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Relationships of temperament, endocrine, reproductive, and behavioral parameters measured during performance testing of bulls

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To the Graduate Council:

I am submitting herewith a thesis written by Sierra Ashley Lockwood entitled "Relationships of temperament, endocrine, reproductive, and behavioral parameters measured during performance testing of bulls." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Animal Science.

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Dedication

This thesis is dedicated to those who matter the most; past, present, and future.

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Abstract

The aim of this study was to examine relationships between bull temperament, behavior, growth, endocrine, and reproductive parameters measured over an 84 d test period. Bulls (n = 65) were reared in 6 pens separated on BW and age. Pen scores were assigned on d -1, 27, 55 and 83. On d 0, 28, 56, and 84, exit velocity, BW, time it took each bull to leave the chute, bull order through chute, and hair and blood samples were recorded or collected. Frame score was calculated, carcass ultrasounds and breeding soundness exams were performed on d 84. ADG was calculated for each period (period 1 = d 0-28; period 2 = d 29-56; period 3 = d 57-84) and over the 84 d. Bulls (n=30; 3 pens) received dataloggers to measure activity during period 1 and 3. Bulls were categorized into three temperament (pen score + exit velocity / 2) categories (docile, intermediate, and temperamental).

Temperamental bulls tended (P=0.07) to enter the chute system prior to docile bulls on d 84 and weighed less (P<0.05) than docile bulls on d 0, 28, and 56. Frame score was less (P<0.05) for temperamental bulls than docile bulls on d 84 (5.88 ± 0.13 vs. 6.34 ± 0.18). Bulls categorized as intermediate on d 56 had greater (P<0.05; 2.10 ± 0.04 kg/d) overall ADG than docile (1.94 ± 0.06 kg/d) and temperamental bulls (1.92 ± 0.06 kg/d).

Docile bulls had greater serum (P=0.06) and hair testosterone (P<0.05) and lower (P<0.05) serum cortisol concentration than temperamental bulls on d 56. Docile and intermediate bulls tended (P=0.07) to have a lower percentage of primary spermatic defects (20.58 ± 4.86 %) than temperamental bulls (38.01 ± 8.06 %) on d 84. Serum testosterone measured on d 28 and 56 was positively correlated to BW (r = 0.47 and

0.39; *P*<0.01). On d 28, BW was positively correlated (r = 0.32; *P*<0.05) to hair testosterone and negatively correlated to hair cortisol (r = -0.31; *P*<0.05).

In conclusion, selecting bulls based on docility could increase BW, frame score, serum and hair testosterone concentration, and lower serum cortisol concentrations.

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Chapter 1. Introduction

The United States beef industry has become more reliant on artificial insemination as compared to using herd sires. However, producers continue to produce and use bulls as a way to service cows and heifers after artificial insemination or as a herd bull, and continue to seek genetically superior bulls. Housing and handling bulls can be very dangerous to personnel, the facility's equipment, and the bull itself. The CDC (2009) reported that, over a span of five years (2003-2008), approximately 108 human fatalities were caused by cattle; of which 48% resulted from handling bulls. As an end result, destruction caused by temperamental bulls can lead to monetary deficits for producers as a result of property damage, personnel injury, injury to the bull, and a decrease in cattle performance (Fordyce et al., 1988b; Grandin, 1993; Voisinet et al., 1997a; Voisinet et al., 1997b).

In Tennessee, the beef industry is the largest revenue generating agricultural enterprise and Tennessee ranks 11th in the nation in cow-calf production. The most common breeding method used by cow-calf producers in Tennessee is "natural service", and more than 14,000 bulls are produced annually by purebred breeders providing half the genetic makeup of the state's calf crop. One current problem the Tennessee cattle industry faces is that the average beef producer is 54 years of age and with an increase in age there is a greater risk for injury. Recognizing that these producers will be in contact with bulls throughout their careers, it is necessary to develop an objective and accurate method for selecting bulls according to temperament to insure the livelihood of producers and increase growth performance of bulls.

The beef industry plays an influential role in the United States' agricultural sector. It is estimated that the beef industry in the United States produced $5.51 billion in exports in 2012; with much of the revenue coming from Canada, Japan, Mexico, South Korea, and China. Canada has been the leading importer of United States' beef with 132,981 metric tons in 2012 and Japan second with 120,979 metric tons (NCBA, 2012b). As of 2012, the beef industry in the United States is comprised of approximately 742,000 herds, with about 90% of the herds consisting of less than 100 cows (NCBA, 2012a). The majority of the beef industry is centralized to the west and mid-west regions of the United States; the top five producing states being Texas, Nebraska, Missouri, Oklahoma, and South Dakota.

The beef industry embraces two species types of cattle, *Bos indicus* and *Bos taurus*. *Bos indicus* cattle include Brahman and Nelore cattle while *Bos taurus* cattle include breeds such as Angus, Simmental, Hereford, and Charolais. The beef industry in the United States is heavily weighted with *Bos taurus* cattle as compared to *Bos indicus* due to their superior meat quality. A study performed by Fordyce et al. (1993) found that cattle that were ¾ *Bos indicus* had faster growth rates than cattle that were ½ *Bos indicus*. The study also showed that Brahman influenced cattle had a faster growth rate than Sahiwal influenced cattle. *Bos indicus* are known for their loose skin structure and short hair that allows heat to dissipate more rapidly. Therefore, incorporating *Bos indicus* into composite breed programs has allowed for heat tolerant cattle, especially in the southern region of the United States.

Chapter 2. Literature Review

Temperament and Scoring Methods

Temperament with regards to cattle can be defined as their behavioral response to the presence of humans or novel environments (Fordyce et al., 1988a; Fordyce et al., 1988b). Cattle with poor temperament are a risk to the livelihood of personnel and pose a threat to the longevity of facilities and equipment (Burdick Sanchez et al., 2013). Therefore, an accurate measure of cattle temperament should be an important tool available to producers to utilize to reduce risk of injuries and facility damage.

Traditionally, both subjective and objective methods for measuring temperament have been employed. Pen scoring is an example of a subjective measurement of cattle temperament obtained by evaluating the reactions of 3-5 animals in a pen to the presence of a human observer (Curley et al., 2006; King et al., 2006). Typically, the same observer will make the pen score determination for all animals on test to insure uniformity. Pen score ratings are assigned on a per animal basis and were as follows: 1 = animal is docile, walks slowly; 2 = runs along fence, standoffish toward observer; 3 = runs along fence, head held up, runs away from observer; 4 = runs, very cautious of observer, may run into fences trying to escape; 5 = very aggressive, easily agitated, runs into fences and possibly observer (Kunkle et al., 1986; Hammond et al., 1996).

Exit velocity is an example of an objective measure of temperament that involves the use of two infrared sensors placed at a specified distance apart in the path that the animal will use upon exiting the chute (Burrow et al., 1988; Curley et al., 2006; King et al., 2006). Once the animal breaks the first beam, the timer starts until the second beam is broken. Thus, a time is recorded for how long the animal takes to cross the specified distance. Exit velocity is then calculated by dividing the distance traveled by the time recorded and typically ranges from 1-5 m/s in cattle (Curley et al., 2006).

Combining pen score and exit velocity has been offered as a more accurate and complete measure of temperament (Curley et al., 2006; King et al., 2006) by providing an average of two different temperament scoring methods. Scoring overall temperament is performed by averaging pen scores and exit velocity (pen score + exit velocity / 2) (Curley et al., 2006; King et al., 2006). Based on this scoring method, animals that receive an overall temperament of 5 are considered highly temperamental.

Role of Temperament in Performance

Temperament is moderately heritable (R^2= 0.25 – 0.46) (Shrode and Hammack, 1971a; Stricklin et al., 1980; Hearnshaw and Morris, 1984; Fordyce, 1985) and previous research has shown that genetics can influence cattle temperament where feedlot cattle with Brahman (*Bos indicus*) breeding are more temperamental than *Bos taurus* cattle (Fordyce et al., 1982; Fordyce et al., 1988a). Previous studies have also found that horned cattle tended to be more docile than hornless cattle (Fordyce and Goddard, 1984; Fordyce et al., 1988a). Based on these findings, breed type and horn status may be useful predictors of temperament in cattle.

Cattle with desirable temperament are not only important for safety, but production as well. Average daily weight gain (ADG) is an important measurement in the beef industry as producers continue to strive to decrease the amount of time needed to reach market weight. A study by Voisinet et al. (1997b) found that docile steers of *Bos taurus* ancestry (Simmental x Red Angus, Angus, Tarentaise x Angus) had a 0.19

kg/d higher ADG than steers of similar breeding that were easily agitated. Busby (2010) also reported that docile cattle have a higher ADG and feed to gain ratios than temperamental cattle. Thus, docile cattle are considered to be faster growers in weight gain as compared to temperamental cattle.

Poor temperament not only affects the ADG, but can also affect carcass and meat quality of cattle (Fordyce et al., 1988b). Cattle housed with temperamental pen mates have a tendency to have more carcass bruising at time of rendering (Voisinet et al., 1997a; Falkenberg et al., 2005). However, these studies cannot ascertain whether the carcass bruising was the result of other cattle or if they were self-inflicted (Fordyce et al., 1988b). The bruising noted on carcasses of temperamental cattle occurred along the back, hook, and pin areas decreasing the value of the meat where higher quality cuts are located (Fordyce et al., 1988b).

Similar studies have shown that temperamental (Voisinet et al., 1997a) and older (Tarrant, 1981) cattle exhibit higher incidences of dark cutting meat. Dark cutting meat is considered to be a product of stress or injury to the muscle before slaughter and is an undesirable quality caused by a reduction in glycogen in the muscle (McVeigh et al., 1982). Dark cutting meat can be caused by transportation stress, injury to the animal, preexisting injuries, and fighting due to mixing unfamiliar cattle (Grandin, 1978; Voisinet et al., 1997a). Temperamental cattle of *Bos indicus* breeding were prone to dark cutting meat at time of slaughter as compared to docile cattle (Voisinet et al., 1997a). This study also reported that meat from temperamental cattle was tougher compared to meat from docile cattle, when measured on d 14 of aging, based upon higher Warner-Bratzler shear force ratings.

A recent study investigated temperament in relation to carcass merit of feedlot beef cattle using chute exit velocity as a predictor of temperament (Schmidt et al., 2013). Cattle with lower exit velocities and considered docile had higher marbling scores, greater BW, hot carcass weight (HCW), back fat (FAT), and yield grade, as compared to temperamental cattle. By selecting for docile cattle, the beef industry could see an increase in meat quality due to a decrease in dark cutting meat, bruised meat, and increase in tenderness and marbling.

Temperament has also been noted to have a negative correlation with pregnancy probability in cows (Cooke et al., 2009). Previous research has found that temperamental cattle have greater basal concentrations of cortisol and adrenocorticotropic hormone (ACTH) than docile cattle (Curley Jr et al., 2008). During periods of elevated ACTH concentrations, luteinizing hormone release is inhibited and ovulation does not occur (Dobson et al., 2000). Therefore, because temperament is moderately heritable, it is important to select against temperamental bulls to prevent the possibility of decreasing the pregnancy rates of his daughters.

Though temperament is often hard to manipulate, some studies have shown that repeated exposure to handling can alter cattle temperament over time. Conditioning cattle to common management practices (i.e. squeeze chute systems, milking parlors, handling, etc.) has been employed to familiarize cattle with future encounters with these practices. Cattle that were handled at an early age tend to have lower temperament scores than cattle that lack handling experience (Fordyce et al., 1985; Fordyce et al., 1988a). Therefore, repeated exposure to handling procedures as calves can help habituate cattle to management and industry procedures and can decrease incidence of adult cattle with poor temperament (Fordyce et al., 1988a).

Stress and Stressors

Stress refers to a non-specific response (i.e. behavioral, physiological) of the body to any demand made upon it, whereas the condition(s) which constitute the demand and result in eliciting a stress response are termed stressors (Selye, 1950). Stress can occur in two forms, one that does not harm the individual, referred to as eustress, and one that can pose a threat to the well-being of the individual, referred to as distress (Selye, 1950).

Selye (1950) describes a cascade of three events that an individual may experience in response to a stressful situation. In the first event, 'Alarm Reaction', the individual perceives a threat to homeostasis. Thereafter, the 'Stage of Resistance' occurs as the individual tries to adapt to the changes caused by the stressor. If the individual is unable to adapt, the 'Stage of Exhaustion' occurs and can lead to mortality. Throughout these events, various alterations in an animal's behavior and/or physiology may be evident.

Stress Response

Three important components involved with the stress response are the behavioral, autonomic nervous system, and neuroendocrine responses (Moberg, 2000). Moberg (2000) describes the behavioral response as the animal's first reaction to a stressor where the animal will try to remove itself from the threat and the autonomic nervous system response as the animal's second reaction to a stressor. The autonomic nervous system response includes physiological alterations which include an increased heart rate, respiratory rate, blood pressure, etc. (Moberg, 2000). Glucocorticoids (i.e. cortisol) are steroid hormones released during the neuroendocrine response that are responsible for initiating specific physiological changes in response to acute and chronic stressors (Möstl and Palme, 2002).

Cortisol is the primary glucocorticoid found in domestic animals and is released by the cortical regions of the adrenal glands during sympathetic arousal (Muller and Wrangham, 2004; Aronson, 2009). Cortisol is released through the Hypothalamic-Pituitary-Adrenal Axis (HPA). The hypothalamus releases corticotrophic releasing hormone (CRH) to stimulate the release of ACTH from the corticotrpohic cells of anterior pituitary gland (Muller and Wrangham, 2004). The release of ACTH then prompts the adrenal cortex to release cortisol into the circulation. After release, cortisol works as a negative feedback mechanism when circulating concentrations are abundant; inhibiting further release of CRH and ACTH from the hypothalamus and anterior pituitary, respectively. Removal of cortisol, in the form of cortisol sulfate, includes excretion through urine or defecation (Pearson Murphy et al., 1980; Möstl and Palme, 2002).

During the stress response, cortisol is considered to be in the biologically active state when it is unbound or loosely bound to albumin (Siiteri et al., 1981; Adcock et al., 2007; Aronson, 2009). Cortisol is considered to be in the inactive state when bound to its specific binding protein, corticosteroid binding-globulin (CBG), that allows for distribution and availability of cortisol throughout the body (Siiteri et al., 1981). Available cortisol can promote gluconeogenesis, the process by which proteins and fat are converted by the liver into metabolites that can be further converted to glucose (Matteri et al., 2000). The resulting elevations in glucose provide the necessary energy for the individual to cope with stress. However, as a result of glucose conversion, the body can suffer from a reduction of proteins after long periods of elevated cortisol concentrations (Matteri et al., 2000).

Behavioral Signs of Stress

Understanding normal behavioral patterns for a particular species and identifying alterations in that behavior can be a way to recognize signs of stress. Behavioral changes (i.e. escaping, fleeing, seeking shelter, etc.) are the first indicator of an animal experiencing stress (Moberg, 2000; Rushen, 2000). Auditory clues, such as vocalization, have also been considered a behavioral alteration as a result of stress (Grandin, 1998). In stressful situations (i.e. isolation from the herd and their calves) cows with greater cortisol concentrations made more vocalizations and spent less time ruminating than cows with lower cortisol concentrations (Bristow and Holmes, 2007). Thus, recognizing normal and abnormal behavioral patterns of cattle will help in the recognition of stressed individuals.

Testosterone Production and Inhibition

The androgenic steroid hormone testosterone is produced in the testes, ovaries, and adrenal cortex (Senger, 2005). Spermatogenesis relies on adequate testosterone concentrations (Hillgarth et al., 1997; Christiansen, 1998). Circulating testosterone concentrations decrease after castration, however, trace amounts are present in steers due to adrenal production. A study performed by Kellaway et al. (1971) found that mean plasma testosterone concentration in steers (0.86 ng/mL) was significantly lower than that in intact males (13.29 ng/mL). Bulls exhibit higher ADG and superior feed efficiency (Turton, 1962; Bailey et al., 1964; Field et al., 1964; Nichols et al., 1964; Bailey et al., 1966) resulting in leaner carcasses and overall higher carcass yield (Cahill, 1964; Warner et al., 1965; Hedrick et al., 1969; Nygaard et al., 1971). However, testosterone is known to have an effect on carcass merit by decreasing carcass quality grade and meat tenderness from bulls as compared to steers (Gortsema et al., 1974).

Testosterone concentrations can vary across breed groups and can influence weight gain, scrotal circumference, and testis size (Lunstra et al., 1978). The authors measured circulating concentrations of testosterone in bulls from the start of puberty, at about 7 mo of age until 13 mo of age. Bull breeds included: Hereford, Angus, Hereford X Angus crossbreds, Angus X Hereford crossbreds, Red Poll, and Brown Swiss. During this time, serum samples were collected bi-weekly and revealed that circulating testosterone increased over the 6-mo period and that Brown Swiss and Red Poll bulls had the highest testosterone concentrations, while Hereford and Hereford X Angus crossbred bulls had the lowest concentrations. At the conclusion of the 6-mo study, Hereford bulls were the lightest of all the bulls, had the smallest testis size, and were the last to reach puberty compared with Brown Swiss bulls (Lunstra et al., 1978).

Cortisol, either naturally produced or administered, can reduce both luteinizing hormone (LH) and testosterone concentrations (Thibier and Rolland, 1976). In bulls, high concentrations of cortisol correlate with low levels of LH and testosterone (Welsh et al., 1979). During a period of stress, CRH and opiates inhibit gonadotropin releasing hormone (GnRH) release from the hypothalamus preventing further release of LH from the anterior pituitary gland (Rasmussen et al., 1983; Sirinathsinghji et al., 1983). Thus, elevated cortisol concentrations coincide with a reduction in testosterone production by the Leydig cells in the testis and may hinder spermatogenesis, fertility, and reproductive behavior (Doerr and Pirke, 1976; Rivier and Vale, 1984).

Steroid Hormone Detection

Several different methods for collection and analysis of cortisol and testosterone concentrations have been employed. Analysis performed on urine or fecal matter is only effective if sample collection occurs frequently in order to gather samples that exhibit short term elevations of metabolites (Palme et al., 2000; Möstl and Palme, 2002). Blood plasma or serum can be used to accurately measure acute fluctuations in adrenal and testicular production of cortisol (Mormède et al., 2007) and testosterone (Yang et al., 1998) if collected frequently.

Recently, analysis of hair samples has been employed as a non-invasive, highly accessible method to measure the chronic production of cortisol, testosterone, and other hormones (Koren et al., 2002). Radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), used for salivary and blood analysis, can determine cortisol and testosterone concentrations in the shaft of the hair (del Rosario González-de-la-Vara et al., 2011; Bryan et al., 2013b). Evaluating hair cortisol is a method of determining if stress is persistent over time and is reflective of chronic stress as seen by elevated hair cortisol concentrations during induced or natural periods of stress (Koren et al., 2002; Burdick et al., 2011b; del Rosario González-de-la-Vara et al., 2011). Steroid hormones (i.e. cortisol, testosterone, estradiol, etc.) are incorporated into the hair shaft through vascular supply to the hair follicle, sweat, sebaceous gland secretions, and environmental contamination (Cone, 1996). Concentrations will increase within the shaft after a period of elevated production (Meyer and Novak, 2012; Bryan et al., 2013b).

Sauvé et al. (2007) reported that hair cortisol concentrations do not differ between hair color, but del Rosario González-de-la-Vara et al. (2011) found that cortisol concentrations are higher in white hair as compared to black hair when collected from the same individual (23.8 pg/mg vs. 14.3 pg/mg, respectively). Similarly, Gleixner and Meyer (1997) reported that hair of different colors possess different concentrations of testosterone. They found that black hair collected from bulls contained higher concentrations of testosterone (12-33 ng/g) than all other hair colors within the same individual (5-14 ng/g).

Changes in steroid hormone circulation can be detected in hair as shown by the accumulation of different concentrations in hair shaft segments (Anielski et al., 2005; Kirschbaum et al., 2009). Studies have examined the effect of ACTH challenge on hair cortisol accumulation and found that the challenged cattle had a significantly greater concentration of hair cortisol 14 and 28 d post challenge (del Rosario González-de-la-Vara et al., 2011). Similarly, Thomson et al. (2009) reported that post treatment hair testosterone concentrations were elevated in hypogonadal human males that received testosterone treatments as compared to hypogonadal males that received no treatment. Thus, circulating levels of testosterone is reflected in the hair shaft. Researchers have also reported that when human hair and serum samples were collected at the same time from an individual, testosterone concentrations were correlated ($r = 0.395$; $P < 0.05$), suggesting that hair testosterone concentrations are a reflection of chronic testosterone production (Yang et al., 1998).

Behavior

The use of behavioral measures as a means of assessing the reactivity of cattle has been of recent interest. Advances in technology have provided an easy and non-invasive way to measure animal behavior. In cattle, the use of accelerometer technology allows for the collection of continuous objective behavioral measurements without the presence of an observer (MacKay et al., 2012). Data collected by an accelerometer is highly correlated with video observation (Munksgaard et al., 2006), and is less time consuming when compared with video analyses (Trénel et al., 2009). One example of a technology that utilizes an accelerometer is a datalogger. Dataloggers are a lightweight accelerometer device typically attached above the fetlock of cattle to collect data regarding the animals movement in the vertical (y), longitudinal (x), and transverse (z) directions, which can then be used to determine total lying and standing time, total steps taken, and lying bout count and duration (MacKay et al., 2012).

Beef cattle spend approximately 12 h/d lying with the majority of lying time occurring between 2100 h and 0600 h regardless of season (Hoffman and Self, 1973). Previous studies have shown that lying duration is an indicator of cattle comfort, and when forced to choose between lying and feeding, cattle will choose to rest (Metz, 1985;

Haley et al., 2000). Fisher et al. (1997) found that cattle in overcrowding situations showed a reduction in total lying time and ADG as compared to cattle in larger pens. A later study suggested that genetic parameters for temperament and feed consumption in cattle are indirectly related as demonstrated by an inverse relationship between exit velocity and feeding head down time (Nkrumah et al., 2007). Research has also found that lying time and cattle temperament are inversely related (Wierenga, 1987). In overcrowding situations, cattle can become more agitated or temperamental due to a reduction in lying space (Wierenga, 1987). It has also been reported that lower social ranked cattle spend less time lying than dominant individuals (Fisher et al., 1997). Based on previous research regarding behavior and temperament, accelerometer technology could be an objective way to assess temperament.

Conclusion Statement

In conclusion, temperamental cattle have lower ADG, feed to gain ratios, and greater incidences of carcass bruising and basal concentrations of circulating cortisol than docile cattle. Thus, the incorporation of hormonal, behavioral, and growth production measures could be used to determine the effect of selecting bulls according to temperament on bull performance.

Chapter 3. Relationships of temperament, behavior, and growth during performance testing of bulls

Abstract

Temperamental cattle are dangerous to personnel and reduce individual and cohort performance. The aim of this study was to explore objective criteria for evaluating temperament while examining relationships between temperament, behavior, and performance of bulls enrolled in an 84 d bull testing program. Bulls (n = 65) were reared in 6 pens separated on BW and age. Pen scores (1: docile - 5: very aggressive) were assigned on d -1, 27, 55 and 83. Exit velocity, BW, latency to leave the chute (Time 1), and bull order through the chute was recorded on d 0, 28, 56, and 84. Frame score and body composition measurements were performed on d 84. The ADG was calculated for each period (period 1 = d 0–28; period 2 = d 29–56; period 3 = d 57–84) and over the 84 d test. Dataloggers were used to measure lying time, steps taken, lying bout duration, and number of lying bouts of bulls (n = 30; 3 pens) during periods 1 and 3. Spearman correlation analysis was performed on temperament and its relationship with the behavioral and performance data with moderate and strong correlations of interest ($r > 0.3$, $r < -0.3$; SAS 9.3). Using PROC UNIVARIATE, bulls were separated into three groups (temperamental, intermediate, and docile) based on the 25th and 75th quantiles for overall temperament (pen score + exit velocity / 2). Mixed model analysis of variance was used to evaluate relationships of overall temperament with behavior and growth performance. During the early phase of period 3, bulls categorized as temperamental on d 56 spent longer ($P = 0.05$) periods of time lying than intermediate bulls. Temperamental bulls tended ($P = 0.07$) to enter the chute system prior to docile bulls on d 84 and weighed less ($P < 0.05$) than docile bulls on d 0, 28, and 56. Frame score was less ($P < 0.05$) for temperamental bulls than that of docile bulls at the

conclusion of the test (5.88 ± 0.13 vs. 6.34 ± 0.18). Bulls categorized as intermediate on d 56 had greater ($P < 0.05$; 2.10 ± 0.04 kg/d) overall ADG than docile (1.94 ± 0.06 kg/d) and temperamental bulls (1.92 ± 0.06 kg/d). Bulls categorized as docile on d 28 had larger ($P < 0.05$; 86.20 cm^2 ± 1.46) ribeye area (REA) than intermediate bulls (81.71 ± 1.05 cm^2), and docile bulls on d 56 had less ($P < 0.05$; 4.22 ± 0.23 %) intramuscular fat percentage (IMF) when compared with intermediate bulls (4.94 ± 0.16 %). It was concluded that selecting bulls based on docility could increase BW and frame score.

Introduction

Handling cattle can be dangerous, especially when working with temperamental bulls, or cows with newborn calves. Between 2003 and 2008, 108 human fatalities were caused by cattle (CDC, 2009). In cattle, temperament is defined as the reactivity, or fear response to humans or novel environments and is considered to be moderately heritable (Fordyce et al., 1988a). Multiple measures have been employed for scoring temperament, with the three most common measurements being chute score, pen score, and exit velocity (Curley et al., 2006). Exit velocity and pen score have been used more commonly for assessing temperament because cattle have shown less adaptation over time to these temperament measures as compared to chute score (Curley et al., 2006).

Temperamental cattle have a lower average daily gain (ADG) and produced tougher meat than docile cattle (Voisinet et al., 1997a; Voisinet et al., 1997b). Nkrumah et al. (2007) found that exit velocity was positively correlated with back fat thickness (FAT; $rg = 0.36$) and ribeye area (REA; $rg = 0.81$), but negatively correlated ($rg = -0.13$)

to marbling score as measured on the live animal by ultrasonography. Behavioral patterns (i.e. lying time, feeding behavior, etc.) differ for cattle of varying temperaments (Wierenga, 1987; Fisher et al., 1997) such that a reduction in lying time can cause cattle to become temperamental and temperamental cattle have a reduction in head down time during feeding when compared with docile cattle .

The objective of this study was to examine behavioral differences between bulls of different temperaments in conjunction with growth performance measured over 84 d and to identify additional objective methods for determining bull temperament and the effect of selecting bulls according to temperament.

Materials and Methods

Animals and Housing

All animal procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee. Bulls (n = 70; 222-311 d of age) born between December 2012 and March 2013 were delivered to the University of Tennessee Bull Testing Station at Middle Tennessee Research and Education Center (MTREC; Spring Hill, TN). Bulls originated from producers located in Tennessee and Kentucky. The majority (n = 60) of bulls were registered Angus. The remaining bulls were Simmental, SimAngus, and Santa Gertrudis breeds. Enrolled bulls were accompanied by a health certificate from a licensed veterinarian, including vaccination records and results of a negative test for Bovine Viral Diarrhea (BVD). All bulls were revaccinated with BRD Shield™ (Bovine Rhinotracheitis - Virus Diarrhea - Parainfluenza 3 - Respiratory Syncytial Virus, Novartis Animal Health) upon arrival to the Bull Testing Station. Bulls (n

= 5) deemed physically unsound or otherwise unsuitable (i.e. a threat to personnel) were eliminated from the testing program. Animals meeting the above criteria (n = 65) were housed in pens (36.58 m X 9.75 m uncovered dirt pad and 14.63 m X 9.75 m covered concrete pad) of 8-12 bulls per pen based on similar BW and age (approximately 63 m^2/bull). Feed bunks and hay rings were located under the covered portion of the pen and automatic waters were located at the boundary of the covered and uncovered portions of the pen. Bulls received *ad libitum* access to hay (orchard grass and tall fescue blend) and a pelleted feed (Table 1). The bulls were habituated to this environment for 14 d before beginning the 84 d testing period.

Temperament Data Collection

On d -1, 27, 55, and 83, bulls were randomly grouped within their pens (3-5 bulls/group) and pen scores were assigned when each bull was approached for approximately 30 s by an observer. The same observer approached each bull and assigned a pen score (1-5 scale) based on the animal's reactivity to the presence of the observer. Scoring criteria was as follows: 1 = docile animal, lets observer approach closely, walks slowly; 2 = runs along fence when observer approaches, standoffish toward observer; 3 = runs along fence, head held up, runs away from observer when approached; 4 = runs, very cautious of observer, may run into fences trying to escape; 5 = very aggressive, destructive, easily agitated, runs into fences and possibly observer (Kunkle et al., 1986; Hammond et al., 1996).

Table 1. University of Tennessee Bull Testing Station feed composition and nutrient analysis.

Feed Composition	**% (As-fed basis)**
Wheat Middlings	33.3
Cottonseed Hulls	20.0
Rice Hulls	6.5
Corn	10
Corn Gluten Pellets	10
Soymill Feed	7.1
Distiller's Grains & Solubles	6.5
Limestone	1.9
Liquid Binder	1.5
Cottonseed Meal	1.3
Salt	0.5
Sodium Bicarbonate	0.4
Vitamin/Mineral Premix	1.1
Nutrient Content[1]	**% (Dry Matter Basis)**
TDN	60.66
CP	12.26
Ca	0.91
P	0.47
K	0.85
CF	16.60
Salt	0.50

[2]Nutrient content of pelleted feed ration fed to all bulls *ad libitum.*

On the following day (d 0, 28, 56, and 84), each pen of animals was worked through a chute system and the order in which the bulls entered the chute was recorded to ascertain if there was any relationship between temperament and willingness to enter the chute. The latency to exit the chute (Time 1) after the head gate was completely opened was recorded to assess the initial reaction time. A sensor on the gate was tripped when the head gate was completely opened, recording the time it took each bull to leave the squeeze chute and reach Beam 1 (Fig. 1).

Exit velocity was recorded for each bull over a fixed distance following exit from the squeeze chute (Burrow et al., 1988; King et al., 2006). Two infrared sensors (Beam 1 and 2), located 1.83 meters apart (Burdick et al., 2011a; Burdick et al., 2011b; Burdick Sanchez et al., 2013), recorded the time it took the bull to cross the fixed distance (Beam 1 to Beam 2 = Time 2; Fig. 1). Exit velocity was calculated thereafter (exit velocity = 1.83 m / Time 2).

An overall temperament score was calculated for each bull using the assigned pen score and exit velocity recorded on each of the four testing periods (pen score + exit velocity / 2) as described previously (Burrow et al., 1988; Curley et al., 2006; King et al., 2006).

Behavioral Accelerometer Data Collection

A datalogger (IceTag, IceRobotics Ltd., Edinburgh, Scotland, UK) was attached to the left rear fetlock of the same 30 bulls (3 pens) during period 1 (d 0-28) and period 3 (d 56-84) of the test. Attachment allowed for continuous behavior measurements that included: total steps taken, total lying time, total number of lying bouts, and lying bout

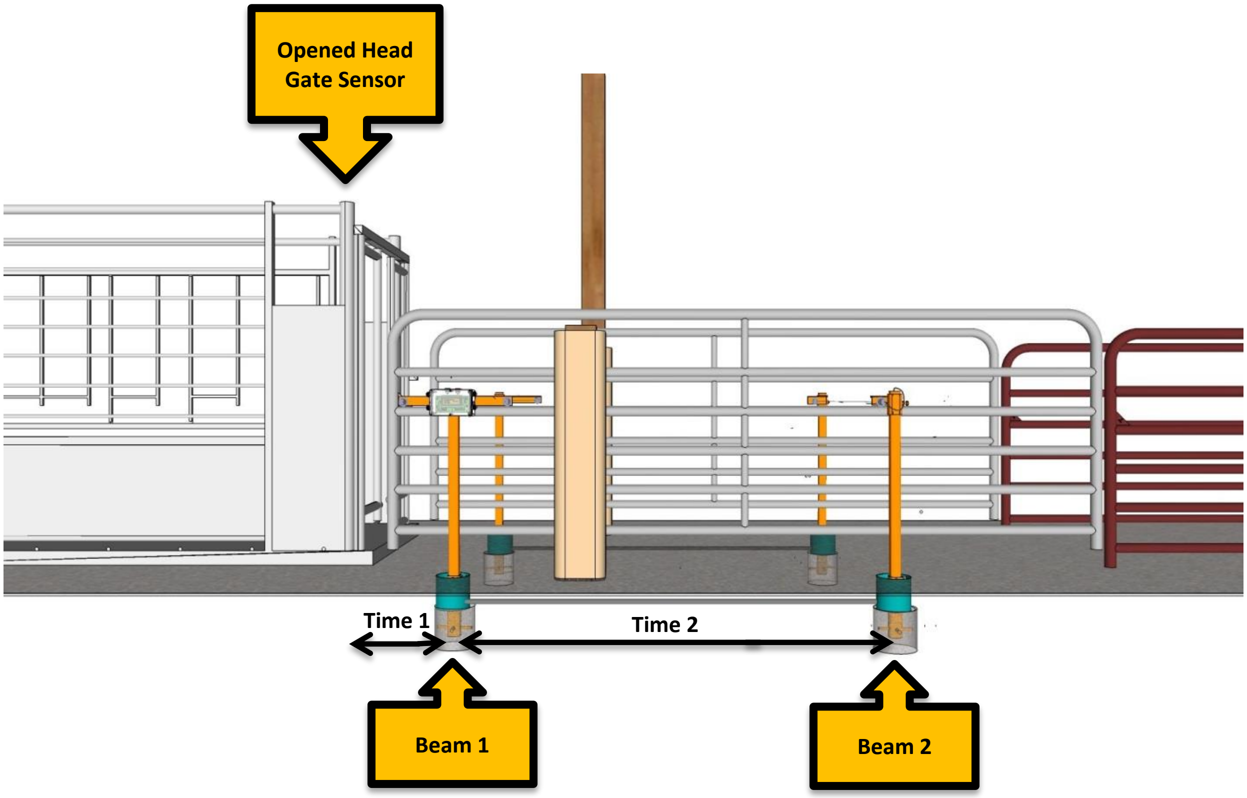

Figure 1. University of Tennessee Bull Testing Station exit velocity set up.

Beam 1 and Beam 2 were placed 1.83 m apart. Time 1 was used to determine the initial reactivity of the bulls leaving the chute. Time 2 was used to calculate exit velocity for each bull.

duration. A 3 d habituation period was implemented after datalogger attachment (MacKay et al., 2012). Data collected during the habituation period was not included in the final analysis.

Performance Data Collection

Hip height and BW were recorded for each bull on d 0, 28, 56, and 84. Frame score (Table 2) was determined on d 84 by combining age of the bull with its hip height recorded on the same day (Vargas et al., 1999). The ADG was calculated over each of the three time periods (period 1 = d 0 – 28, period 2 = d 29 – 56, period 3 = d 57 – 84). On d 84, FAT, REA, and intramuscular fat percentage (IMF) were measured on each bull by ultrasonography. Adjusted 365 d weight was calculated for each bull at the end of the 84 d testing period.

Statistical Analyses

Bull behavior data captured by IceTag dataloggers were split into four phases (early phase of period 1 = d 3 – 15, late phase of period 1 = d 16 – 28, early phase of period 3 = d 59 – 70, late phase of period 3 = d 71 – 84) to better relate to measures of exit velocity, pen score, and overall temperament on d 0, 28, 56, and 84 (± 1 d).

All statistical analyses were performed in SAS 9.3 (SAS Institute, Cary, NC). Using PROC UNIVARIATE, bulls were separated into three groups (temperamental, intermediate, and docile) based on the 25th and 75th quantiles for each measure of temperament (pen score, exit velocity, and overall temperament). A Spearman correlation analysis was performed on overall temperament and its relationship with the

Table 2. Frame score chart used by the University of Tennessee Bull Testing Station.

Frame Score[1]	3	4	5	6	7	8
Age[2]			Hip Height, cm			
5 months	96.52	101.6	106.88	111.76	116.84	121.92
6 months	99.06	104.14	109.22	114.3	119.38	124.46
7 months	101.6	106.88	111.76	116.84	121.92	127.00
8 months	104.14	109.22	114.3	119.38	124.46	129.54
12 months	114.30	119.38	124.46	129.54	134.62	139.70

[1]Frame score (3-8) based on age and hip height according to Beef Improvement Federation recommendations.
[2]Age of bull when hip height was measured.

behavioral patterns measured via dataloggers and performance data with moderate and strong correlations of interest ($r > 0.3$, $r < -0.3$).

Fisher's Exact Test and Kendall's Tau-b correlations were used to determine repeatability of pen score, exit velocity, and overall temperament categories. Exit velocity and overall temperament categories (docile, intermediate, and temperamental) were dummy variables used in a dummy regression analysis to compare alterations in response variables (overall temperament and exit velocity scores) over the 84 d testing period. A log transformation was performed on exit velocity and overall temperament.

Mixed model analysis of variance was utilized to evaluate the relationships of overall temperament on bull behavior and growth performance data collected over the 84 d testing period. Pen number was included in the model as a random effect. Overall temperament groupings were used as a fixed effect to analyze for differences in behavior and growth performance. Fisher's Least Significant Differences were used to separate means ($P < 0.05$).

Results

Temperament Validation

Bulls categorized as temperamental according to exit velocity (quadratic regression; $P < 0.001$), became more docile ($R^2 = 0.60$) over time as determined by dummy regression analysis (Fig. 2). Bulls initially deemed intermediate and docile remained unchanged ($P > 0.10$) over time. Pen score of bulls, assigned on d -1 and 27, based on Fisher's Exact Test and Kendall's Tau b Correlation, were found to be repeatable ($P < 0.05$; $r = 0.34$). Thereafter, pen scores showed low repeatability ($P >$

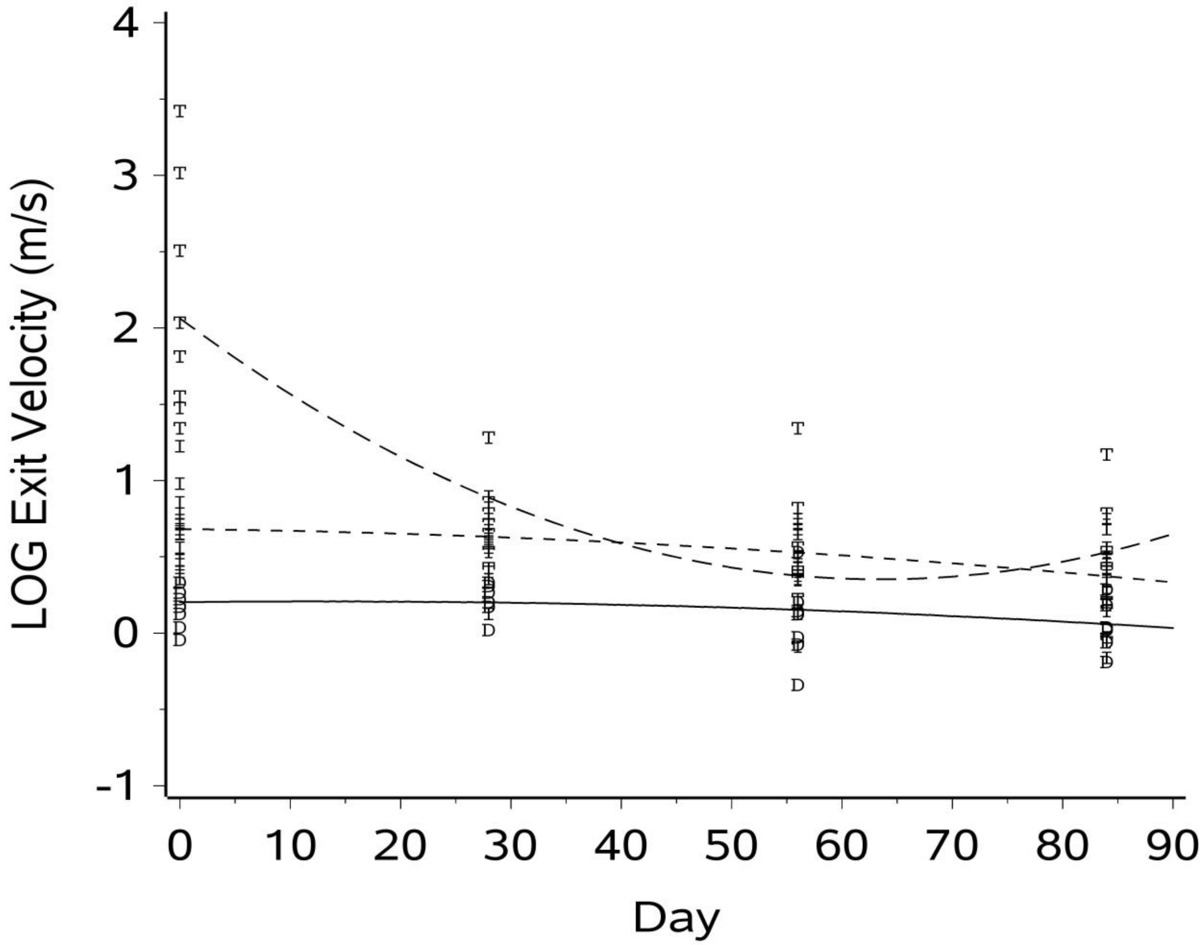

Figure 2. Dummy regression analysis of exit velocity categories[1] and exit velocity scores over time (SAS 9.3).

[1]Exit velocity categories were determined using PROC UNIVARIATE in SAS 9.3 based on the 25th and 75th quantiles. Docile (——) and intermediate (- - - -) categories were linear regressions. The temperamental (– – – –) category was a quadratic regression.

0.05; r = 0.13 – 0.17) as bulls became less temperamental. Based upon overall temperament score (pen score + exit velocity / 2), bulls categorized as temperamental (quadratic regression; P < 0.001) and intermediate (linear regression; P < 0.01) became more docile (R^2 = 0.52) over the 84 d testing period as determined by a dummy regression analysis (Fig. 3). For both temperamental and intermediate categories, bulls appeared to have habituated after d 28 as demonstrated by a leveling of the regression slopes. Bulls initially categorized as docile according to overall temperament remained the same (P > 0.10) over time. Exit velocity was correlated (P < 0.01) with pen score on d 28 (r = 0.38) and 84 (r = 0.34). Based on these results, overall temperament score was used when designating temperament category in the subsequent analyses of behavior and growth performance during the 84 d testing period.

Behavior

Based on Spearman correlations, overall temperament was not correlated (-0.3 < r < 0.3) with behavioral data collected from the data loggers over the 84 d testing period. A moderate negative correlation (r = -0.30; P < 0.05) between overall temperament and the time it took the bulls to exit the chute (Time 1) was noted on d 84.

Mean (± SEM) total time lying, total steps taken, lying bout duration, and total number of lying bouts recorded during period 1 (d 3 – 28) and late phase of period 3 (d 71 – 84) did not differ (P > 0.10) between bulls categorized as docile, intermediate, and temperamental on d 0, 28, and 84 (Table 3). On d 56, bulls categorized as temperamental, in comparison to intermediate on d 56, tended (P = 0.06) to have longer lying bout durations and fewer total number of lying bouts during the early phase of

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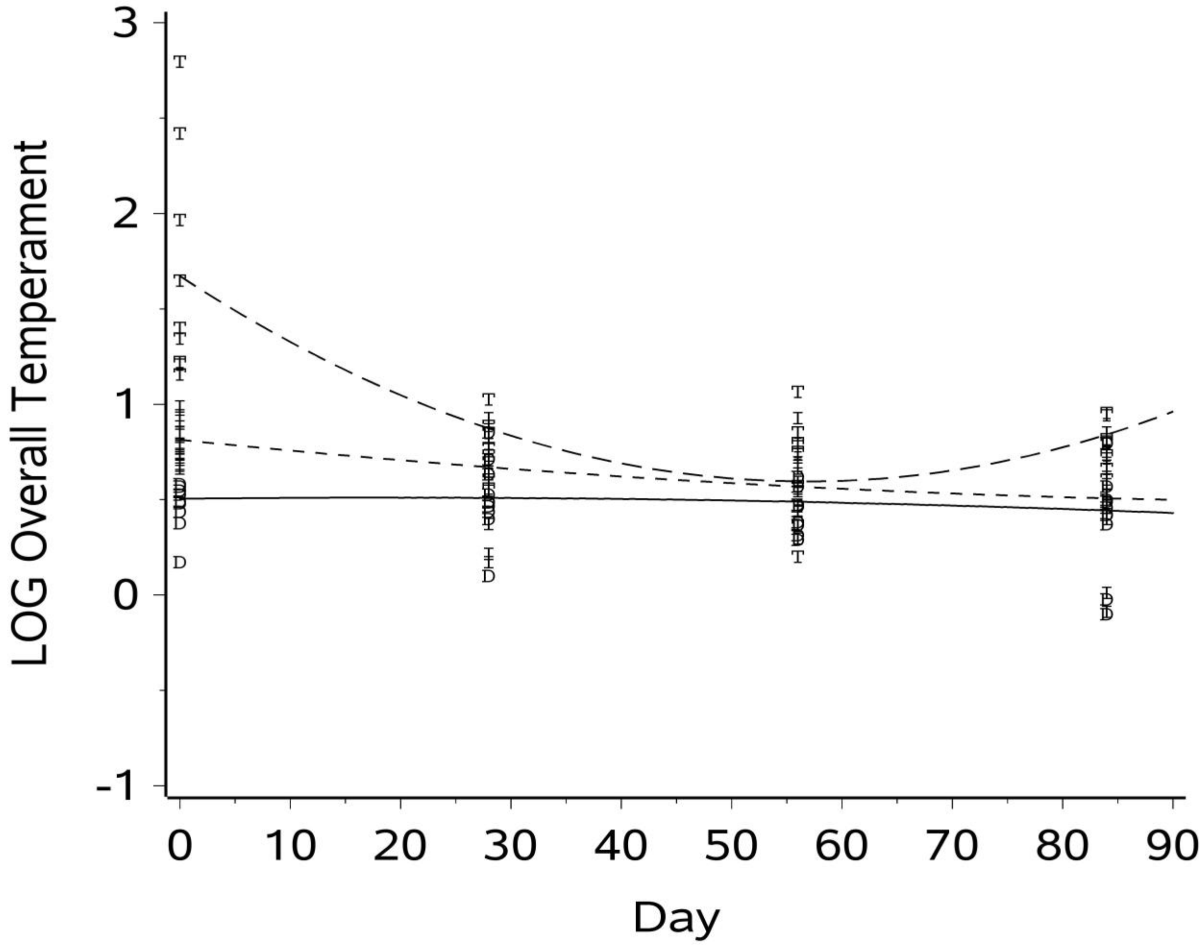

Figure 3. Dummy regression analysis of overall temperament scores by temperament category[1] over time (SAS 9.3).

[1]Overall temperament categories were determined using PROC UNIVARIATE in SAS 9.3 based on the 25th and 75th quantiles. Docile (——) and intermediate (- - - -) categories were linear regressions. The temperamental (– – – –) category was a quadratic regression.

period 3 (d 58 – 70; Table 3). Mean lying bout duration and total number of lying bouts for the docile bulls was not different from intermediate or temperamental bulls during this same time period (Table 3).

Bull order through the chute recorded on d 0, 28, and 56 was unrelated ($P >$ 0.10) to temperament category. On d 84, docile bulls tended ($P = 0.07$) to enter the chute system later in order than the temperamental bulls (7.07 ± 0.86 vs. 4.47 ± 0.83, respectively). Mean order through the chute for bulls deemed intermediate (6.48 ± 0.56) on d 84 did not differ from either the docile or temperamental bulls. Initial bull reactivity to exiting the chute (Time 1) was negatively correlated ($r = -0.30$; $P < 0.05$) to overall temperament only on d 84.

Performance

Overall, BW was positively correlated ($P < 0.001$) to hip height ($r = 0.46 – 0.63$) and hip height was positively correlated ($r = 0.49 – 0.78$) to frame score measured on d 84. Bulls categorized as docile were heavier on d 0, 28, and 56 as compared with temperamental bulls (Table 4). Similarly, frame score measured on d 84 was greater ($P < 0.05$) for docile bulls compared with intermediate or temperamental bulls. On d 0 and 56, BW differed between overall temperament categories such that docile and intermediate bulls had greater ($P < 0.05$) BW than temperamental bulls. On d 28, docile bulls had a greater ($P < 0.05$) mean BW than both intermediate and temperamental bulls. By d 84, BW did not differ ($P > 0.10$) between bulls in the three temperament categories.

Table 3. Behavioral variables measured via dataloggers attached to bulls (n = 30) during period 1(d 3 – 28) and 3 (d 59 – 84).

		Overall Temperament Category[1]		
Behavioral Variables[2]	**Stage and Period[3]**	**Docile**	**Intermediate**	**Temperamental**
Lying Bout Duration, hrs	Early phase of period 1	0.96 ± 0.08	0.92 ± 0.06	1.03 ± 0.08
	Late phase of period 1	0.94 ± 0.05	0.93 ± 0.05	0.99 ± 0.06
	Early phase of period 3	1.07^{cd} ± 0.08	0.93^{d} ± 0.05	1.15^{c} ± 0.09
	Late phase of period 3	1.13 ± 0.12	1.24 ± 0.08	1.11 ± 0.09
Total Number of Lying Bouts	Early phase of period 1	15.02 ± 1.26	16.12 ± 0.97	14.29 ± 1.34
	Late phase of period 1	14.96 ± 0.93	13.57 ± 0.85	14.71± 1.04
	Early phase of period 3	14.04^{cd} ± 1.15	16.12^{c} ± 0.90	13.15^{d} ± 1.10
	Late phase of period 3	14.08 ± 1.56	12.97 ± 0.10	13.31 ± 1.15
Total Steps Taken	Early phase of period 1	1575.38 ± 143.80	1458.58 ± 101.25	1426.64 ± 143.66
	Late phase of period 1	1316.44 ± 75.03	1316.45 ± 68.86	1195.99 ± 83.82
	Early phase of period 3	1203.41 ± 102.43	1302.95 ± 78.52	1308.50 ± 114.01
	Late phase of period 3	1423.84 ± 154.41	1421.50 ± 105.71	1295.50 ± 118.65
Total Time Lying, hrs	Early phase of period 1	14.30 ± 0.39	14.42 ± 0.22	14.19 ± 0.39
	Late phase of period 1	13.93 ± 0.25	13.97 ± 0.24	14.15 ± 0.27
	Early phase of period 3	14.47 ± 0.31	14.68 ± 0.22	14.95 ± 0.32
	Late phase of period 3	13.59 ± 0.39	13.90 ± 0.24	14.07 ± 0.28

[a,b] Means within a row without common superscripts differ (*P*< 0.05).
[c,d] Means within a row without common superscripts differ (*P* < 0.10).
[1] Overall temperament was determined by averaging pen score and exit velocity. Overall temperament categories were determined using PROC UNIVARIATE in SAS 9.3 based on the 25th and 75th quantiles.
[2] Behaviors were measured through accelerometer technology of IceTag dataloggers.
[3] Early phase of period 1 = d 3 – 15; Late phase of period 1 = 16 - 28; Early phase of period 3 = 59 - 70; late phase of period 3 = 71 - 84.

Table 4. Growth performance variables over all days according to overall temperament category.

		Overall Temperament Category[1]		
Growth Performance Variables[2]	**Day**	**Docile**	**Intermediate**	**Temperamental**
Adjusted 365 d Weight, kg	0	$573.57^{a} \pm 10.56$	$561.92^{a} \pm 7.21$	$531.59^{b} \pm 10.56$
	28	$585.06^{c} \pm 10.29$	$545.45^{d} \pm 7.28$	$550.99^{d} \pm 10.29$
	56	562.82 ± 11.27	562.07 ± 7.60	541.27 ± 11.27
	84	574.71 ± 11.12	554.20 ± 7.24	559.30 ± 10.74
Body Weight, kg	0	$394.83^{a} \pm 19.43$	$378.88^{a} \pm 17.98$	$354.00^{b} \pm 19.40$
	28	$446.49^{a} \pm 16.54$	$414.13^{b} \pm 15.13$	$413.92^{b} \pm 16.53$
	56	$502.07^{a} \pm 14.75$	$499.13^{a} \pm 12.49$	$465.87^{b} \pm 14.96$
	84	561.29 ± 17.86	541.28 ± 15.42	546.17 ± 17.47
Frame Score	84	$6.34^{a} \pm 0.18$	$5.84^{b} \pm 0.13$	$5.88^{b} \pm 0.17$
Hip Height, cm	0	120.93 ± 0.89	121.42 ± 0.69	121.02 ± 0.89
	28	123.65 ± 0.88	123.11 ± 0.69	123.80 ± 0.88
	56	125.14 ± 0.80	125.42 ± 0.55	124.57 ± 0.81
	84	$130.49^{a} \pm 0.83$	$128.10^{b} \pm 0.59$	$128.60^{ab} \pm 0.80$

[a,b]Means within a row without common superscript differ ($P < 0.05$).
[c,d]Means within a row without common superscripts differ ($P < 0.01$).
[1]Overall temperament was determined by averaging pen score and exit velocity. Overall temperament categories were determined using PROC UNIVARIATE in SAS 9.3 based on the 25th and 75th quantiles.
[2]Growth performance was measured every 28 d as part of the bull testing program.

Hip height did not differ ($P > 0.10$) between temperament categories on d 0, 28, and 56 (Table 4). Docile bulls had greater ($P = 0.04$) hip height as compared with intermediate bulls measured on d 84; however, neither docile nor intermediate bulls differed in hip height from the temperamental bulls. Similar to hip height, frame score calculated on d 84 did not differ ($P > 0.10$) between temperament categories assigned on d 0, 28, and 56. On d 84, docile bulls had a larger ($P < 0.05$) frame score than intermediate and temperamental bulls.

Adjusted 365 d BW of bulls categorized on d 0 as docile and intermediate was greater ($P < 0.05$) than that calculated for the temperamental bulls (Table 4). Bulls that were deemed as more docile on d 28 had greater adjusted 365 d BW when compared to temperamental bulls. Adjusted 365 d BW was not different between the three temperament categories on d 56 or 84.

The ADG over the test period was greater ($P < 0.05$) in bulls categorized as intermediate on d 56 when compared to docile and temperamental bulls (Table 5). Bulls categorized as intermediate on d 56 had greater ($P < 0.05$) ADG during period 2 (d 28 – 56) than did temperamental bulls. During this same time, mean ADG for docile bulls was similar to that of the bulls in the other two categories. The ADG measured during all other periods was not different ($P > 0.10$) among bulls due to temperament category.

There was no difference ($P > 0.10$) in mean FAT between temperament categories assigned on d 0, 28, 56, and 84 (Table 6). Ribeye area did not differ due to overall temperament category assigned on d 0, 56, and 84. Intramuscular fat did not differ ($P > 0.05$) due to overall temperament category assigned on d 0, 28, and 84.

Table 5. Growth performance variables over all days according to overall temperament category.

		Overall Temperament Category[1]		
Growth Performance Variables[2]	**Day**	**Docile**	**Intermediate**	**Temperamental**
ADG Period 1[3], kg/d	0	1.50 ± 0.14	1.79 ± 0.10	1.64 ± 0.14
	28	1.57 ± 0.14	1.73 ± 0.12	1.64 ± 0.14
	56	1.45 ± 0.14	1.72 ± 0.10	1.71 ± 0.14
	84	1.63 ± 0.14	1.67 ± 0.11	1.82 ± 0.14
ADG Period 2[4], kg/d	0	2.29 ± 0.12	2.27 ± 0.09	2.07 ± 0.12
	28	2.22 ± 0.12	2.19 ± 0.09	2.18 ± 0.12
	56	2.18^{ab} ± 0.12	2.30^{a} ± 0.09	2.00^{b} ± 0.12
	84	2.35 ± 0.12	2.14 ± 0.09	2.25 ± 0.12
ADG Period 3[5], kg/d	0	2.10 ± 0.12	2.08 ± 0.08	2.02 ± 0.12
	28	2.10 ± 0.11	2.08 ± 0.08	2.07 ± 0.11
	56	2.08 ± 0.12	2.12 ± 0.08	1.97 ± 0.12
	84	1.96 ± 0.11	2.18 ± 0.07	2.08 ± 0.11
Overall ADG, kg/d	0	2.02 ± 0.07	2.06 ± 0.05	1.95 ± 0.07
	28	2.02 ± 0.07	2.00 ± 0.05	2.04 ± 0.07
	56	1.94^{b} ± 0.06	2.10^{a} ± 0.04	1.92^{b} ± 0.06
	84	2.05 ± 0.07	1.99 ± 0.04	2.11 ± 0.06

[a,b]Means within a row without common superscripts differ (*P* < 0.05).
[1]Overall temperament was determined by averaging pen score and exit velocity. Overall temperament categories were determined using PROC UNIVARIATE in SAS 9.3 based on the 25^{th} and 75^{th} quantiles
[2]Growth performance was measured every 28 d as part of the bull testing program.
[3]Period 1 ADG was calculated for d 0 - 28.
[4]Period 2 ADG was calculated for d 29 - 56.
[5]Period 3 ADG was calculated for d 57 - 84.

Table 6. Body composition measurement means according to overall temperament category.

		Overall Temperament Category[1]		
Body Composition Measurement[2]	**Day**	**Docile**	**Intermediate**	**Temperamental**
Back Fat (FAT), cm	0	0.83 ± 0.05	0.79 ± 0.03	0.82 ± 0.05
	28	0.84 ± 0.04	0.76 ± 0.03	0.84 ± 0.04
	56	0.76 ± 0.04	0.81 ± 0.03	0.81 ± 0.05
	84	0.77 ± 0.05	0.80 ± 0.03	0.83 ± 0.05
Rib Eye Area (REA), cm^2	0	86.25 ± 1.67	83.07 ± 1.12	82.93 ± 1.67
	28	86.29^{a} ± 1.46	81.71^{b} ± 1.05	84.61^{ab} ± 1.56
	56	85.07 ± 1.55	82.59 ± 1.11	84.41 ± 1.61
	84	85.21 ± 1.71	83.52 ± 1.07	82.76 ± 1.64
Intramuscular Fat (IMF), %	0	4.61 ± 0.27	4.74 ± 0.18	4.66 ± 0.27
	28	4.75 ± 0.23	4.58 ± 0.17	4.93 ± 0.25
	56	4.22^{b} ± 0.23	4.94^{a} ± 0.16	4.66^{ab} ± 0.24
	84	4.34 ± 0.25	4.87 ± 0.16	4.81 ± 0.24

[a,b]Means within a row without common superscripts differ ($P < 0.05$).
[1]Overall temperament was determined by averaging pen score and exit velocity. Overall temperament categories were determined using PROC UNIVARIATE in SAS 9.3 based on the 25th and 75th quantiles.
[2]Body composition measurements were obtained via ultrasonography on d 84.

Bulls categorized as docile on d 28 had a greater (*P* < 0.05) REA on d 84 than intermediate bulls (Table 6). The IMF measured on d 84 was greater (*P* < 0.05) for bulls categorized as intermediate on d 56 when compared to docile bulls (Table 6).

Discussion

Over the 84 d testing period, bulls became more docile in regards to pen score, exit velocity, and overall temperament score. Likewise, Curley et al. (2006) reported that over a 120 d period, cattle became more docile in regards to pen score, chute score, and exit velocity, which the authors suggested was due to the animals adapting to the presence of humans. We speculate that the bulls in the present study also became familiarized with the University of Tennessee Bull Testing Station personnel and facilities as shown by a reduction in exit velocity, pen score, and overall temperament scores over the testing period.

Contrary to the results of Wierenga (1987), which reported finding an inverse relationship between lying time and bull temperament, we found that bulls categorized as temperamental on d 56 had longer lying bout durations and fewer lying bouts during the early phase of period 3 when compared to intermediate bulls. When comparing the difference in lying bout duration and number of lying bouts however, the difference was biologically irrelevant since total lying time did not differ between temperament categories.

Throughout the 84 d testing period, bulls spent between 13 and 14 h/d lying regardless of overall temperament category. Hoffman and Self (1973) reported that feed lot steers spend approximately 12 h/d lying regardless of season, which was slightly less than the total lying time in the present study. We speculate that environmental conditions, management routine, gender, and pen size could have attributed to differences in total lying time between the two studies. Feedlot steers in the study conducted by Hoffman and Self (1973) were provided less lying space per steer than the bulls in the present study which could have reduced total lying time. Hoffman and Self (1973) also reported that steers were housed on concrete and were not provided bedding throughout the study, whereas the bulls in our study were provided a dirt pad which could contribute to the conflicting results seen in lying behavior.

Although previous research has not examined the relationship between temperament and order through a chute system, we hypothesized that due to their flighty nature, temperamental bulls would be hesitant to enter the chute system and would be the last to be worked through the chute. Contrary to our hypothesis, bulls that did not habituate and were deemed temperamental according to their overall temperament category assigned on d 84 entered the chute system before the docile bulls. The present study was limited in regards to the number of temperamental bulls since bulls considered to be a threat to personnel were removed prior to the start of the testing period. Future studies involving larger groups of cattle with a greater variation in temperament may be necessary to determine if there is truly a relationship between temperament and the order to which animals repeatedly enter a chute system.

Schmidt et al. (2013) reported that BW was greater in docile cattle compared with temperamental cattle when temperament category was based solely upon exit velocity. Hoppe et al. (2010) also reported that there was a negative correlation between exit velocity and BW. However, it has not been determined if exit velocity is hindered by a greater BW. Analogous to the results of Schmidt et al. (2013), BW reported in the present study differed between temperament categories on d 0, 28, and 56, with docile cattle having greater BW than temperamental bulls. We also found that frame score measured on d 84 was also greater for docile bulls as compared to temperamental bulls. These results suggest that when selecting for docility, we may be selecting for bulls with greater BW. In addition, if we select for docility at the conclusion of the 84 d test, we may be selecting for bulls with larger frame scores.

The ADG results of the present study differed slightly from those reported previously that found that docile cattle had higher ADG than temperamental cattle (Voisinet et al., 1997b; Fell et al., 1999; Müller and von Keyserlingk, 2006). Our study found that bulls scored as intermediate temperament on d 56 had a greater ADG over the 84 d testing period and during period 2 when compared to temperamental bulls. Differing results from our study and those reported previously may be attributed to varying temperament scoring methods. In the previous studies, temperament scores were assigned according to flight speed and/or chute score, whereas we used the average of exit velocity and pen score to determine temperament. On d 56, our overall temperament category scores were as follows: docile = 0 - 1.5; intermediate = 1.6 – 2.2; temperamental = 2.3 – 4.0. Thus, bulls categorized as intermediate in our study may have been considered docile based upon flight speed and/or chute score used by others.

Previous studies have not examined the relationship between cattle temperament and adjusted 365 d BW. Based on our data, bulls categorized as docile and intermediate on d 0 had greater adjusted 365 d BW than temperamental bulls.

Knowing that docile cattle had greater ADG than temperamental cattle (Voisinet et al., 1997b; Fell et al., 1999; Müller and von Keyserlingk, 2006) we would expect docile bulls to have greater adjusted 365 d BW as compared to temperamental bulls.

In the present study, FAT measured by ultrasonography on d 84 did not differ between bulls of different overall temperament categories. It was previously found that both FAT and marbling scores were greater in docile feedlot cattle when compared with temperamental feedlot cattle (Schmidt et al., 2013) and that marbling scores were directly correlated with the degree of IMF (Hale et al., 2013). We found that bulls categorized as intermediate on d 56 had greater IMF than docile bulls. These conflicting results may be due to differences in gender, nutrition, and genetics between our study and theirs, all of which have been noted to influence FAT (Smith et al., 1984; Crouse et al., 1989; Charagu et al., 2000) and IMF (Field et al., 1966; Crouse et al., 1989; Pethick et al., 2004). Camfield et al. (1997) reported that cattle with larger frame scores possess less marbling as compared to cattle with smaller frame scores. We found that docile cattle had larger frame scores and less IMF than temperamental cattle. Thus, we believe that the reduction in IMF seen here in docile cattle may be a reflection of frame size, not temperament.

The REA was larger for bulls categorized as docile on d 28 when compared to intermediate bulls, but REA did not differ between overall temperament categories assigned on d 0, 56, and 84. Black et al. (2013) assessed temperament in heifers of different breed types every 2 wk by evaluating both pen score and exit velocity with regard to REA and concluded that REA did not differ due to temperament when assessed over a 70 d period. Gender and breed differences may explain these conflicting results. Bulls are faster growers and have larger REA than heifers of the same age and sire (Hedrick et al., 1969), and accelerated growth rate may have led to larger variations in REA among the bulls in our study. The heifers in the study conducted by Black et al. (2013) were a mixture of *Bos taurus* and *Bos indicus* breed types, whereas the bulls in the present study were mostly *Bos taurus* and were a uniform group of animals for comparison of body conformation measurements.

In the present study, bulls became docile in regards to pen score, exit velocity, and overall temperament over the 84 d testing period. By the end of the testing period, bulls deemed temperamental by our ranking system might have been considered docile to other researchers. However, selecting bulls based on initial (d 0) temperament score may serve as a means for selecting bulls with greater BW. As a result of bulls becoming more docile over the 84 d testing period, additional studies utilizing bulls exhibiting a greater variation in temperament are needed to further examine the relationships of bull temperament, behavior, and growth performance.

Chapter 4. Relationships of temperament, cortisol, testosterone, and reproductive soundness during performance testing of bulls

Abstract

The aim of this study was to examine relationships among bull temperament, endocrine, and reproductive parameters to better understand the impact of selecting bulls based on temperament. Relationships between endocrine parameters, growth performance, and behavioral data were also examined. Bulls (n=65) were reared in 6 pens and grouped by age and BW. Overall temperament (pen score + exit velocity /2) was assigned, hair and blood samples were collected, and BW was measured for each bull on d 0, 28, 56, and 84. Bulls were categorized as docile, intermediate, or temperamental based on the 25^{th} and 75^{th} quantiles of overall temperament. Dataloggers were attached to bulls (n=30; 3 pens) to measure animal activity during period 1(d 3-28) and 3 (d 59-84). Breeding soundness exams (BSE) and body conformation measurements were performed on d 84. Spearman correlation analyses and mixed model analysis of variance were performed in SAS 9.3. Docile bulls tended to have greater (P = 0.06) serum testosterone and lower (P < 0.05) serum cortisol concentration than temperamental bulls on d 56 (2.32 ± 0.35 ng/mL vs. 1.30 ± 0.36 ng/mL, respectively). Hair testosterone was lower (P < 0.05) in temperamental bulls as compared to docile and intermediate bulls on d 56 (7.12 ± 0.59 pg/mg vs. 10.04 ± 0.82 pg/mg, respectively). Docile and intermediate bulls tended (P = 0.07) to have a lower percentage of primary spermatic defects (20.58 ± 4.86 %) as compared to temperamental bulls (38.01 ± 8.06 %) on d 84. Serum testosterone on d 0 was positively correlated (r = 0.34; P < 0.05) to the percentage of normal sperm and negatively correlated (r = 0.45; P < 0.01) to primary defects on d 84. Serum testosterone measured on d 28 and 56 was positively correlated to BW (r = 0.47 and

0.39; P < 0.01) and adjusted 365 d BW (r = 0.38 and 0.36; P < 0.01). However, as serum testosterone concentration increased on d 56 and 84, less intramuscular fat (IMF) was present on d 84 (r = -0.36 and r = -0.32; P <0.05, respectively). On d 28, BW was positively correlated (r = 0.32; P < 0.05) to hair testosterone and negatively correlated to hair cortisol (r = -0.31; P < 0.05). Total lying time during the later phases of periods 1 and 2 decreased as hair and serum cortisol increased on d 28 (r = -0.50; P < 0.01) and 84 (r = -0.35; P = 0.08). During the early phase of period 3, time spent lying was positively correlated (r = 0.43; P < 0.05) with hair testosterone concentration measured on d 56. In conclusion, bulls that are selected for docility may have higher serum and hair testosterone, lower serum cortisol concentrations, and lower IMF.

Introduction

Working with temperamental cattle can be dangerous to the livelihood of personnel and may jeopardize the longevity of facilities and equipment. Temperament, with regard to cattle, can be defined as the behavioral response to the presence of humans or novel environments (Fordyce et al., 1988a; Fordyce et al., 1988b). However, early exposure to common management practices has been proven to decrease incidences of temperamental adult cattle (Fordyce et al., 1985; Fordyce et al., 1988a). Similarly, Curley et al. (2006) found that cattle become more docile after repeated exposure to a particular routine. In Chapter 3, it was reported that selecting bulls around a year of age with larger BW and frame scores may coincide with the selection of docile bulls with greater adjusted 365 d BW.

Previous research that examined cattle temperament and circulating cortisol concentrations over a 120-d period has shown that temperamental cattle, in regards to pen score and exit velocity, repeatedly have higher cortisol concentrations when compared with docile cattle (Curley et al., 2006), and cortisol concentrations will decrease with repeated exposure to a particular management practice (Crookshank et al., 1979).

Circulating testosterone concentrations have been linked to poor temperament and aggressive behavior in both male humans (Olweus et al., 1988; Chichinadze et al., 2010) and Asian elephants (Lincoln and Ratnasooriya, 1996). However, there are no similar studies reported for cattle. Corticosteroids, either naturally produced or administered, can reduce both luteinizing hormone (LH) and testosterone concentrations within the male (Thibier and Rolland, 1976). In bulls, an elevated concentration of circulating corticosteroids (i.e. cortisol) correspond with lower concentrations of LH and testosterone (Welsh et al., 1979).

Hair has been used as a non-invasive method for measuring concentrations of cortisol and testosterone (Koren et al., 2002). Steroid hormones can accumulate into the hair shaft via the vascular supply to the hair follicle, sweat, sebaceous gland secretions, and environmental contamination (Cone, 1996; Meyer and Novak, 2012; Bryan et al., 2013b). Thus, measuring hair cortisol and testosterone concentrations may be indicative of chronic cortisol and testosterone production.

Thus far, there have been no studies focusing on the relationship between temperament measured over an 84 d bull testing program and breeding soundness of bulls at the conclusion of the test. In addition, the relationship between serum and hair cortisol concentration and temperament has not been fully examined. The aim of the present study was to examine whether temperament has an effect on acute and chronic cortisol and testosterone concentrations and breeding soundness in bulls examined over an 84 d testing period. Relationships between endocrine and reproductive parameters and the behavioral and growth performance data presented in the previous chapter were further investigated.

Materials and Methods

Animal Selection and Housing

Bull selection and housing was the same as that reported in Chapter 3. Briefly, consigned bulls (n = 65) were reared in pens (8-12 bulls/pen) based on age and weight. All bulls received *ad libitum* access to pelleted feed, hay, and water as described previously.

Tissue Collection and Analysis

All animal procedures were pre-approved by the University of Tennessee Institutional Animal Care and Use Committee. Approximately 10 mL of blood was collected in serum vacutainer tubes via tail vein puncture from each bull on d 0, 28, 56, and 84. Samples were centrifuged at 930 x *g* for 15 m. The serum was aliquoted in two microcentrifuge tubes and stored at -20 ˚C until later analyses for cortisol and testosterone concentrations as described below.

Serum total cortisol concentration (ng/mL) was determined by following the radioimmunoassay (RIA) kit procedures of Coat-A-Count Cortisol (Siemens Medical

Solutions Diagnostics, Los Angeles, CA) as performed previously in our lab (Doherty et al., 2007). Intra- and inter- assay coefficient of variation (CV) were 10.09 and 7.07 % respectively for low (9.5 ng/mL) and 7.09 and 10.18 % respectively for high (44.5 ng/mL) cortisol standards. Total testosterone concentration (ng/mL) was determined by the RIA kit procedure of ImmuChem Double Antibody Testosterone (ICN Biomedicals, Inc., Costa Mesa, California). Intra- and inter- assay CV were 5.86 and 13.04 % respectively for low (1.7 ng/mL) and 4.18 and 9.01 % respectively for high (4.3 ng/mL) testosterone standards.

Hair samples were collected from each bull on d 0, 28, 56, and 84 using electric clippers (#40 blades) over the same 20 cm x 30 cm area located between the tuber ischii and tuber coxae region of each bull. Once clipped, hair samples were placed in zip-lock plastic bags and stored at room temperature for later analysis (del Rosario González-de-la-Vara et al., 2011). Clipper blades were cleaned with absolute ethanol between each bull sampled (del Rosario González-de-la-Vara et al., 2011).

Cleaning procedures were similar to those previously described (Kirschbaum et al., 2009; Bryan et al., 2013a; Bryan et al., 2013b; Ghassemi Nejad et al., 2013). Samples of hair (200mg) were weighed and placed into a 15 mL disposable polypropylene tube and washed four times (3 min per wash) with 3 mL isopropanol to remove manure and environmental debris. After washing, samples were placed on weighing paper and allowed to dry at room temperature, wrapped in aluminum foil, and stored for later analysis (Paulsen et al., 2001; Davenport et al., 2006).

Based on recommendations from Omni-International, Inc. representatives, hair samples (50 mg) were placed in 2 mL reinforced centrifuge tubes with four 2.4 mm metal grinding beads (Omni-International Inc., Kennesaw, GA). Hair samples were then ground to a powder at room temperature in an Omni Bead Ruptor 24 Bead Mill Homogenizer (Omni-International Inc., Kennesaw, GA) in two 50 s cycles of 6.95 m/s with a 15 s break between cycles (Davenport et al., 2006; Kirschbaum et al., 2009; Bryan et al., 2013a; Bryan et al., 2013b).

Hair hormone extraction procedures were performed similarly to those described previously (Bryan et al., 2013b). All extraction procedures were performed at room temperature. Each ground hair sample (30 mg) was placed in a glass vial with 3 mL of HPLC-grade methanol and allowed to extract for 24 h with gentle shaking (Davenport et al., 2006). Tubes were then centrifuged for 30 m at 3724 x *g*. Aliquots of the supernatant were pipetted into separate borosilicate tubes for testosterone (100 uL) and cortisol (2000 uL) analyses and evaporated under a stream of air. Samples were reconstituted in 6.5 uL HPLC-grade methanol and 123.5 uL assay diluent (Salimetrics, Philadelphia, Pennsylvania, USA) prior to steroid hormone analysis.

Reconstituted hair samples were analyzed according to Salimetrics Salivary Cortisol and Testosterone ELISA (Salimetrics, Philadelphia, Pennsylvania, USA) kit procedures as described previously (Gow et al., 2010; Bryan et al., 2013a; Bryan et al., 2013b; Ghassemi Nejad et al., 2013). Cortisol ELISA intra- and inter-assay CV was 4.35 and 7.34 % respectively for low (7.15 pg/mg) and 4.51 and 6.15 % respectively for high (67.60 pg/mg) cortisol standards. Testosterone ELISA intra- and inter-assay CV was 6.04 and 11.54 % respectively for low (2.68 pg/mg) and 8.00 and 8.84 % respectively for high (26.19 pg/mg) testosterone standards.

Cortisol ELISA Validation

Procedures for the validation of sample cleaning, extraction, and the Salimetrics Salivary Cortisol ELISA kit were similar to those used to validate the Salimetrics Salivary Testosterone ELISA kit. Cortisol extracted from the ground hair sample was similar to the concentration of cortisol extracted from the cut hair sample (2.60 pg/mg vs. 2.54 pg/mg, respectively). The validation also showed that there was little difference between cortisol concentrations from samples that were extracted for 24 h versus a 48 h extraction period (2.60 pg/mg vs. 2.99 pg/mg). Serial dilutions of reconstituted hair samples (1:2, 1:4, 1:8) showed a linear reduction in cortisol concentration (1.76, 1.04, 0.59 pg/mg, respectively).

Testosterone ELISA Validation

Previously mentioned hair preparation, extraction, and Salimetrics Salivary Testosterone ELISA procedures were validated prior to analyzing samples for this study. Hair processing techniques (cut vs. ground) were compared to determine the efficiency of hormone extraction. Testosterone concentrations from ground hair samples (15.33 pg/mg) were greater than that measured from samples cut with scissors (12.73 pg/mg). There was also little difference in concentrations between hair samples that were allowed to extract for 24 h compared with those following a 48 h extraction period (12.78 pg/mg vs. 13.15 pg/mg). Serial dilutions of samples (1:2, 1:4, and 1:8) showed a linear reduction in testosterone concentration (5.96, 3.28, 1.83 pg/mg, respectively).

Breeding Soundness Exams

Scrotal circumference was measured on d -14 and 84 of the test period. All bulls were subjected to a breeding soundness exam (BSE) on d 84 to assess physical breeding soundness, sperm motility, and sperm morphology (i.e. primary and secondary spermatic defects). Electroejaculation was used to collect semen to assess sperm motility and morphology. Primary spermatic defects (i.e. pyriform heads, distal midpiece reflex, etc.) were classified as abnormalities associated with spermatogenesis, while secondary defects (i.e. decapitated defect) more commonly occurring in the epididymis (Youngquist and Threlfall, 2007). Physical abnormalities (i.e. sheath cover, persistent frenulum, developmental deformities, etc.) warranted a BSE failure.

Performance and Behavioral Data Collection

Performance and behavioral data were collected as described in Chapter 3. Briefly, weight and hip height was measured for all bulls on d 0, 28, 56, and 84. The ADG for each 28 d period (periods 1 - 3), overall ADG (d 0 - 84), and frame score on d 84 were calculated. Dataloggers were attached to bulls (n=30; 3 pens) during period 1 (d 3 – 28) and 3 (d 59 – 84) to measure animal activity.

Statistical Analysis

All statistical methods were performed in SAS 9.3 (SAS Institute, Cary, NC). Using PROC UNIVARIATE, bulls were separated into three groups (temperamental, intermediate, and docile) based on the 25th and 75th quantiles for overall temperament (pen score + exit velocity / 2). Spearman correlation analysis was performed on temperament and hormone (i.e. cortisol and testosterone), BSE, performance, and behavioral data with moderate and strong correlations of interest ($r > 0.3$, $r < -0.3$).

Mixed model analysis of variance was utilized to evaluate the relationships of overall temperament (docile, intermediate, and temperamental) as defined in Chapter 3 with serum and hair cortisol and testosterone concentrations and breeding soundness data collected during the testing period. Pen number was included in the model as a random effect. Overall temperament groupings were used as a fixed effect to analyze differences in behavior and growth performance. Fisher's Least Significant Differences were used to separate mean differences ($P < 0.05$).

Results

Based on results provided in Chapter 3, the average of pen score and exit velocity was used to assess relationships between the endocrine and reproductive parameters measured with regards to bull temperament over the 84 d testing period.

Temperament and Hormone Concentrations

Serum cortisol concentration tended ($P = 0.06$) to be greater in the temperamental bulls when compared with docile bulls on d 56 of the testing period (Table 7). Conversely, serum testosterone concentration on d 56 was greater ($P < 0.05$) in docile bulls when compared to temperamental bulls. No other differences ($P < 0.05$) were found between the three temperament categories and circulating cortisol and testosterone concentrations on any other day of test.

Table 7. Mean hormone concentrations over all days for overall temperament categories.

		Overall Temperament Category[1]		
Sample Type and Hormone	**Day**	**Docile**	**Intermediate**	**Temperamental**
Serum Cortisol, ng/mL	0	6.30 ± 1.49	9.89 ± 1.68	10.68 ± 2.52
	28	6.18 ± 1.59	6.93 ± 1.26	10.38 ± 2.68
	56	7.57^{d} ± 2.12	10.97^{cd} ± 1.43	14.92^{c} ± 2.12
	84	9.21 ± 1.70	9.18 ± 1.12	11.77 ± 2.18
Serum Testosterone, ng/mL	0	2.12 ± 0.40	1.64 ± 0.30	1.74 ± 0.40
	28	2.38 ± 0.34	2.35 ± 0.26	1.68 ± 0.34
	56	$^{a}2.32$ ± 0.35	$^{ab}1.63$ ± 0.28	$^{b}1.30$ ± 0.36
	84	2.27 ± 0.34	1.99 ± 0.23	2.01 ± 0.37
Hair Cortisol, pg/mg	0	5.60 ± 0.90	5.10 ± 0.68	5.77 ± 0.92
	28	3.51 ± 0.63	3.55 ± 0.59	3.84 ± 0.70
	56	2.99 ± 0.50	2.57 ± 0.38	2.57 ± 0.43
	84	2.83 ± 0.39	2.58 ± 0.25	2.38 ± 0.30
Hair Testosterone, pg/mg	0	12.79 ± 1.95	10.52 ± 1.40	12.86 ± 2.00
	28	9.46 ± 0.94	9.47 ± 0.70	8.99 ± 0.90
	56	$^{a}10.04$ ± 0.82	$^{a}9.92$ ± 0.61	$^{b}7.12$ ± 0.59
	84	8.78 ± 0.93	8.84 ± 0.64	7.95 ± 0.81

^{a,b} Means within a row without common superscripts differ ($P < 0.05$).
^{c,d}Means within a row without common superscripts differ ($P < 0.10$).
[1]Overall temperament was determined by averaging pen score and exit velocity. Overall temperament categories were determined using PROC UNIVARIATE in SAS 9.3 based on the 25^{th} and 75^{th} quantiles.

Hair cortisol concentrations were repeatable over the 84 d testing period (r = 0.31 – 0.55; P < 0.05) and were not different (P > 0.10) between overall temperament category assigned on the day of hair collection (Table 7). No relationship (-0.30 < r < 0.30) was found between serum and hair cortisol concentrations over the testing period.

Hair testosterone concentrations were repeatable over the 84 d testing period (r = 0.38 – 0.60; P < 0.05) and only on d 28 did hair testosterone concentration correlate (r = 0.35; P < 0.01) to serum testosterone concentration. On d 56, hair testosterone concentrations differed between overall temperament categories (Table 7). Bulls categorized as docile and intermediate according to overall temperament score on d 56 had greater (P < 0.05) hair testosterone concentrations as compared to temperamental bulls. Also on d 56, hair testosterone concentration was negatively correlated (r = -0.34; P < 0.01) to overall temperament.

Bulls categorized as docile on d 0 tended (P = 0.08) to have greater hair testosterone concentration than intermediate bulls in samples collected on d 28 (Table 8). Bulls categorized as docile and intermediate on d 28 had greater (P < 0.05) hair testosterone concentrations than temperamental bulls in samples collected on d 56 (Table 8). Also on d 56, hair testosterone was negatively correlated (r = -0.30; P < 0.05) to overall temperament assessed on d 28. Hair testosterone concentrations on d 28 differed between overall temperament categories assigned on d 56 such that docile and intermediate bulls had greater (P < 0.05) hair testosterone concentrations than temperamental bulls.

Table 8. Mean hair cortisol and testosterone concentrations in comparison to overall temperament category assigned 28 d earlier.

			Overall Temperament Category[1]		
Hair Hormone	**Hair Collection Day**	**Temperament Day[2]**	**Docile**	**Intermediate**	**Temperamental**
Cortisol, pg/mg	28	0	3.86 ± 0.73	3.57 ± 0.62	3.48 ± 0.66
	56	28	2.71 ± 0.45	2.69 ± 0.40	2.48 ± 0.41
	84	56	2.72 ± 0.34	2.34 ± 0.21	3.10 ± 0.40
Testosterone, pg/mg	28	0	10.74^{c} ± 1.14	8.36^{d} ± 0.62	10.17^{cd} ± 1.04
	56	28	9.36^{a} ± 0.86	9.74^{a} ± 0.68	$7.7\,0^{b}$ ± 0.69
	84	56	8.98 ± 0.93	8.82 ± 0.64	7.47 ± 0.74

[a,b] Means within a row without common superscripts differ ($P < 0.05$).
[c,d]Means within a row without common superscripts differ ($P < 0.10$).
[1]Overall temperament was determined by averaging pen score and exit velocity. Overall temperament categories were determined using PROC UNIVARIATE in SAS 9.3 based on the 25[th] and 75[th] quantiles.
[2]Day overall temperament categories were assigned.

Temperament and Reproductive Parameters

Scrotal circumference measured on either d -14 or upon completion of the 84 d testing period was not correlated to overall temperament ($-0.30 < r < 0.30$). Only on d 28 was there a difference between overall temperament categories and scrotal circumference. Bulls categorized as docile on d 28 tended ($P = 0.07$) to have a larger scrotal circumference on d -14 than intermediate bulls (28.48 ± 1.39 cm vs. 26.22 ± 1.28 cm), but neither differed from the scrotal circumference of temperamental bulls (27.13 ± 1.42 cm). On d 0 and 84, overall temperament was negatively correlated with secondary spermatic defects ($r = -0.32$ and $r = -0.36$; $P < 0.05$, respectively) measured upon the completion of the 84 d testing period. Bulls categorized as docile on d 0 had a greater ($P < 0.05$) percentage of secondary spermatic defects as compared to intermediate and temperamental bulls (Table 9). Mean percentage of secondary spermatic defects did not differ ($P > 0.10$) between overall temperament categories during the remainder of the testing period. On d 84, bulls categorized as temperamental tended ($P = 0.07$) to have a greater percentage of primary spermatic defects than both docile and intermediate bulls. Primary spermatic defects did not differ ($P > 0.10$) between overall temperament categories assigned on all other days of test (Table 9). Sperm motility did not differ ($P > 0.10$) between overall temperament categories assigned throughout the 84 d testing period.

Serum Cortisol and Testosterone

Serum cortisol concentration measured on d 84 was positively correlated ($r = 0.36$; $P < 0.001$) to primary spermatic defects and tended to negatively correlate ($r =$

Table 9. BSE measurements collected on d 84 in comparison to overall temperament categories assigned throughout the testing period.

		Overall Temperament Category[1]		
BSE Measurement[2]	**Temperament Day[3]**	**Docile**	**Intermediate**	**Temperamental**
Primary Defects[4], %	0	26.96 ± 6.43	29.44 ± 4.97	27.27 ± 6.42
	28	19.96 ± 4.39	27.50 ± 4.61	28.07 ± 6.18
	56	26.67 ± 6.10	25.41 ± 4.26	25.24 ± 5.63
	84	20.58[d] ± 4.86	23.15[d] ± 3.56	38.01[c] ± 8.06
Secondary Defects[5], %	0	7.89[a] ± 1.31	4.73[b] ± 0.93	3.53[b] ± 1.31
	28	4.78 ± 1.14	3.94 ± 0.68	3.66 ± 0.92
	56	4.87 ± 1.21	4.22 ± 0.82	3.38 ± 0.84
	84	5.34 ± 1.49	4.05 ± 0.64	3.63 ± 1.01

[a,b] Means within a row without common superscripts differ ($P < 0.05$).
[c,d] Means within a row without common superscripts differ ($P < 0.10$).
[1] Overall temperament was determined by averaging pen score and exit velocity. Overall temperament categories were determined using PROC UNIVARIATE in SAS 9.3 based on the 25th and 75th quantiles.
[2] Breeding Soundness Exams (BSE) were performed on all bulls on d 84.
[3] Day overall temperament categories were assigned.
[4] Sperm developmental defects were classified as primary spermatic defects.
[5] Secondary spermatic defects were defects caused by storage in the testes.

-0.33; $P = 0.07$) to sperm motility, and primary spermatic defects and sperm motility were negatively correlated ($r = -0.32$; $P < 0.05$). Data collected during BSE showed no relationship ($-0.30 < r < 0.30$) with serum cortisol concentration on all other days of test. Only on d 0 did serum testosterone concentration relate to BSE data collected on d 84. Serum testosterone concentration was positively correlated to the percentage of normal sperm ($r = 0.34$; $P < 0.05$) and secondary spermatic defects ($r = 0.30$; $P < 0.05$), but was negatively correlated to primary spermatic defects ($r = -0.45$; $P < 0.01$).

Serum cortisol concentrations throughout the 84 d testing period showed no relationship ($-0.30 < r < 0.30$) with growth performance or ultrasound data. Serum testosterone concentrations measured on d 0 and 84 showed no relationship with growth performance. However, on d 28, serum testosterone concentration was positively correlated with BW ($r = 0.47$; $P < 0.001$) and adjusted 365 d weight ($r = 0.38$; $P < 0.01$). Serum testosterone on d 56 was positively correlated with both BW ($r = 0.39$; $P = 0.002$) and adjusted 365 d weight ($r = 0.36$; $P < 0.01$), but was negatively correlated ($r = -0.36$; $P < 0.01$) to IMF measured on d 84. Similarly, serum testosterone was negatively correlated ($r = -0.32$; $P < 0.05$) to IMF on d 84.

On d 0, serum cortisol concentration was positively correlated ($r = 0.36$; $P < 0.01$) to bull order through the chute. However, on d 56, serum cortisol was found to be negatively correlated ($r = -0.35$; $P < 0.01$) to bull order through the chute. On d 84, there was a tendency for serum cortisol to be negatively correlated ($r = -0.35$; $P = 0.08$) to lying time during the later phase of period 3. No relationship was found between behavioral measures and serum testosterone concentration throughout the 84 d testing period.

Hair Cortisol and Testosterone

Hair cortisol and testosterone concentrations were positively correlated on d 0 (r = 0.68; P < 0.001) and 56 (r = 0.34; P < 0.01), but showed no relationship (-0.30 < r < 0.30) on d 28 and 84. Hair cortisol and testosterone concentrations obtained from samples collected throughout the 84 d testing period showed no relationship with data collected from the BSE on d 84.

Hair cortisol concentration and BW measured on d 28 were shown to be correlated (r = -0.31; P < 0.05). All other performance data collected on d 0, 28, 56, and 84 showed no relationship (-0.30 < r < 0.30) with hair cortisol concentration sampled on the same day. Body weight was positively correlated with hair testosterone concentrations on d 28 (r = 0.32; P < 0.05) and 56 (r = 0.32; P < 0.01), all other growth performance data measured on these two days showed no relationship with hair testosterone (-0.30 < r < 0.30). On d 84, hair testosterone concentration was negatively correlated with frame score (r = -0.30; P < 0.05).

Hair cortisol concentration on d 28 had a negative correlation (r = -0.50; P < 0.01) with lying time during the late phase of period 1. All other behavior data (i.e. lying time, total steps taken, lying bout duration, total number of lying bouts, and order through the chute) collected during the 84 d testing period were not related (-0.30 < r < 0.30) to hair cortisol concentration.

Hair testosterone concentration on d 0 and 28 showed no relationship (-0.30 < r < 0.30) with bull behavior during the early and late phase of period 1. However, there tended to be a positive correlation between testosterone concentration in hair collected on d 56 (r = 0.35; P = 0.06) and 84 (r = 0.36; P = 0.09) and the total number of lying bouts during the later phases of periods 2 and 3, respectively. Also on d 56, hair testosterone concentration showed a positive correlation ($r = 0.43$; $P < 0.05$) with lying time.

Neither hair cortisol nor hair testosterone showed any relationship ($-0.30 < r < 0.30$) with ultrasound data collected on d 84.

Scrotal Circumference and Body Weight

On d -14 and 84, BW showed a positive correlation ($r = 0.66$ and $r = 0.45$, respectively; $P < 0.001$) with scrotal circumference.

Discussion

Bulls categorized as temperamental tended to have greater serum cortisol concentrations as compared to docile bulls when measured on d 56. Thibier and Rolland (1976) and Welsh et al. (1979) reported that elevated cortisol concentrations will cause a reduction in circulating testosterone. This was true in the present study only on d 56 as demonstrated by higher concentrations of serum testosterone in docile bulls when compared to temperamental bulls. On all other days sampled, neither serum cortisol or testosterone concentration differed between overall temperament categories.

Hair cortisol concentrations did not vary between overall temperament categories on any day of test. It has been reported that temperamental cattle have higher concentrations of circulating cortisol when compared to docile cattle (Stahringer et al., 1990; Curley et al., 2006). However, because serum is an acute measure of cortisol concentration within the body, the question remains if elevated circulating cortisol concentration found in temperamental cattle were only an acute response to handling. Circulating cortisol concentration in cattle begins to increase after 2 min of restraint (Hopster et al., 1999) and thus, may be a reflection of the agitation towards humans and restraint of temperamental bulls in previous studies. Perhaps after release from confinement and removal from the presence of humans, circulating cortisol in temperamental bulls decreased as shown by equal hair cortisol concentration between overall temperament categories.

Hair testosterone concentration on d 56 was greater in bulls categorized as docile and intermediate when compared with temperamental bulls. When examining relationships between temperament and testosterone concentrations in hair samples collected one period following temperament assessment, hair testosterone concentrations varied between temperament categories assigned on d 28 and 56. Testosterone concentration in hair collected on d 56 was lower in bulls categorized as temperamental on d 28 when compared with docile bulls. These results contradict those previously reported in humans and Asian elephants where higher concentrations of circulating testosterone coincide with aggressive behavior (Olweus et al., 1988; Lincoln and Ratnasooriya, 1996; Chichinadze et al., 2010). Previous research has not reported on relationships between bull temperament and hair and serum testosterone concentrations. Knowing that an elevation in circulating cortisol will inhibit LH release and suppress testosterone production (Welsh et al., 1979), we suspect that a greater concentration of testosterone was available for incorporation into the hair shaft of docile bulls than temperamental bulls on d 56 due to lower circulating cortisol concentrations in docile bulls. As mentioned in the previous chapter, docile bulls were heavier than temperamental bulls on d 56. We also found that heavier bulls had larger scrotal circumferences than lighter weight bulls. The difference in BW could be indicative that docile bulls have begun to reach puberty as shown by a greater concentration of serum and hair testosterone. We also speculate that the initiation, or stage of puberty, may have attributed to differences in testosterone concentrations between overall temperament categories because testosterone concentrations rise until one year of age (Lunstra et al., 1978).

Serum and hair cortisol concentrations were not correlated at any time over the 84 d testing period, which may be due to frequent fluctuations of serum cortisol as part of its natural circadian pattern and in response to acute stressors (Thun et al., 1981; Möstl and Palme, 2002). Yang et al. (1998) reported that serum testosterone was related to hair testosterone in women when sampled nine times over the course of a month. Only on d 28 did we find that serum testosterone positively correlated to testosterone concentrations in hair collected on the same day. Differences in our results and those reported by Yang et al. (1998) may be attributed to species and gender difference and the time of serum collection. Blood samples in the present study were obtained from bulls between 0700 and 1200 h and may have been collected during different stages of episodic secretion (Kiser et al., 1978). Circulating hormone concentrations can fluctuate rapidly and may not be reflected in hair samples since hormone levels measured in hair represent an overall accumulation over time (Koren et al., 2002).

Scrotal circumference measured on d -14 was greater in bulls categorized as docile versus intermediate only on d 28. Similarly, Burrow (2001) reported that selecting bulls based on exit velocity would result in the selection of bulls with larger scrotums. Selecting bulls based on temperament may also be an indicator of sperm quality as shown by a lower percentage of primary spermatic defects as compared to temperamental bulls measured on d 84 in our study. Presence of primary spermatic defects hinder fertility and are a result of physiological changes and/or genetics that may be passed to offspring (Barth and Oko, 1989; Chandler and Adkinson, 1990; Chenoweth, 2005). In regards to secondary spermatic defects, we found that bulls categorized on d 0 as docile had a greater percentage of secondary defects than temperamental bulls. However, the relationship between d 0 overall temperament category and percentage of secondary spermatic defects remains questionable since BSE was performed on d 84.   We also found that the percentage secondary spermatic defects and normal sperm examined on d 84 increased with serum testosterone concentration collected on 0. We speculate that secondary spermatic defects and normal sperm percentages increased with serum testosterone concentration because bulls were further along in reaching puberty at the start of the testing period and could have produced more sperm than bulls with lower serum testosterone concentration. Because electroejaculation was performed to assess sperm characteristics, total sperm concentration was not assessed as part of the BSE in our study.

Serum cortisol concentration measured in bulls on d 84 was positively correlated with primary defects. Similarly, a study performed by Barth and Bowman (1994) found that the administration of dexamethasone, a synthetic corticosteriod, will increase the occurrence of primary spermatic defects in bulls. These researchers noted an increase in primary defects (i.e. knobbed acrosomes, mitochondrial sheath distributions, midpiece defects, and proximal droplets) by d 8 post dexamethasone injection. Barth and Bowman (1994) concluded that because dexamethasone will inhibit LH secretion from the anterior pituitary, insufficient concentrations of testosterone hindered proper sperm maturation. In our study serum cortisol concentration on d 84 was negatively correlated to sperm motility. Since primary spermatic defects increased with serum cortisol concentration, we would expect sperm motility to decrease due to developmental abnormalities that might affect normal motility.

Serum cortisol concentrations over the 84 d testing period were not related to growth performance or body confirmation measurements examined via ultrasonography on d 84. Similarly, hair cortisol concentration was not related to data collected during the BSE on d 84. In regards to growth performance, our results contradict those reported by Theis et al. (2002) who found that elevations of circulating cortisol concentration in steers coincided with a reduction in ADG. However, we did find that chronic concentrations of cortisol reflected in hair samples were negatively correlated to BW on d 28. During a period of elevated circulating cortisol concentrations, circulating leptin concentrations are also elevated and cause appetite suppression in an rats and mice (Matteri et al., 2000). Therefore, we suspect that the negative correlation between hair cortisol concentration and BW may have been attributed to appetite suppression caused by elevated cortisol concentrations.

On d 28, we found that both serum and hair testosterone concentration were positively correlated to BW. Serum testosterone concentration on d 28 and 56 was positively related to adjusted 365 d BW. On d 56 and 84, serum testosterone concentration was negatively related to IMF. Similarly, serum testosterone concentration was positively correlated to BW on d 56 and on d 84 and negatively correlated to IMF and frame score. These data suggest that bulls with larger BW were closer to puberty as compared to lighter weight bulls as demonstrated by a greater concentration of serum testosterone. However, this data also suggest that a tradeoff for superior growth performance may be a hindrance to carcass quality as shown by a reduction in IMF. The same findings are observed when comparing bulls to steers. In a review by Field (1971), the author noted that bulls converted feed to live weight more efficiently than steers and had lower marbling scores.

In the present study, bulls with higher serum cortisol concentration on d 0 entered the chute later than bulls with lower serum cortisol concentration. However, on d 56, bulls with higher serum cortisol concentration entered the chute system before bulls with lower serum cortisol. Similarly, we found that the temperamental bulls on d 84 entered the chute before the docile bulls. Grandin (1993) reported that behavioral agitation in bulls and steers is persistent when handled every 30 d for 5 mo and that temperamental bulls tried to escape while in the chute system. Thus, we suspect that changes in the order of bulls with higher cortisol concentrations were due to their familiarity of the chute system and recognition of the escape route.

Hair and serum cortisol concentrations were inversely related to total lying time on d 28 and 84, respectively. Lying time has been shown to be an indicator of cattle comfort (Fisher et al., 2003); therefore, we expect hair cortisol concentrations to be lower in bulls that spend more time lying. During both the early and late phases of period 3, total number of lying bouts increased with testosterone concentrations in hair collected on d 56 and 84. Also during the early phase of period 3, lying time increased along with hair testosterone concentration measured on d 56. These data suggest that bulls with greater hair testosterone concentrations spend more time lying than bulls with lower concentrations. During this time (d 56), hair cortisol and testosterone concentrations were negatively correlated and indicate that a reduction in lying time may hinder testosterone production in bulls as a result of elevated cortisol production. However, further studies need to be conducted to determine if investigating bull lying behavior can provide insight to bull testosterone production.

In conclusion, no direct relationship was found between hair cortisol concentration and bull temperament. Based on the data presented in this study, bulls that were categorized as docile on d 28 and 56 were closer to puberty than temperamental bulls as shown by a greater concentration of testosterone in hair and serum. Further studies should be conducted to confirm if selecting bulls based on temperament will hinder any other aspects of production and indeed enhance reproductive performance.

Chapter 5. General Conclusions

Previous research has not examined relationships between temperament and data collected in a standard post-weaned bull test, but has found that cattle with mild temperaments are faster growers and produce more desirable carcasses at the time of rendering (Fordyce et al., 1988b; Voisinet et al., 1997a; Voisinet et al., 1997b; Schmidt et al., 2013). Recognizing that temperament is moderately heritable (Shrode and Hammack, 1971b; Stricklin et al., 1980) and the majority of Tennessee beef producers still rely on a herd bull to service their cows and heifers, we need to fully understand the benefits and consequences associated with selecting cattle according to temperament.

Based on the data presented in Chapter 3 and 4, and recognizing that production traits are moderately to highly heritable (Gosey, 2004), selecting docile bulls to use as herd sires may increase progeny BW, frame score, and adjusted 365 d BW. Likewise, BW and frame score may be useful indicators of temperament when selecting a bull to use in a breeding program. Further studies examining a larger variation in bull temperament is needed to conclude if the above relationships of temperament with growth performance are precise indicators of temperament that may be used to assess bulls of similar age.

Since previous research has not addressed the relationship between temperament and the order to which bulls enter a chute system, the question remains if bull order could be used as an indicator of temperament. Additional studies to address this question would include assessing overall temperament (pen score + exit velocity / 2) of several groups of bulls with varying ages that are introduced to a novel environment (i.e. sale barn, research facility, etc.) and evaluating the order to which they enter a chute system. With this information, producers may be able to identify temperament within their own stock or make informed decisions about future purchases.

Lying behavior and the observed difference in ADG between intermediate and temperamental bulls needs further investigation. In regards to overall temperament, we found that intermediate bulls had higher ADG and a tendency for shorter lying bout durations, but higher lying bout frequencies than temperamental cattle. Whether or not pen behavior (i.e. lying bout duration and total number of lying bouts) was influenced by feeding behavior and thus, altered ADG between the overall temperament categories, remains to be answered. Recently, GrowSafe automated feeding systems (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) have been used to examine feeding behavior, ADG, and feed efficiency in cattle. GrowSafe feeding systems are capable of recording feeding behavior and feed and water consumption per each feed bunk visit. The University of Tennessee Plateau Research and Education Center (PREC) recently implemented the GrowSafe technology. A future study should involve the incorporation of IceTag datalogger and GrowSafe technology to examine bulls with varying temperaments to determine if the pen behavior observed in the present study was influenced by feeding behavior and was reflected in the variation of ADG between intermediate and temperamental bulls. An additional study could also examine the relationships of pen behavior, feeding behavior, growth performance, and temperament in heifer development at PREC.

Even though the results from the present study suggest that selecting bulls according to temperament may be indicative of higher testosterone production, lower cortisol production, and a decrease in primary spermatic defects, the question remains if these results are repeatable in all groups of bulls of similar ages. A valuable follow-up study would include selecting two groups of post-pubertal bulls according to overall temperament (docile and temperamental) and evaluating the same reproductive and endocrine parameters as in the present study. Lunstra et al. (1978) reported that testosterone production increases until a year of age in bulls. Therefore the effects of selecting bulls according to temperament may be clearer when examined in sexually mature bulls.

Overall, the main weakness of this study is the lack of variation in bull temperament. Specifically, the lack of temperament variation at the conclusion of the study where temperamental bulls based on our ranking system might be considered docile to another researcher or within a larger group of bulls. The take home message from this study and practicality for producers is that culling bulls of this age based on initial temperament scores may not be indicative of habituated temperament. Providing a 28 d habituation period before reassessing temperament may provide better insight to an individual bull’s demeanor.

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Appendices

Appendix A. Preparation of hair for cortisol and testosterone analysis

A. Procedure

1. Wash 100 mg of hair 4 times with 1.5 mL of isopropanol in a 50 mL disposable polypropylene tube (Davenport et al., 2006; Bryan et al., 2013a; Bryan et al., 2013b).

2. Let hair dry completely in a fume hood for approximately 4 days (Paulsen et al., 2001).

3. Grind 50 mg of dried hair to a powder (Bryan et al., 2013b) using an Omni Bead Ruptor 24 Bead Mill Homogenizer with four 2.4 mm metal beads (Omni International, Inc., Kennesaw, GA) at 6.95 m/s for two 50 second periods with a 15 second rest between cycles.

4. Add 30 mg of powdered hair to a glass tube with 3 mL of HPLC grade methanol (Bryan et al., 2013b).

5. Extract for 24 hours at room temperature with slow rotation (Ghassemi Nejad et al., 2013).

6. Centrifuge tubes for 30 minutes at 4000 rpm (Bryan et al., 2013b) at room temperature.

7. In two separate borosilicate tubes, aliquot the supernatant (100 uL for testosterone and 2000 uL for cortisol) and evaporate under a stream of air (Bryan et al., 2013b).

8. Reconstitute samples in 6.5 uL of methanol followed by 123.5 uL of assay diluent (Bryan et al., 2013b) and measure using the Salimetrics Salivary Testosterone and Cortisol ELISA (Salimetrics, Philadelphia, Pennsylvania, USA) kit procedures (Bryan et al., 2013a; Bryan et al., 2013b; Ghassemi Nejad et al., 2013).

Appendix B. Glassware cleaning procedure

A. Procedure

1. Wash glassware 5 times with detergent and tap water.

2. Soak glassware overnight in an acid bath (5% HCl) to remove detergent and remaining debris.

3. Rinse 5 times with deionized water and allow to air dry.

Vita

Sierra A. Lockwood was born in Syracuse, New York in July of 1990. Sierra grew up on her family's farm in Canandaigua, New York and graduated from Canandaigua Academy in 2008. She graduated with a Bachelor's of Science in Animal Science from the University of Tennessee, Knoxville in May 2012 and started her Masters in Animal Science at the University of Tennessee, Knoxville under the guidance of Dr. Henry Kattesh, in August of 2012.