-	-	0.13	0.74
NEFA	0.78	0.51	0.66	0.79	0.01	0.84	0.36

<sup>1</sup> See Table 5.2. for abbreviations

Table 5. 4. Effects of the OCS by monensin interaction on ADG, DMI, G:F conversion, leptospirosis titer responses, and serum NEFA concentrations (n = 40)

	Control		Monensin		SEM <sup>1</sup>
	Low OCS	High OCS	Low OCS	High OCS	
ADG, kg/d					
d 0 to 28	1.24	1.27	1.11	1.26	0.056
d 28 to 56	1.48 <sup>a</sup>	1.44	1.30 <sup>b,c</sup>	1.41 <sup>d</sup>	0.038
d 0 to 56	1.36 <sup>a</sup>	1.36	1.20 <sup>b,c</sup>	1.33 <sup>d</sup>	0.034
DMI, kg/d					
d 0 to 28	6.88	6.95	5.86	6.07	0.136
d 28 to 56	8.46	8.55	7.58	7.63	0.159
d 0 to 56	7.66	7.76	6.71	6.85	0.138
Intake, % BW					
d 0 to 28	2.45	2.51	2.10	2.18	0.043
d 28 to 56	2.66	2.71	2.42	2.41	0.038
d 0 to 56	2.55	2.61	2.26	2.31	0.036
G:F					
d 0 to 28	0.179	0.175	0.191	0.208	0.009
d 28 to 56	0.175	0.17 <sup>a</sup>	0.169 <sup>c</sup>	0.185 <sup>b,d</sup>	0.0050
d 0 to 56	0.178	0.173 <sup>a</sup>	0.179 <sup>c</sup>	0.195 <sup>b,d</sup>	0.0040
Leptospirosis titers, ln	5.96	6.48 <sup>a</sup>	6.32	5.89 <sup>b</sup>	0.231
NEFA concentrations, mEq/L	0.45	0.48	0.34	0.38	0.036

<sup>a,b</sup> Monensin effects within OCS (P < 0.10)

<sup>c,d</sup> OCS effects with monensin (P < 0.10)

<sup>1</sup> SEM – standard error of the means

Table 5. 5. Main effects of exit velocity on ADG, DMI, G:F conversion, and morbidity (n = 40)

	Slow	Fast	SEM <sup>1</sup>
ADG, kg/d			
d 0 to 28	1.23	1.21	0.039
d 28 to 56	1.45	1.36	0.026
d 0 to 56	1.34	1.28	0.026
DMI, kg/d			
d 0 to 28	6.53	6.31	0.094
d 28 to 56	8.18	7.92	0.111
d 0 to 56	7.35	7.12	0.097
Intake, % BW			
d 0 to 28	2.33	2.29	0.030
d 28 to 56	2.58	2.53	0.028
d 0 to 56	2.45	2.41	0.026
G:F			
d 0 to 28	0.203	0.207	0.006
d 28 to 56	0.183	0.179	0.003
d 0 to 56	0.191	0.191	0.002
Morbidity, number of Animals Treated			
Once	4	10	-
Twice	2	2	-
Total	6	12	-

<sup>1</sup> SEM = standard errors of the means

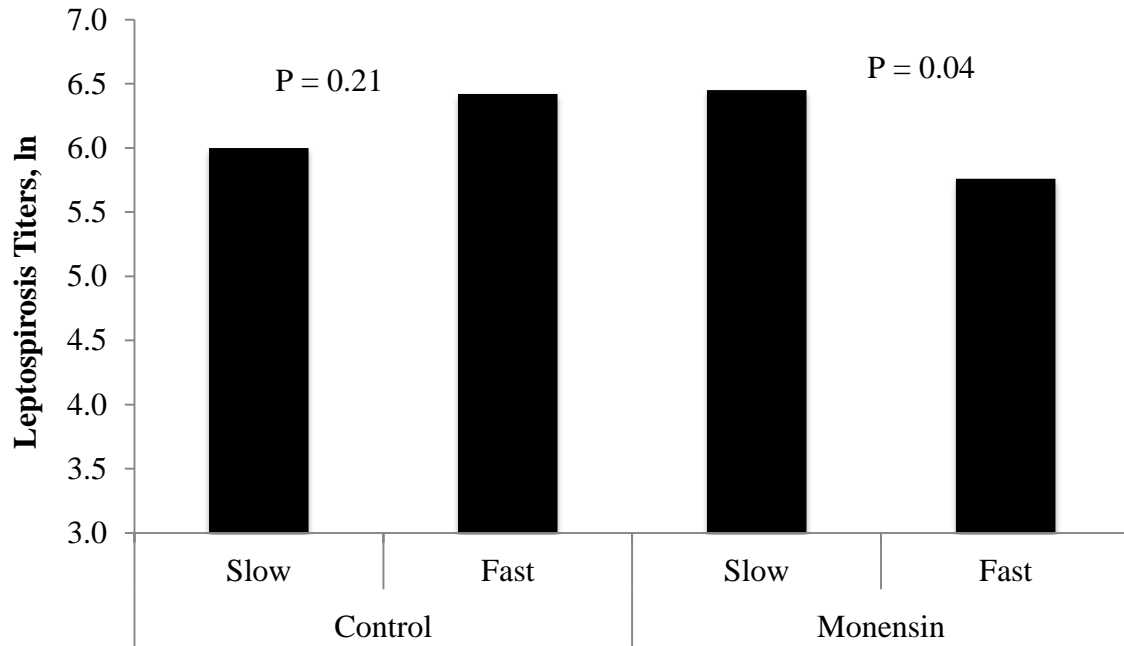


Figure 5. 8. Least square means of leptospirosis titers between EV (slow or fast) and monensin (control or monensin) treatments (n = 158). Although there was no difference between EV rankings on the control diet, on the monensin diet slow EV animals had a higher response than fast EV animals.

## CHAPTER 6

### CONCLUSIONS AND IMPLICATIONS

Behavioral measures of beef cattle have been demonstrated to have some relationship to productivity. Exit velocity was consistently related to ADG, where slow EV animals had higher ADG than fast EV animals. We suggest that differences in gain between EV treatments are primarily linked to differences in intake, rather than differences in efficiency. Additionally, although there are detectable behavioral differences between groups, behavior is probably not the driving factor for differences in intake, rather an inherent biological mechanism is regulating intake.

Because OCS and EV were associated with health measures and production traits differently, we suggest that the two measure different underlying traits of beef cattle and should not be combined. Additionally, while there is a positive correlation between OCS and SCS, general relationships to production appear to differ. We suggest that these differences stem from differences in time used for measurement, where initial measures will relate differently than averaged measures over time.

Antibody levels represent how well animals respond to the vaccines they are given and how well said vaccines will protect them from morbidity. Our results provide some additional evidence that slow EV animals will have a greater response to vaccines than will their fast EV counterparts. Additionally, the difference in response between fast and slow EV animals in chapters 4 and 5 shows how different housing strategies can possibly impact how animals' immune systems respond. Thus, if some groups of cattle can be identified as having an altered immune response, management practices can be altered to reduce the differences, such as altering housing strategies. Additionally, the

results of morbidity analysis showed that there is some relationship between EV and incidence of illness, where fast EV animals were more likely to be treated for illness than their slow EV counterparts. Also, the relationship between OCS and morbidity suggested that although there is not a difference in illness between treatments, that high OCS animals might be more likely to show clinical signs of illness, without the associated temperature. Further research is needed to investigate the differences in health related measures between cattle with different exit velocities and objective chute scores to effectively and appropriately alter management practices.

Temperament can be measured with a number of different tests and generally each test has been devised with specific objectives in mind. While in some cases the objective may be to improve human safety during handling (Boivin et al., 1992; Grandin, 1993), the objective of others has been to relate behavior to production (Voisinet et al., 1997; Petherick et al., 2002). Our objectives were to relate behavior to productivity using testing techniques that are cheap and easy to perform so that they may be applicable to a production setting.

As a result, each test tends to measure aspects of behavior that are specific to the experiment and, commonly, little effort is made to relate them to one another (Kilgour et al., 2006). A large difference in the behavior of individual animals to various challenging situations still remains (Kilgour et al., 2006). Particular testing methods may be specific to certain behavioral responses, such as agitation, social isolation, or avoidance of humans, and examining correlations between different measures may pinpoint which general behaviors they are associated with. Additionally, the differences in individual behavioral responses to aversive or challenging situations may represent distinctly

different coping styles that are a characteristic of an individual animal (Hessing et al., 1993). Kilgour et al. (2006) identified and correlated eight behavioral tests that can be discriminated between three components, two of which were named as general agitation and close human contact.

Some recent literature has combined exit velocity and subjective chute score into one score used to represent “temperament” (Cooke et al., 2009; Hulbert et al., 2011). However, if each measure represents a fundamentally different behavioral response, and we suggest that they are, then tests should not be combined. Although exactly which response each behavior represents is purely speculative, our results confirm that each “temperament” measuring technique stimulates a difference response. Thus, measures should be kept separate and related to production as independent measures, rather than temperament in general.



## APPENDICES

## Appendix 1

Protocol for viewing video records to calculate dominance hierarchies:

1. Using Remote Desktop Connection from your computer, look for and connect to IP address: **128.163.200.48**
2. After the connection has been established, use the user name and password that has been provided to you
3. Double click the **Axis Camera Station icon**, then click connect
4. After Axis Camera has started, click the Navigate to Recordings icon (located in the upper left; looks like a movie reel)
5. Enter a start and end date and/or time for the videos you wish to view
6. There will be a list tab of video available. Click the Timeline tab (next to the list tab), and you will see a list of the recordings you had selected by camera. Each camera covers two pens in the barn, and are as follows:
  - a. **Camera 44 - pens 115 and 116**
  - b. **Camera 45 – pens 11 and 112**
  - c. **Camera 46 – pens 113 and 114**
  - d. **Camera 47 – 117 and 118**
7. You can view the videos in this program or they can be saved from the program for future use. You can use VLC media player (available as a free download) to view the recordings. If you are choosing to play the video in the Axis program, choose which camera you wish to watch by placing your cursor on the line next to your camera choice and double click to begin playback. To export the videos, highlight each line next to the camera and press the Export Video icon at the top.

8. Below the video there are icons similar to a DVD player for play, stop, etc. there is also an indicator for playback speed, from 1X for normal viewing and up to 8X. Each video will have a date/time stamp in the top left corner of the screen.
9. You may also drag the RED icons on the blue timeline (just above the video control icons, below the video) forward or backward to anyplace in the video.
10. You should have a notebook for recording observations. Each entry in the notebook should include:
  - a. **Date of the video you're watching**
  - b. **The camera you are watching**
  - c. **Which pen you are recording**
  - d. **The steer id for the animal times (ear tag color or collar color)**
11. You are now ready to begin watching videos. Click the play icon. When you observe a steer doing something, you can use the pause button, write down your observation in your notebook. You can abbreviate the collar colors with the letter of the color (i.e. green = G).
12. You can also record any other behaviors you find necessary.
13. When done watching the video, close the Axis Camera Station
14. Logoff the remote desktop (DO NOT shut down): Start -> logoff -> confirm yes

The video analysis was used for later analysis to characterize social dominance behavior by individual animals by measuring numerous dyad interactions to create a dominance hierarchy. Five behaviors were used to characterize the outcomes of each dyad interaction:

- Butting – a steer uses head to head, head to neck, or head to flank contact to displace another steer from the feed area
- Pushing – a steer forcefully enters the feed area and displaces another steer from the feed area
- Threatening – A steer takes up a threatening posture by presenting the head in the direction of the recipient steer, no contact occurs
- Defending – a steer used physical contact to push the recipient steer to the back wall of the pen and keeps threatening posture to ensure steer stays there
- Mounting – a steers mounts another recipient animal and forces the recipient steer away from the feed area

These behaviors were observed between dyad pairs where individual animals were identified by their colored ear tag or colored collar. A “winner” and “loser” were identified from each interaction. The method for recording interactions was written as follows:

G vs. R(**R**) or G vs. R(**G**)

The first letter represents the color ear tag or collar of the animal that initiated the interaction and the second letter represents the color ear tag or collar of the animal being challenged. The circled letter represents the winner of the interaction. The above examples would be read as “green initiated an interaction with red where red was the winner and green was the loser” or “green initiated an interaction with red where green was the winner and red was the loser”.

## Appendix 2

Table A1.1. Calculation test using data from Hemelrijk et al., 2005

	G	Y	R	C	X		ADI	$\sum_j w_{ij}$	w2	L	L2	DS					
G	-	6	9	8	5	G	-	1	1	0.888889	1	0.972	3.89	4.98	0.11	0.31	8.44
Y	0	-	4	6	0	Y	0	-	0.666667	1	-	0.556	1.67	2.20	1.33	0.93	1.61
R	0	2	-	4	7	R	0	0.333333	-	0.444444	0.777778	0.389	1.56	1.64	2.44	3.08	-2.33
C	1	0	5	-	3	C	0.111111	0	0.555556	-	0.5	0.292	1.17	1.66	2.83	3.66	-3.67
X	0	0	2	3	-	X	0	-	0.222222	0.5	-	0.241	0.72	0.93	2.28	3.43	-4.06
						L	0.11	1.33	2.44	2.83	2.28						
						L2	0.31	0.93	3.08	3.66	3.43						
						$\sum_j w_{ij}$	3.89	1.67	1.56	1.17	0.72						

Table A1.2. Calculator for EV1402

	G	Y	R	C	X		G	Y	R	C	ADI	$\sum_j w_{ij}$	w2	L	L2	DS
G	0	0	0	0	0	G	-	0	-	0	0.000	0.00	0.00	2.00	1.17	-3.17
Y	5	0	0	1	6	Y	1	-	-	0.142857	0.571	1.14	0.38	0.86	0.27	0.40
R	0	0	0	1	1	R	-	-	-	0.166667	0.167	0.17	0.45	0.83	0.26	-0.48
C	1	6	5	0	12	C	1	0.857143	0.833333	-	0.897	2.69	1.12	0.31	0.26	3.24
X	6	6	5	2	19											
						L	2.00	0.86	0.83	0.31						
						L2	1.17	0.27	0.26	0.26						
						$\sum_j w_{ij}$	0.00	1.14	0.17	2.69						

### Appendix 3

Table A1.3. Effect of temperament and monensin on ADG, DMI, G:F, immunological response, and serum NEFA concentrations

	Control				Monensin				SEM
	Low OCS <sup>1</sup>		High OCS		Low OCS		High OCS		
	Slow EV <sup>2</sup>	Fast EV	Slow EV	Fast EV	Slow EV	Fast EV	Slow EV	Fast EV	
Exit Velocity <sup>3</sup> , m/s	1.86	3.36	2.24	3.83	-	-	-	-	0.1084
OCS <sup>3</sup> , %	1.35	1.37	2.72	3.00	-	-	-	-	0.1318
ADG, kg/d									
d 0 to 28	1.26	1.22	1.25	1.28	1.09	1.12	1.31	1.21	0.079
d 28 to 56 <sup>a</sup>	1.54	1.45	1.49	1.40	1.32	1.28	1.47	1.34	0.053
d 0 to 56 <sup>b</sup>	1.39	1.33	1.37	1.34	1.21	1.20	1.39	1.28	0.094
DMI, kg/d									
d 0 to 28	7.03	6.72	6.91	7.02	5.99	5.74	6.26	5.888	0.192
d 28 to 56	8.61	8.31	8.61	8.49	7.54	7.63	7.79	7.29	0.225
d 0 to 56	7.82	7.50	7.76	7.76	6.75	6.68	7.11	6.59	0.195
Intake % BW									
d 0 to 28	2.50	2.40	2.47	2.55	2.10	2.06	2.23	2.13	0.06
d 28 to 56	2.69	2.63	2.70	2.71	2.40	2.44	2.50	2.33	0.05
d 0 to 56	2.59	2.52	2.60	2.63	2.26	2.26	2.26	2.24	0.05
G:F									
d 0 to 28	0.195	0.196	0.194	0.195	0.201	0.209	0.225	0.228	0.013
d 28 to 56 <sup>c</sup>	0.181	0.181	0.181	0.181	0.181	0.177	0.189	0.192	0.006
d 0 to 56 <sup>d</sup>	0.187	0.197	0.186	0.180	0.189	0.191	0.203	0.206	0.005
Leptospirosis titers <sup>ef</sup> , ln	5.47	6.44	6.55	6.41	6.60	6.04	6.30	5.48	0.326
NEFA, mEq/L	0.43	0.47	0.48	0.47	0.35	0.33	0.40	0.36	0.052

<sup>1</sup>OCS (objective chute score) = the coefficient of variation of the 5 prerecorded weight values across the 10 s interval while the animal is restrained by the head in the a chute.

<sup>2</sup>EV (exit velocity) = time taken for steers to travel 1.68m upon exiting the chute

<sup>3</sup> Average initial (d -7) measures of OCS and EV of animal by treatment

<sup>a</sup> Monensin effect within low OCS (P <0.01); monensin effect within high OCS (P = 0.48)

OCS effect within control (P = 0.54); OCS effect within monensin (P = 0.05)

<sup>b</sup> Monensin effect within low OCS (P <0.01); monensin effect within high OCS (P = 0.64)

OCS effect within control (P =0.93); OCS effect within monensin (P =0.01)

<sup>c</sup> Monensin effect within low OCS (P = 0.29); monensin effect within high OCS (P = 0.03)

OCS effect within control (P = 0.39); OCS effect within monensin (P = 0.02)

<sup>d</sup> Monensin effect within low OCS (P = 0.81); monensin effect within high OCS (P < 0.001)

OCS effect within control (P = 0.36); OCS effect within monensin (P < 0.01)

<sup>e</sup> Monensin effect within low OCS (P = 0.27); monensin effect within high OCS (P = 0.08)

OCS effect within control (P =0.12); OCS effect within monensin (P = 0.20)

<sup>f</sup> Monensin effect within slow EV (P = 0.18); monensin effect within fast EV (P = 0.05)

EV effect within control (P = 0.21); EV effect within monensin (P = 0.04)



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