EVALUATION OF THE INTER-RELATIONSHIPS OF TEMPERAMENT, STRESS RESPONSIVENESS AND IMMUNE FUNCTION IN BEEF CALVES

A Thesis

by

RYAN ALLAN OLIPHINT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

May 2006

Major Subject: Physiology of Reproduction

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ABSTRACT

Evaluation of the Inter-relationships of Temperament, Stress Responsiveness and Immune Function in Beef Calves. (May 2006)

Ryan Allan Oliphint, B.S., Texas Tech University

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A series of *in vivo* and *in vitro* approaches were followed to assess the inter-relationships of temperament, stress responsiveness and immune function in beef bulls and steers. In experiment one, Brahman bull calves were weaned at approximately six months of age when pen score and exit velocity were measured to sort calves into groups with extremes in temperament (calm n = 10 and temperamental n = 10). The calves were vaccinated on day 0 and 42 of the study with serial blood samples colleted for 11 weeks. Calm calves tended to have higher primary and secondary immune responses as indicated by increased serum concentrations of immunoglobulin G following Clostridial vaccination.

In vitro lymphocyte cultures were performed on day 0 and 42 to measure proliferation and IgM production. Calm calves had significantly higher proliferative responses on both day 0 and 42. Lymphocyte IgM production was significantly higher in calm calves on day 0 and tended to be higher on day 42 than temperamental calves.

In experiment two, weaned and yearling steers were arrayed by pen score and exit velocity, to assign steers to groups with extremes in temperament (trial 1: calm n = 7

and temperamental n = 5; trial 2: calm n = 5 and temperamental n = 5). In both trials, temperamental steers had higher proliferative responses than calm steers. Immunoglobulin M production did not differ in either trial.

The effects of stress responsiveness on animal performance and health are considerable because they affect the profitability of the cattle industry. Investigations into animal temperament can help cattle producers identify animals that may be more susceptible to decreased performance and immunosuppression. The effectiveness of vaccines given to calves is important in conferring immunity to common diseases at times when they are at a higher risk for infection. If we can identify temperamental animals that will not perform as well as their cohorts, management procedures can be altered to reduce the risks associated with decreased performance and morbidity.

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INTRODUCTION

Cattlemen have been aware of the connections between animal behavior and animal performance for decades. It has been common knowledge that an animal's behavior can indirectly affect several physiologic systems of economic significance. However, the intricacies of these relationships have been investigated only minimally. With more importance being placed on carcass quality and consumer demand, additional aspects of these relationships are being researched to determine specific markers of animal temperament that can be managed in order to deliver a more uniform and profitable product.

Animal temperament, in normal production situations, has been described as the degree of fearfulness and reactivity to humans, as well as reactivity to strange, novel or threatening environments (Grandin, 1993; Murphy et al., 1994; Burrow, 1997). Temperament is a measure of many behavioral characteristics, exhibited by animals, which can be quantified by observation of a behavior departing from a normal, non-threatening environment. Stressors have been described as environmental factors or conditions that can induce a physiological response (stress) in an animal. Stressors, such as standard management processes for handling cattle, can induce these responses making it important to understand their effects on cattle. Animals with extremes in temperament usually react differently to stressors. Specifically, calmer animals will have less of a stress response, whereas wilder animals predominately have a greater stress

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This thesis follows the style and form of the Journal of Animal Science.

response due to their excitability. These responses can be used as a measure of temperament and the level of fear or excitement experienced by the animal.

The concentration of cortisol, a steroid produced by the adrenal gland, has long been a measure of stress responsiveness in people and in animals. Negative or undesirable temperaments have been associated with increased serum cortisol concentrations in cattle (Stahringer et al., 1990; Curley, 2006). Release of cortisol from the adrenal cortex is associated with increased stress stimuli and has been shown to have a significant negative impact on animal performance (Voisinet et al., 1997a), immunity (Fell et al., 1999), meat quality (Lacourt and Tarrant, 1985), and beef tenderness (King et al., 2006). For example, Brown-Borg et al. (1993) demonstrated that pigs with an elevated stress response to restraint had a 60% lower mitogen-induced lymphocyte proliferative response. Animals with impaired humoral mediated responses may have difficulty in providing a sufficient immune response when challenged with pathogens in production or feedlot situations. Greater rates of morbidity in cattle decrease: a) average daily gain (ADG), and b) carcass quality grade [which increases total cost of gain due to added labor, antibiotic costs, and smaller carcasses (Gardner et al., 1998, Smith, 1998, Baker, 2002)]. The proposed experiments, with recently weaned beef steers and bulls, will assess to what degree, if any, that animal temperament, stress responsiveness, and immune function are inter-related. The resultant data will provide insight into different ways cattle respond to stress and lead to improvement in animal welfare and health which consequently should increase efficiency of producing higher quality beef.

LITERATURE REVIEW

Temperament in Beef Cattle

Animal Temperament. Changes in the behavior of animals have been noted by humans since the domestication of wild animals several thousand years ago. The domestication process has led to a decreased appreciation of environmental influences on animals and an increased psychogenic tolerance of stressful stimuli (Hemmer, 1990). Put more simply, domestic animals evolved to tolerate human interaction and thus, their behavior patterns shifted. With these changes, there still remains a marked range of behaviors displayed by beef cattle, giving us the opportunity to make breeding decisions based on this potential selection differential.

Scott and Fredericson (1951) characterized mouse behavior toward humans as savage, wild or tame. Tame behavior was referred to as the absence of conflict behavior, wild as the tendency to escape and savage as an aggressive or attacking behavior. These behavior differences have commonalities with different species, including cattle, making it possible to select animals with more desirable temperaments.

Temperament has been used to describe many characteristics of cattle such as nervousness, skittishness, quietness, excitability, individuality, libido, constitution or emotionality of animals (Stricklin and Kautzscanavy, 1984). Although there has been confusion among researchers on a common definition of temperament, in beef production scenarios it refers to those characteristics encompassed as an animal's reaction to standard animal handling practices. More specifically, an animal's behavior is the product of its experience and environment. Novel or threatening situations could

cause the animal to display behaviors of self defense, fear, curiosity, etc. A more pronounced or exacerbated response is interpreted as an increased excitability or fear response. These behaviors would be indicative of a poor or undesirable temperament. It is important to understand the roles that animal temperament plays in beef production scenarios to better comprehend its effects on traits of importance to cattle producers.

Temperament Assessment. In order to understand differences in cattle temperament, a system was needed to correlate a scale of measure to different temperaments. The first attempt to assess temperament was conducted by Tulloh (1961) who created a numerical scoring system to summarize an animal's behavior. Specifically, observations were taken as the animal entered the chute, entered the head gate (bail), and while the animal was restrained in the head gate. The temperament scores taken while the animal was in the head gate were based on a scale of 1 to 6, with 1 representing an animal that showed little movement and a calm disposition, to 6 representing an animal that was aggressive and moved violently when restrained. In the same study, Tulloh (1961) also noted that contemporaries with lower chute scores had significantly higher body weights which led him to the notion that docile animals grew better than nervous or aggressive animals. Subsequent investigations refined Tulloh's system, and devised new tests to assess temperament in different production situations.

An approachability test (Murphey et al., 1980) was created to measure the proximity (in meters) that an observer could approach before the animal would move or react. Variations of this method using a scoring system (pen score) to measure an animal's temperament when approached were developed to aid in the ease of

measurement. Many tests have been created and they have been termed differently over the past few decades, but many measure the same characteristics of cattle behavior. Examples include the approach/avoidance behavior test (Murphey et al., 1981), chute (crush) score (Vanderwert et al., 1985) and docility score (Le Neindre et al., 1995). These tests, while beneficial, are subjective, time consuming and often difficult to measure in normal production scenarios. In an effort to devise an easier system to measure temperament, it was noted by Burrow (1988) that cattle which remained calm while in the chute, exited at a different rate than some of their more aggressive contemporaries. This observation led to the employment of an electronic timing system to measure an animal's velocity over a given distance (1.7 m). These exit velocities (EV) were found to be significantly correlated to flight distance scores, designating the fastest animals as those that were the least approachable in a pen or pasture. The resultant data showed that exit velocity was a useful method to measure temperament in cattle.

Heritability of Temperament. Many tests on the heritability of temperament have been conducted and reviewed by Burrow (1997). It was noted that although each of the various tests actually measure different behaviors, the effects of breed, sex, age and experience have not all been taken into account. However, the average estimates for heritability of temperament were 0.36 and 0.23 for unrestrained and restrained tests respectively. These estimates are lowly to moderately heritable, indicating that selection pressure can be applied to improve the trait.

Temperament and Beef Production. There is a perception in the cattle industry that animals with poor temperament affect the profitability of the beef enterprises by

increasing production costs and decreasing performance (Burrow, 1997). Not only are cattle with poor or undesirable temperaments difficult and potentially dangerous to work with, the relationship with other traits makes it important to assess temperament to try to improve production efficiency. A study by Grandin (1993) showed that animals which become behaviorally agitated have consistent temperaments over time. Because of some variability in behaviors, temperament assessments should be made over multiple observations to provide a clearer picture. If there is consistency in temperament, then it is possible to relate differences in behavior to other production traits that are of economic importance to cattle producers.

In feedlot situations, contact with humans occurs more frequently than it does for cattle in pasture conditions. Any differences in production traits, due to temperament, might be more pronounced as a result to an animal's increased exposure with human handling. Voisinet et al. (1997a) reported that animals with the calmest temperaments had a significantly higher ADG than steers with the highest temperament scores. Across all breed types studied, there was a 0.15 kg/d difference in ADG. They concluded that the differences in ADG were a product of calm animals gaining more weight, not excitable animals gaining less. These findings were supported by Burrow and Dillon (1997) who reported that cattle with lower exit velocities had higher ADG and carcass weights. However, no differences were found in dressing percentage or bruising score.

In order to identify animals more susceptible to yielding carcasses with poor meat quality (i.e., dark cutting and tough), an additional study was conducted by Voisinet et al. (1997b). They found that carcasses from animals with poor temperament

had a significantly higher proportion of borderline dark cutters, which in turn led to quality grade discounts. Warner-Bratzler shear force measurements also showed a significant difference in meat tenderness, with excitable animals yielding tougher meat. These deficiencies in performance and end-product quality are cost prohibitive and warrant further study into their causes.

Stress in Beef Cattle

Stress. There are many misconceptions about the nature and scope of stress. It was once thought, and perhaps still is today, that stress is mental strain, tension or anxiety, but research has taught us that stress is actually the body's response to stimuli. It can be characterized as a series of events which begins with a stimulus (stressor) and causes a reaction of certain physiological events in the body (stress response) (Dhabhar and McEwen, 1997). The stressor can be either physical or psychological, and can lead to physiological changes in the body. There have been many attempts to define stress, but researchers today have yet to agree on one. Perhaps this is why Selye (1956) found it easier to define what stress is not, rather than limit it to an absolute. In defining an animal's response to a stressful stimulus, Selye characterized the body's response to the damage independent of the stressor itself. He termed the condition the "general adaptation syndrome" or GAS. It is in this state, or the body's reaction to the stressor, that stress is manifested. The GAS consists of three stages: 1) the general alarm reaction by the organism, 2) the stage of resistance or adaptation, 3) the stage of exhaustion or loss of resistance. It was in this adaptation that Selye (1950) proposed that there was a general, nonspecific response by the body, independent of the nature of the stressor. He

found that this nonspecific response involved the autonomic nervous, neuroendocrine and the endocrine systems. This concept was later challenged by Mason (1968) and further explained by Pacak (1998) stating that neuroendocrine responses were specific to the type and magnitude of the stressor and that the measurement of a single biological response to that stressor fails to provide a complete physiological picture (Moberg, 1987).

Stress Physiology. In experiments with mice, Selye (1936) observed intense shrinkage of the thymus and lymph nodes, formation of gastric ulcers and an enlargement of the adrenal glands in alarmed mice. The enlargement of the adrenal glands was accompanied by the loss of cortical lipoids and chromaffin substance. Subsequent experiments involving adrenalectomy failed to produce similar results; however, when the same mice were treated with adrenal extracts, similar results to the alarm reaction were found (Selye 1956). This led him to believe that adrenal secretions were a necessary component of the GAS and therefore important in stress response. Kendall (1949) found that the steroid nature of the compounds isolated from the adrenal cortex, later termed corticosteroids, had a marked effect on mineral and water balance, but no effect on lipid or protein metabolism. However, he did know of other hormones that did have this effect and also "increased resistance to stress, toxic compounds and infections".

Stress induced activation of the hypothalamic-pituitary adrenal (HPA) axis induces corticotrophin-releasing hormone (CRH) to be released from the hypothalamus which then releases adrenal corticotropin hormone (ACTH) from the adenohypophysis

(Vale et al., 1981). The primary targets for circulating concentrations of ACTH are the adrenal glands. The adrenal glands are bilaterally positioned at the superior poles of the kidneys. They are highly vascularized and contain morphologically distinct regions. It is divided into an inner, catecholamine producing region containing chromaffin tissue termed the medulla (Pohorecky and Wurtman, 1971), and an outer steroid producing region termed the cortex. The cortex is further divided into three distinct regions; the zona glomerulosa, fasciculata and reticularis. Cortisol (CS), or hydrocortisone, is the primary glucocorticoid involved in the stress response in humans and cattle, and is produced primarily in the zona fasciculata (Stachenko and Giroud, 1959). Cortisol's presence at normal, basal concentrations, allows for normal expression of certain responses to stress. This was in contrast to Selye's theory that glucocorticoid (GC) secretion was needed for the body to build a resistance to stress (Ingle, 1952).

Implications of Stress. While the entire picture of cortisol's effect during stress is unknown, it has important roles in carbohydrate, lipid and protein metabolism (Sapolsky, 2000). Mobilization of amino acids and fats from cellular stores for rapid energy availability are a result of increased cortisol concentrations. The mobilization of amino acids from cellular stores shows a shift in the partitioning of nutrients away from growth to cellular maintenance and energy usage (i.e., gluconeogenesis in the liver) in the presence of a stressor (Black et al., 1982). Glucocorticoids have been shown to increase protein degradation as evidenced by amino acid efflux from the muscle in a catabolic state (Wilmore and Shabert, 1998). While these effects are initiated by other hormones [catecholamines, growth hormone (GH), glucagon, etc.], cortisol helps to

mediate the metabolic effects of the stress response (Sapolsky, 2000). However, prolonged activation of the stress axis and consequent increased systemic cortisol concentrations have been shown to have a negative effect on average daily gain and protein/muscle accretion in cattle (Fell et al., 1999). These effects are mediated by loss of appetite and subsequent decreased feed intake following prolonged stress. Rather than enhancing the stress response, cortisol limits its magnitude (through its suppressive negative feedback actions) and contributes to recovery from it. But often times this occurs to the detriment of economically important traits such as performance and end-carcass quality.

Immune Function in Beef Cattle

Immune System Overview. A complete overview of the immune system is beyond the focus of this discussion; however, a brief synopsis is provided with an emphasis on the role of glucocorticoids with respect to the immune system. The role of the immune system is to recognize organisms and molecules foreign to the body and provide a response to remove, destroy or neutralize them. The body has two different systems to accomplish this: the natural or innate system, and the acquired or adaptive system (Janeway and Travers, 1994). Both of these are necessary and they work in concert to defend the host against invading pathogens.

The innate immune system is comprised of physical barriers to infection, normal microbial flora, soluble factors (maternal immunoglobulin G passed through placenta and complement), inflammatory responses and a host of different phagocytic cells. They have low specificity, diversity, and do not invoke a memory response. The molecules

responsible for the coordination and trafficking of immune cells and molecules throughout the body are a series of small proteins called cytokines. These proteins invoke a variety of responses from cells involved in immunity and inflammation (Akira et al., 1990). Conversely, acquired immunity is highly specific, diverse and has memory cells that provide a basis for the efficacy of vaccinations. The cells of this system are of a lymphoid lineage and are composed of B and T lymphocytes (Janeway and Travers, 1994). During an active infection, circulating B cells are activated by helper T cells and antigen presenting cells (APC) during the acute phase response. These APCs stimulate $CD4^+$ T cells to differentiate into Th1 and Th2 phenotypes depending on the antigen encountered (Yang et al., 2005). These helper T cells direct cytokine expression leading to a cascade of events to invoke many immunological responses to include: lymphocyte proliferation, macrophage activation, MHC expression, and immunoglobulin (Ig) class switching. The acute phase response is characterized by the production of pro-inflammatory cytokines: interleukin (IL)-1, IL-6 and tumor necrosis factor (TNF)-α (Klasing 1988; Wherling et al., 1996). Tissue injury, inflammation, stress and acquired immune responses can activate the acute phase response which is viewed as a protective measure taken by the body to overcome infection.

Immunity and Glucocorticoids. The many roles that stress hormones play in the immune system can be confusing, considering the fact that they can have both stimulatory and suppressive actions (Sapolsky, 2000). In the HPA cascade, Jain et al. (1991) and Pawlikowski (1988) demonstrated that CRH-induced cortisol release can decrease T cell proliferation and natural killer (NK) cell cytotoxicity. However, CRH

can also be an immune stimulant by directly enhancing B cell proliferation by increasing interleukin IL-2 receptor numbers (Singh, 1989). But, the overwhelming effects of GCs and GC-stimulating hormones on the immune system are their ability to inhibit synthesis, release, and/or efficacy of cytokines and other mediators that promote immune and inflammatory reactions (Brown et al., 1982). While the acute effects of cortisol may be beneficial in mediating the stress/inflammatory response, chronic, high doses can have adverse effects on many physiological systems.

It was first suggested by Munck et al. (1984) that the role of adrenal steroids was to prevent the immune system from entering an autoimmune state caused by injury or infection. Immune system activation and regulation is controlled in part by cytokines. At the onset of infection or inflammation, pro-inflammatory cytokines act to control activation and differentiation of lymphocytes, lymphokine production as well as other non-immunological functions (Maizel et al., 1981). It was known that these effects were mediated by glucocorticoids, but the exact effects of cytokines on the pituitary-adrenal axis were not well understood.

Besedovsky et al. (1986) found that the pro-inflammatory cytokine IL-1 worked in an immunoregulatory feedback circuit with the HPA axis. Other cytokines were found to have similar roles to IL-1 in stimulating ACTH expression in the anterior pituitary gland and subsequent increases in systemic cortisol concentrations. Lymphoid cells have receptors for GCs (Cake and Litwack, 1975) as well as many other hormones; however, they are expressed in different amounts, depending on cell type and state of activation (Landmann et al., 1989). This preferential expression shows that GCs can be targeted at

specific immune cells and at different stages of their activation. This becomes important in limiting immune responses during infection, but stress-induced increases in systemic cortisol can potentially limit the body's effectiveness in fighting pathogens. The down-regulatory effects of cortisol on lymphocyte proliferation and antibody response to challenge in rats were first noted by Joasoo and McKenzie (1976) and Solomon (1969). In addition, there was also a marked decrease in natural killer (NK) cell activity and decreased cytokine (IL-2) and cytokine receptor expression. IL-2 is an important cytokine in inducing proliferation of resting B cells and *in vitro* antibody secretion. To date, GCs have been shown to inhibit the cytokines IL-1α, 1β, 2, 3, 5, 6, 8, 12, 13, IFN-γ, TNF-α, GM-CSF, MIP-1α as reviewed by Munck et al. (1984) and Sapolsky et al. (2000). The roles of GCs and the neuroimmunological relationships are tightly interwoven and further understanding of these relationships will help to reduce morbidity and mortality in the cattle industry and increase profitability.

Objectives

In order to test if an inverse relationship exists between animal temperament and immune function in beef cattle, measurements such as exit velocity and pen score were used to relate animal temperament to immune responses *in vitro* and *in vivo*. Lymphocyte proliferative responses and immunoglobulin (IgM) production were measured (*in vitro*) to test if differences occur in animals with different temperaments. Response to weaning vaccinations (*in vivo*) were evaluated to test these same differences.

INFLUENCE OF TEMPERAMENT ON LYMPHOCYTE PROLIFERATION AND IMMUNIZATION RESPONSE OF WEANED BRAHMAN BULL CALVES

Introduction

Psychoneuroimmunology is an emerging field in human medicine, and it deals with the connection between the mind and the immune system. More specifically, it deals with the physiological systems influenced by psychosocial events and behavior. Its importance is relevant to current beef cattle production in that their effects can have a strong influence on animal health and performance.

Animal temperament, an animal's reactivity to human handling and common management practices, has become an area of recent research into its effects on stress responsiveness and areas of economic significance to cattle producers. Measures of animal temperament have been developed to identify cattle that respond differently to novel stimuli, leading researchers to determine how physiological responses affect systemic adrenal, somatotropic and immune responses.

Humoral immune responses to weaning vaccinations are vital in conferring immunity to pathogens encountered by cattle at times of increased susceptibility. Finding practical and efficient ways to identify cattle, which may be at a higher risk to profit, is important to the economics of the beef industry.

This experiment was conducted to test multiple post-weaning measurements of temperament and stress responsiveness in Brahman bull calves. These tests were coupled

with testing *in vitro* immune responses, *in vivo* responses to weaning vaccinations and the effects of glucocorticoids on these immune parameters. Stocker growth rate and feedlot performance characteristics were also included.

Materials and Methods

Animals and Temperament Assessment. A contemporary group of spring born (2004) Brahman bull calves (n=45, weighing 190 ± 5.89 kg at weaning) were pastured with dams until weaning (day 0) at the Texas Agricultural Experiment Station, Overton. Temperament was assessed to sort the calves into good and bad temperament groups. After weaning, calves were chosen (calm = 10; temperamental = 10) for extremes in temperament based on EV and PS. From weaning until the conclusion of the vaccination trial, the calves had EVs taken at the beginning, middle and end of the trial. Calves were then placed on pasture for a five-month (12/04-5/05) stocker phase before being shipped to the feedyard at West Texas A&M University, Canyon for finishing.

Weaning vaccinations of Fortress 8 (Pfizer, Exton, PA), Clostridial and Titanium 5 (Diamond Animal Health, Des Moines, IA) respiratory complex were given on days 0 and 42. Animals were kept in a pen (9x35 m), but were moved to small contemporary groups (n=4) to facilitate collection of blood samples. Each group of four contained two calves with good temperament, and two with bad temperament. Castration and dehorning were delayed until after the immune portion of this study to gain a more accurate picture of stress response. At the conclusion of the immune portion, calves were commingled and put on rye and ryegrass pasture for a stocker phase

Data Collection. The immunological aspect of the project began at weaning (day 0) and continued for 11 weeks. At day 0, the calves had one 10 mL tube of blood taken to harvest serum for cortisol quantification and immunoglobulin (IgG) response to the Clostridial vaccine. Serial blood samples (Figure 1) were taken for the duration of the

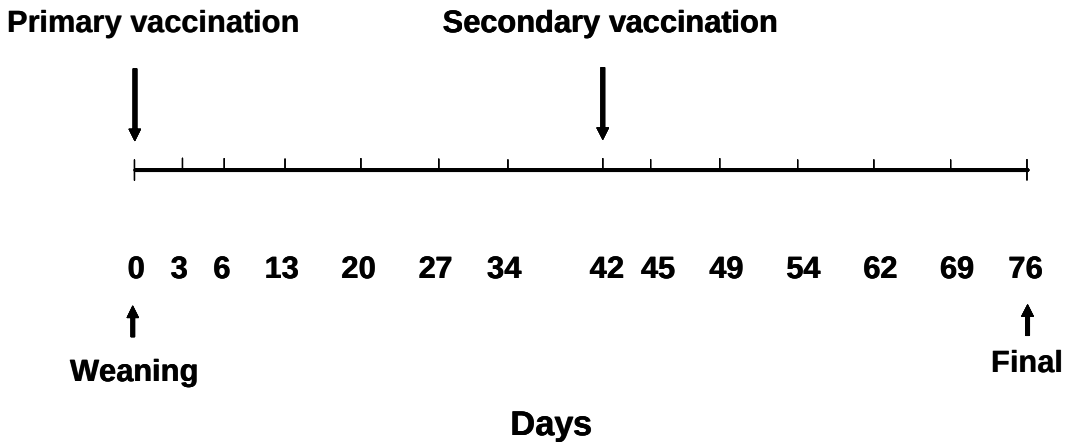

Figure 1.
Vaccination and blood collection schedule for the Brahman bull calf immune trial (n = 20).

trial with more frequent sampling (Figure 1) one week following weaning (day 0) and revaccination (day 42) to get a clearer picture of primary and secondary responses to the vaccine. Two additional EDTA coated, 10 mL tubes of blood were collected at day 0 and 42 for lymphocyte isolation and culture to test *in vivo* proliferative responses and IgM production.

Cortisol RIA. Systemic cortisol concentrations were determined from duplicate 100 μL serum samples by a cortisol RIA (DSL-2100, Diagnostic Systems Laboratory, Webster, TX) antibody coated tube kit. Unknown cortisol concentrations were analyzed

using Assay Zap software (Biosoft, Cambridge, UK). The area under the response curve from day 0 through day 76 was determined utilizing a method described by Lay et al. (1996): $AUC = \Sigma [(\{(CS_n + CS_{n+d}) / 2\} \cdot d)]$ where d is the time in days between CS samplings. The inter-assay CV was 8.4% and the intra-assay CV was 8.6%.

Lymphocyte Isolation. Blood collected from 2, 10 mL EDTA tubes was pooled into a 50 ml conical tube and the volume was increased to 25 mL with Hank's Balanced Salt Solution (HBSS) without $Ca2^+$ or $Mg2^+$ (Hyclone Inc., Logan, UT). The blood/HBSS solution was layered onto 15 mL Histopaque-1077 (Sigma-Aldrich, St. Louis, MO; specific density 1.077 g/mL) and centrifuged at 800 x g for 30 min. The buffy coat layer was removed and placed in a clean 50 mL tube and diluted up to 40 mL with HBSS. Cells were pelleted by centrifugation (400 x g for 15 min) and the supernatant was aspirated and discarded. To remove any red blood cell contamination, the pellet was washed with 5 mL of 0.2% NaCl solution and titrated for 1 min. Five mL of 1.6% NaCl solution was added to normalize osmolarity and HBSS was added up to 40 mL total volume. The tube was centrifuged (400 x g for 10 min) to pellet the cells and the supernatant was aspirated. The pellet was re-suspended in 1 mL of media that consisted of: DMEM F-12 HAM medium (Sigma-Aldrich, St. Louis, MO) with 15mM HEPES, pyridoxine and $NaHCO_3$, 5% horse serum (Sigma-Aldrich, St. Louis, MO) that was heat inactivated at 56° for 1 hr and 0.45 micron sterile filtered, 1 % Penicillin-Streptomycin, 1% L-Glutamine (Life Technologies, Grand Island, NY), 0.007% β-mercaptoethanol (BioRad Laboratories, Hercules, CA). Media was sterile filtered

through a 0.22 micron filter. Suspended cells were kept on ice until concentrations could be determined.

Cell Counts. Ten µL of cell suspension was diluted 1:5 with culture media and pipetted into a 2.5 mL microcentrifuge tube. When the cells were ready to be counted, the suspension was diluted 1:1 with Trypan Blue (Life Technologies Inc., Grand Island, NY). The cell suspension was added to a hemacytometer and counted two times using light microscopy to get an average count. The original cell suspension was then diluted with culture media to yield a final concentration of $2x10^6$ cells/mL. Cells were kept on ice until pipetted onto culture plates.

Cell Culture. Cell cultures were performed at the midpoint of the steers feeding period. Cultures were carried out on two (one for IgM and one for proliferation) 96 well plates allowing for: 4 animals on each plate, 8 rows of treatments (including zero control) and 3 repetitions of each treatment. Treatments consisted of the blastogenic mitogen Concanavlin (ConA, lot# 033K8936, Sigma-Aldrich, St. Louis, MO) beginning at 10 µg/mL, and serially diluted with culture media 1:1 to 0.16 µg/mL. Fifty µL of the treatments and cell suspension were added to the wells to yield a total volume of 100 µL and a final concentration of $1x10^5$ cells/well. Incubation conditions were kept at 37^o C, 5% CO_2, and 50% relative humidity for 72 hrs.

Proliferation and IgM Analysis. ConA induced lymphocyte proliferation was measured by the CellTiter 96 cell proliferation assay (lot 191851, Promega, Madison, WI) after 72 hrs of incubation. IgM plates were frozen (-80^o C) until assayed. ConA induced lymphocyte IgM production was measured by a bovine IgM ELISA

quantication kit (Bethyl Inc., Montgomery, TX). A checkerboard ELISA was used to determine sample dilution (1:1). Plates were read using a microplate spectrophotometer (Biotek Instruments, Inc., Winooski, VT) at 405 nm.

IgG Analysis. Serial serum samples (diluted 1:10) were analyzed for vaccine specific IgG by a double sandwich, enzyme linked immunosorbant assay. The samples were diluted in phosphate buffered saline (PBS) containing 0.05% Tween-20 and 1.0% bovine serum albumin (BSA). Nunc immunoplates (Nalge Nunc International; Denver, CO) were incubated for one hr. on an orbital shaker with 100 µL/well of diluted Fortress 8 vaccine (1:20 in 0.1 M Na_2CO_3, pH 8.2). Plates were washed three times with PBS containing 0.05% Tween (wash buffer). To prevent non-specific binding, 100 µL/well of PBS containing 0.05% BSA (blocking buffer) was added and the plates were incubated for 30 min. Plates were then washed three times with wash buffer, the standards and diluted samples added (100 µL/well), and the plates incubated for one hr. on an orbital shaker. Plates were washed four times with wash buffer and the detecting antibody (sheep anti-bovine IgG-Fc conjugated to horseradish peroxidase; diluted 1:1600 in sample diluent; Bethyl Laboratories; Montgomery, TX) was added. Plates were incubated for one hr. on an orbital shaker and then washed four times with the wash buffer. Enzyme-substrate buffer (2,2'-azino-bis[3-thylbenzthiazoline-6-sulfonic acid] + 0.05% H_2O_2; pH 4.5; Sigma Chemical Co.; St. Louis, MO) was added (100 µL/well) and the plates were incubated in darkness for 10-15 min. Optical densities were read using a microplate spectrophotometer at 405 nm. Sample IgG concentrations were determined

by comparison to a standard curve generated from purified IgG (Bethyl Laboratories; Montgomery, TX.

Statistical Analysis. Analysis of variance procedures for repeated measures were used (GLM of SAS) to determine differences in serum concentrations of cortisol and antibody profiles, temperament measures The GLM procedure and LSM were used to analyze temperament, ADG, avg. cortisol, *in vitro* proliferative response, antibody production, ED50 and AUC.

Results and Discussion

Temperament and Growth. During the immunological aspect of the project, PS and EV were obtained at weaning (day 0) to assign bull calves into temperament groups. Pen score differed ($P < 0.01$) between C and T bull calves scoring 1.83 ± 0.31 and 3.70 ± 0.31 respectively (Figure 2). Measures of PS taken at weaning were repeatable with T bulls ($P > 0.33$); however, midpoint measurements for C bulls were higher ($P < 0.01$) than their weaning measurement. This increase could be due to a number of environmental or managerial factors, but a similar increase was not seen in the T bulls, possibly due to T bulls already scoring close to the highest values on the scoring system. Final PS for C bulls did not differ ($P > 0.15$) from previous readings. Although the midpoint measurement was higher for C bulls, the overall effect of time in the model was not significant ($P > 0.21$), demonstrating that PS measurements were repeatable throughout the trial period.

Exit velocity differed ($P < 0.01$) between C and T bulls and averaged 1.36 ± 0.20 m/s and 2.90 ± 0.20 m/s respectively (Figure 3). Temperamental bulls did not differ ($P >$

0.58) from weaning to midpoint, but did decrease ($P < 0.04$) in EV at the final measurement supporting the data of Curley (2004) stating that cattle can become acclimated to handling and therefore show less of a response to common cattle management procedures. Calm bulls showed a significant increase ($P < 0.01$) from weaning (0.77 ± 0.18 m/s) to midpoint (2.03 ± 0.29 m/s) and a decrease ($P < 0.01$) at the final observation (1.27 ± 0.25 m/s); however, the final observation was significantly higher than the weaning measurement. These differences can account for the effect of time ($P < 0.01$) in the model. The increases in EV seen at the midpoint correspond with the increased PS measurements taken at the same time. It could be suggested that calmer cattle are more susceptible to an increased temperament response than temperamental cattle due to the increases shown at the midpoint measurement. Although the increases may be larger, the means are still significantly higher in the T bulls and distinguishable between the groups at all measurement points.

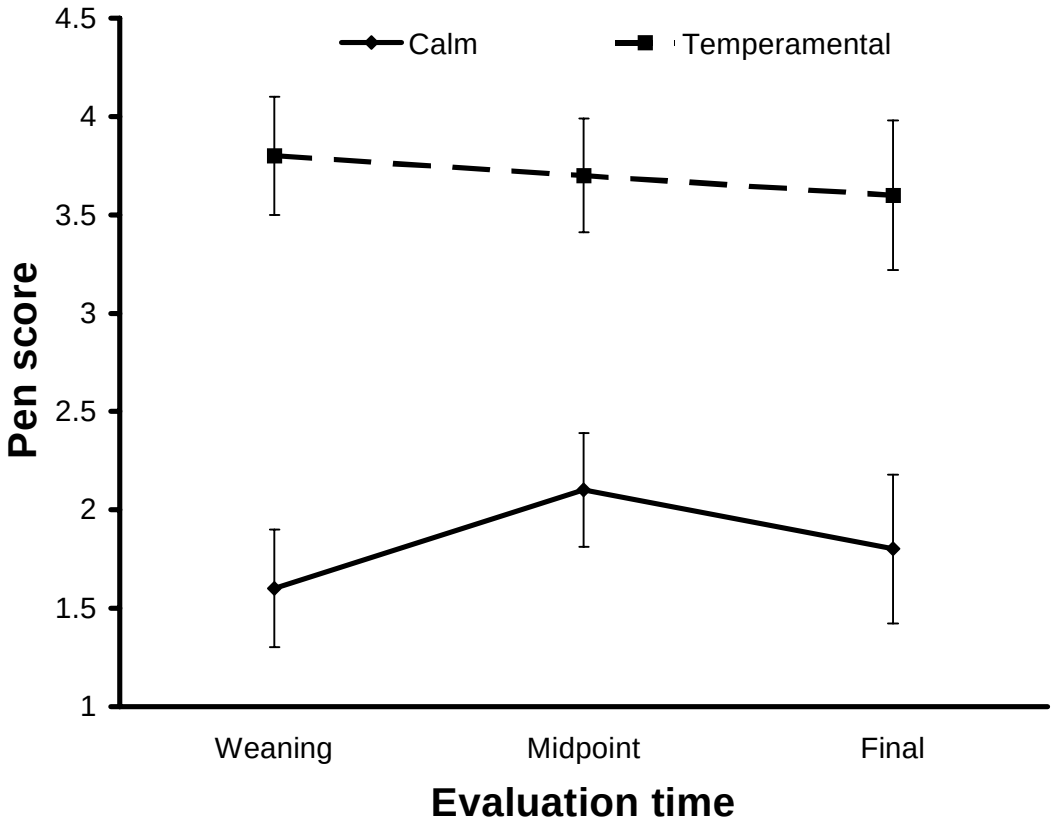

Figure 2.
Least-squares means for pen score evaluation for calm (n = 10) and temperamental (n = 10) bulls at three measurement points. (Interaction Pr > F = 0.072)

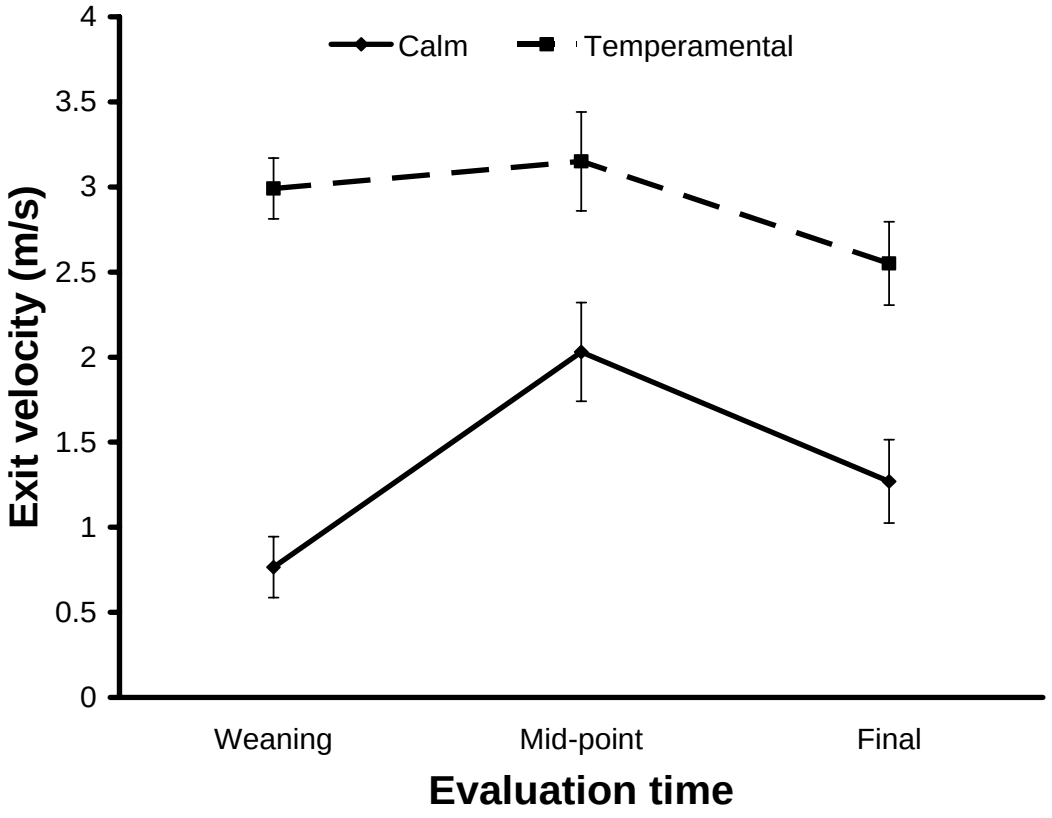

Figure 3.
Least-squares means for exit velocity for calm (n = 10) and temperamental (n = 10) bulls at three measurement points. (Interaction Pr > F = 0.010)

Table 1 shows the Pearson correlation coefficients between the measures of temperament and serum cortisol concentrations at the three measurement points. The initial measurements were used as the base of comparison to subsequent time points because they have been found to be indicative of subsequent measurements of temperament (Grandin, 1993; Fell et al., 1999; Curley, 2004). Weaning PS was highly correlated to midpoint EV ($r = 0.78$, $P < 0.01$), CS ($r = 0.65$, $P < 0.01$) and final EV ($r = 0.84$, $P < 0.01$), CS ($r = 0.79$, $P < 0.01$). Similarly, initial EV was correlated to midpoint PS ($r = 0.83$, $P < 0.01$), CS ($r = 0.69$, $P < 0.01$) and final PS ($r = 0.80$, $P < 0.01$), CS ($r = 0.62$, $P < 0.01$). Initial CS concentrations were also highly correlated to midpoint EV ($r = 0.69$, $P < 0.01$) and final EV ($r = 0.61$, $P < 0.01$). These measures of animal temperament show a strong relationship to each other and can be used as a measure of stress responsiveness in cattle. The endocrine reactions associated with stress responsiveness can affect the somatotropic axis, making it important to investigate their effects on animal growth. In the vaccination trial, ADG (Figure 4) differed ($P = 0.01$)

Table 1.
Pearson correlation coefficients (and associated P values) of temperament measurements and cortisol. (n = 20)

Measurements taken at Time 1	Time 2 PS	Time 2 EV	Time 2 CS	Time 3 PS	Time 3 EV	Time 3 CS
Pen Score (PS)	0.91	0.78	0.65	0.90	0.84	0.79
P =	<.01	<.01	<.01	<.01	<.01	<.01
Exit Velocity (EV)	0.83	0.60	0.69	0.80	0.79	0.62
	<.01	0.01	<.01	<.01	<.01	<.01
Cortisol (CS)	0.60	0.69	0.53	0.68	0.61	0.57
	0.01	<.01	0.02	<.01	<.01	0.01
Tempermant Assessment [a]	0.68	0.54	0.50	0.62	0.66	0.52
	<.01	0.01	0.03	<.01	<.01	0.02

[a] Temperament groups (C and T) based on initial pen score and exit velocity

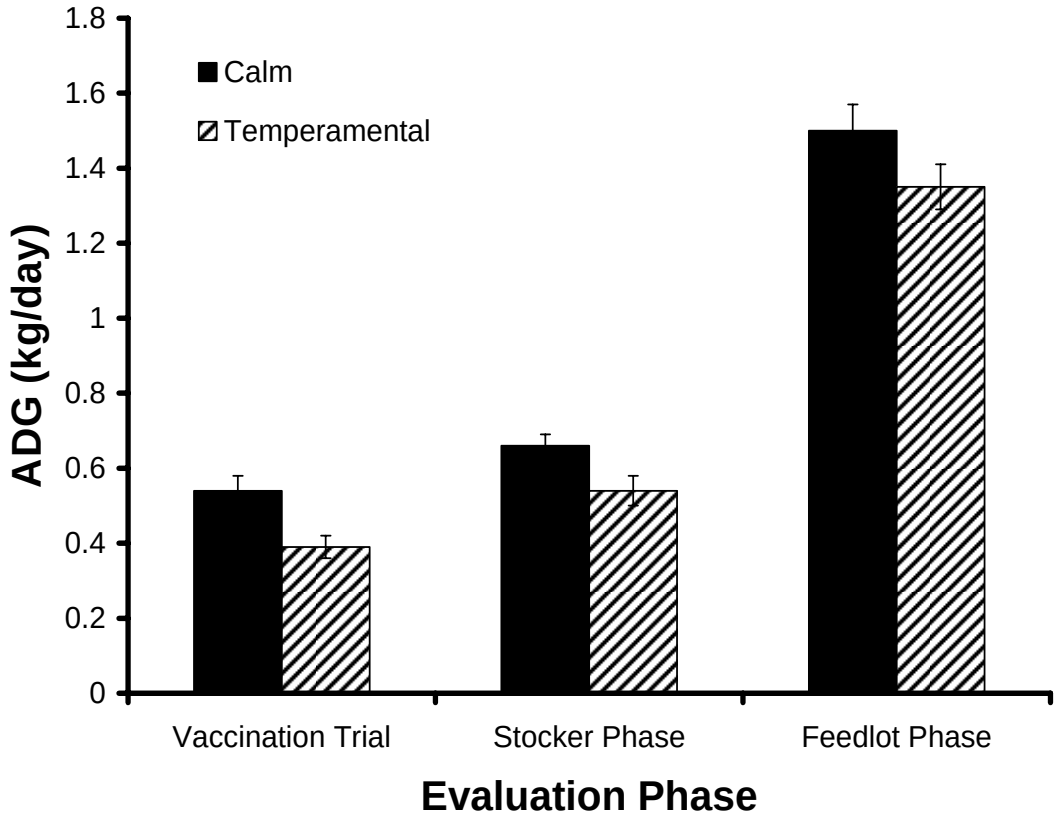

Figure 4.
Least-squares means for average daily gain at each evaluation phase of growth in Brahman bull calves. (Calm n = 10, Temperamental n = 10)

between C and T bulls and averaged 0.54 ± 0.04 kg/day and 0.39 ± 0.03 kg/day respectively. During the stocker phase, C bulls gained significantly more (P = 0.04) weight than T bulls averaging 0.66 ± 0.03 kg/day and 0.54 ± 0.04 kg/day respectively. During the feedlot phase, C bulls did not significantly out gain (P = 0.13) T bulls averaging 1.50 ± 0.07 kg/day and 1.35 ± 0.06 kg/day, respectively. Burrow and Dillon (1997) found that cattle ranked by EV had differences in ADG in feedlot situations with calm cattle gaining more weight (P < 0.05) per day than cattle with excitable temperaments. Voisinet et al. (1997a) reported similar results with *Bos indicus* crossed steers and heifers differing by 0.1 kg/day ADG. Although the feedlot ADG was not significantly different (possibly due to low n), the trend (+ 0.15 kg/day) fits previous literature and small differences in gain can have a significant economic impact when the cattle are sold.

In Vitro Lymphocyte Proliferation. The *In vitro* lymphocyte culture on day 0 of the study (Figure 5), resulted in a ConA dose dependent (P < 0.01) increase in blastogenesis for both C and T bulls. There was a significant treatment effect (P < 0.05) with C bulls averaging a stimulation index (SI) of 3.13 ± 0.11 and T bulls 2.88 ± 0.12 across all concentrations of ConA. As expected, the highest stimulatory effects were seen at 10 μg/mL of ConA (SI of 5.10 ± 0.25 and 4.87 ± 0.35 for C and T bulls respectively). There was not an interaction (P = 0.96) of treatment group and ConA showing that the differences in stimulation between groups did not differ among the increasing ConA concentrations. Similarly, on day 42 of the study (Figure 6), ConA produced a dose dependent (P < 0.01) increase in blastogenesis for both C and T bulls.

There was a significant treatment effect ($P < 0.01$) with C bulls averaging a SI of 2.75 ± 0.09 and T bulls 2.40 ± 0.07 across all levels of ConA. The highest stimulatory effects were seen at 10 μg/mL of ConA (SI of 4.23 ± 0.15 and 3.45 ± 0.16 for C and T bulls respectively). There was a significant interaction ($P < 0.01$) between treatment group and ConA resulting in greater stimulatory effects for C bulls at increasing concentrations of ConA.

Circulating CS concentrations on Day 0 tended to be different ($P = 0.07$) with C bulls averaging 4.82 ± 1.52 ng/mL and 9.02 ± 1.52 ng/mL for T bulls. It is possible that at the time of weaning, stress associated changes in immune function had yet to take suppressive actions on lymphocyte activation and cytosis. Dhabhar and McEwen (1997) suggested that the novel experience of weaning may have imposed an acute stressor that acted as an adaptive response to prepare the body for possible immunologic challenges. However, studies have shown that stress induced suppression of bovine lymphocytes can occur at as little as 5 minutes after exposure to the stressor (Blecha et al., 1983). Circulating CS concentrations on day 42 were significantly different ($P < 0.05$) with the C bulls averaging 6.39 ± 1.70 ng/mL vs. 12.26 ± 1.70 ng/mL for the T bulls. These results agree more closely with many published reports that an increased concentration of cortisol suppresses ConA-stimulated lymphocyte proliferation in different livestock species (Blecha et al., 1984; Coppinger et al., 1991). Beginning at 1.25 ng/mL of ConA, there was a 15% increase in proliferation for C bulls. This trend continued at each subsequent concentration of ConA up to 5 μg/mL, increasing to 20%, and then leveling off at a 23% enhancement in proliferation. These differences could be attributed to the

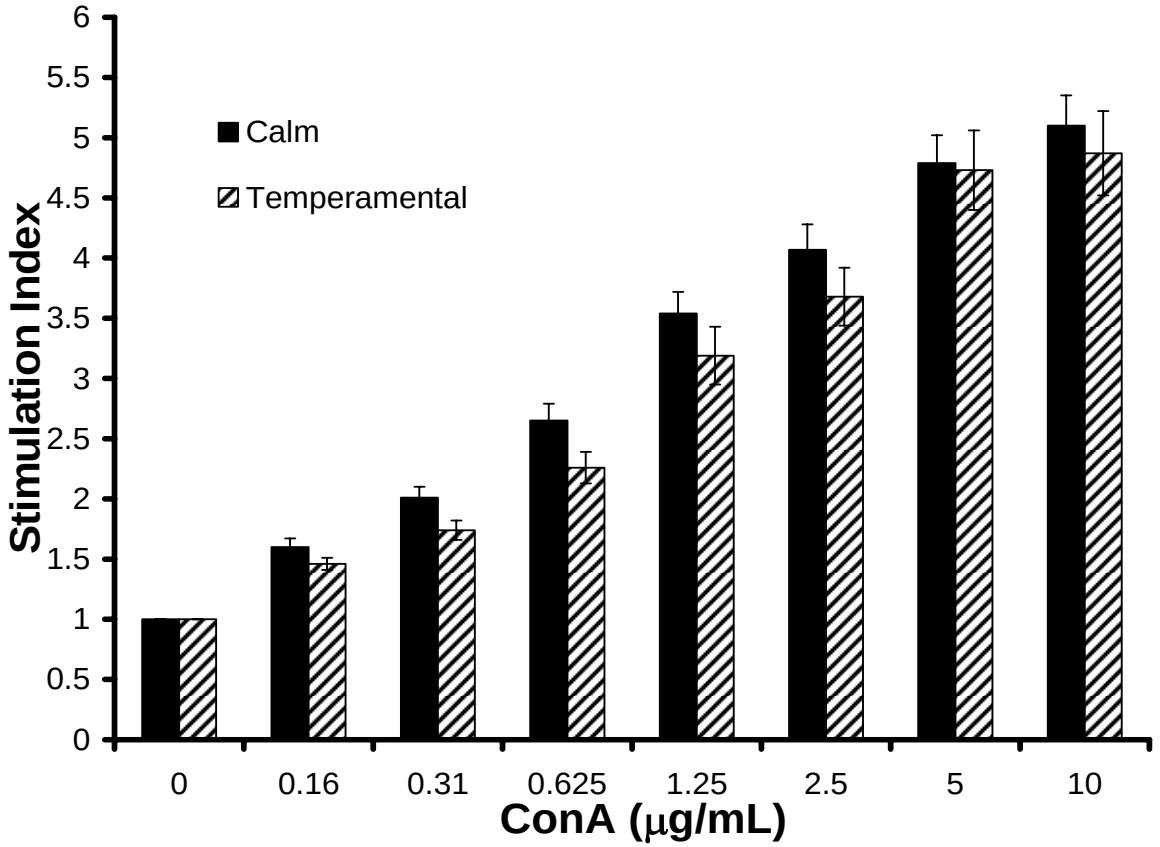

Figure 5.
Day 0 lymphocyte proliferation for calm (n = 10) and temperamental (n = 10) bulls with ConA as the stimulatory mitogen. (Interaction Pr > F = 0.959)

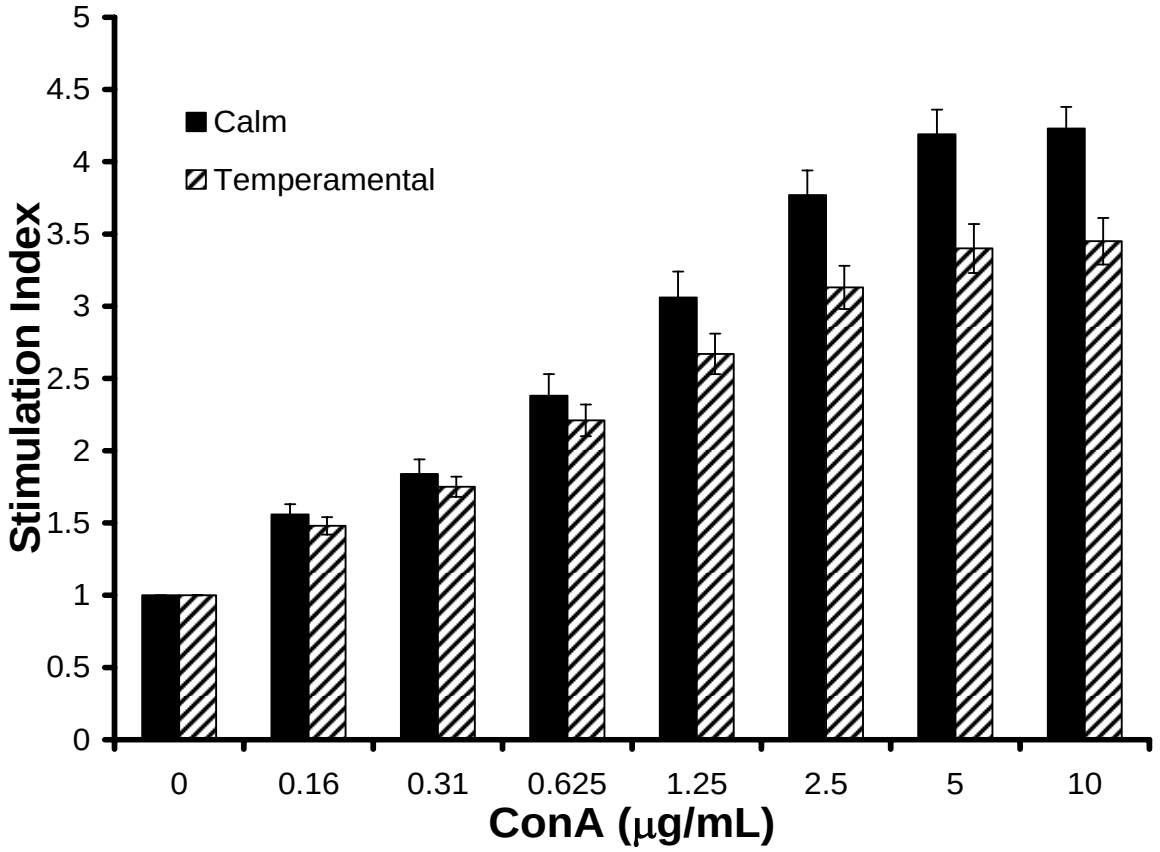

Figure 6.
Day 42 lymphocyte proliferation for calm (n = 10) and temperamental (n = 10) bulls with ConA as the stimulatory mitogen. (Interaction Pr > F = 0.003)

chronic stress of handling multiple times a week and the individual animal variation in adapting to stressful stimuli. The immnosuppressive effects of chronic stress may have affected lymphocyte function in the 6^{th} week (day 42) of the study.

Analysis of the ED_{50} for the proliferation curves on day 0 (Table 2) tended to be different ($P < 0.10$) with the C bulls averaging 0.97 ± 0.10 ng/mL of ConA and 1.62 ± 0.31 ng/mL for the T bulls. An opposite effect occurred on day 42 with C bulls averaging higher, but not significantly ($P = 0.16$), than the T bulls with 1.07 ± 0.19 ng/mL and 0.79 ± 0.12 ng/mL respectively. Relative sensitivities of lymphocytes to thymus dependent stimulatory mitogens are reliant on $CD4^+$ T cell activation and IL ligand/receptor relationships (Wiegers et al., 2004). Many of these ligand/receptor relationships are influenced by glucocorticoids leading to an initial enhancement and an eventual suppression of immune function. As $CD4^+$ T cell status was not assessed in this study, it may be more difficult to thoroughly consider their role in this experiment.

Strong correlation coefficients were not seen between ED_{50} and EV or CS (Table 3). The ED_{50} values were positively correlated with EV on day 0 ($r = 0.33$, $P = 0.159$). However, an opposite relationship was noted on day 42 for ED_{50} and EV ($r = -0.39$, $P = 0.09$). The ED_{50} values were negatively correlated to CS on day 0 and 42 ($r = -0.12$, $P = 0.62$; $r = -0.39$, $P < 0.10$ respectively), but only the day 42 correlation tended to be significant.

Table 2.
Least-squares means for ED_{50} ± SE (ng/mL) for day 0 and 42 lymphocyte cultures on calm (n = 10) and temperamental (n = 10) bulls.

	Temperament Group[a]		
	C	T	Pr > F
ED_{50} day 0	0.97 ± 0.10	1.62 ± 0.31	0.09
ED_{50} day 42	1.07 ± 0.19	0.79 ± 0.12	0.16

ED_{50} = Effective dose required to provide half of stimulatroy effects observed

[a]C = calm, T = temperamental

Table 3.
Pearson correlation coefficients for EV, CS and ED_{50} in Brahman bull calves. (n = 20)

Variable Pairing			Correlation	Pr < |r|
ED_{50} day 0	/	ED_{50} day 42	0.20	0.40
ED_{50} day 0	/	EV day 0	0.33	0.16
ED_{50} day 0	/	CS day 0	-0.12	0.62
ED_{50} day 42	/	EV day 42	-0.39	0.09
ED_{50} day 42	/	CS day 42	-0.39	0.09

EV = exit velocity, CS = Cortisol, ED_{50} = Effective dose of ConA required to provide half of stimulatory effects observed.

In Vitro Lymphocyte IgM Production. ConA dose dependently increased production of IgM ($P < 0.01$) by the *in vitro* lymphocyte culture on day 0 of the study (Figure 7) for both the C and T bulls. A significant treatment effect ($P < 0.01$) was detected with C bulls averaging 208.80 ± 13.06 ng/mL and T bulls 156.54 ± 10.23 ng/mL across all concentrations of ConA. As expected, the highest concentrations of IgM were induced by the highest dose (10 µg/mL) of ConA (351.24 ± 44.37 ng/mL and 305.03 ± 36.89 ng/mL for C and T bulls respectively). Similar to the proliferation results on day 0, there was no interaction ($P = 0.73$) between treatment group and ConA. ConA produced dose-dependent increases ($P < 0.01$) in lymphocyte IgM production on day 42 (Figure 8). The effect of treatment tended to be significant ($P < 0.10$) with C bulls averaging 241.64 ± 13.33 ng/mL and T bulls 224.30 ± 12.40 ng/mL across all concentrations of ConA. Lymphocytes from C bulls reached their highest IgM production at 10 µg/mL of ConA and T bulls at 1.25 µg/mL. Subsequent higher doses of ConA induced less of a response with a 22% decrease in T bulls. There was no observed interaction ($P = 0.33$) between C and T treatment groups. Day 42 IgM production was the only culture in this study in which cortisol was a significant covariate ($P < 0.01$). On day 0 cultures, C bulls IgM production was 52 % higher at 1.25 µg/mL of ConA. Subsequent higher doses of ConA provided sufficient stimulation for both treatment groups to lessen this difference, but even at 10 µg/mL of ConA, C bull's lymphocytes produced 15% more IgM than T bull's. It is conceivable that at lower levels of antigenic challenge, animals with better temperaments could better produce IgM to fight pathogens by opsonization or to have enhanced $CD4^+$ T cell activation of naive B

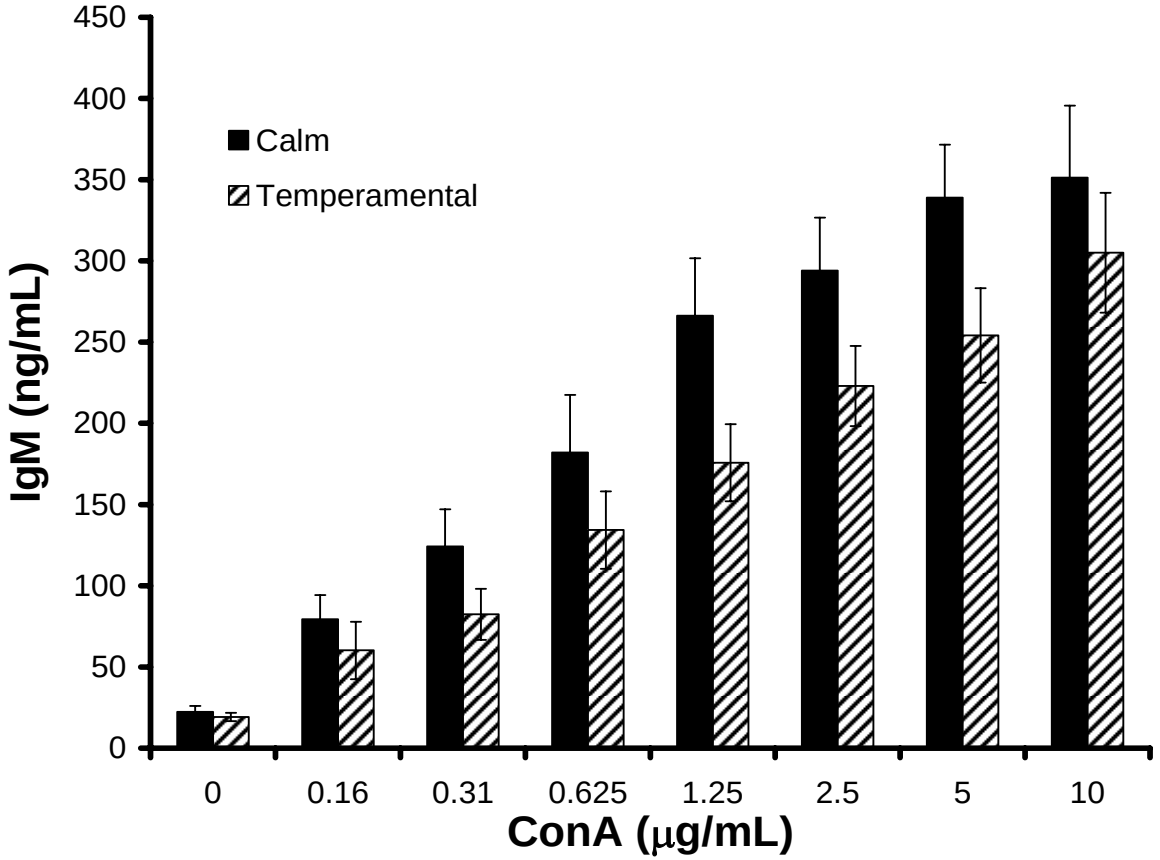

Figure 7.
Day 0 lymphocyte IgM production for calm (n = 10) and temperamental (n = 10) bulls with ConA as the stimulatory mitogen. (Interaction Pr > F = 0.734)

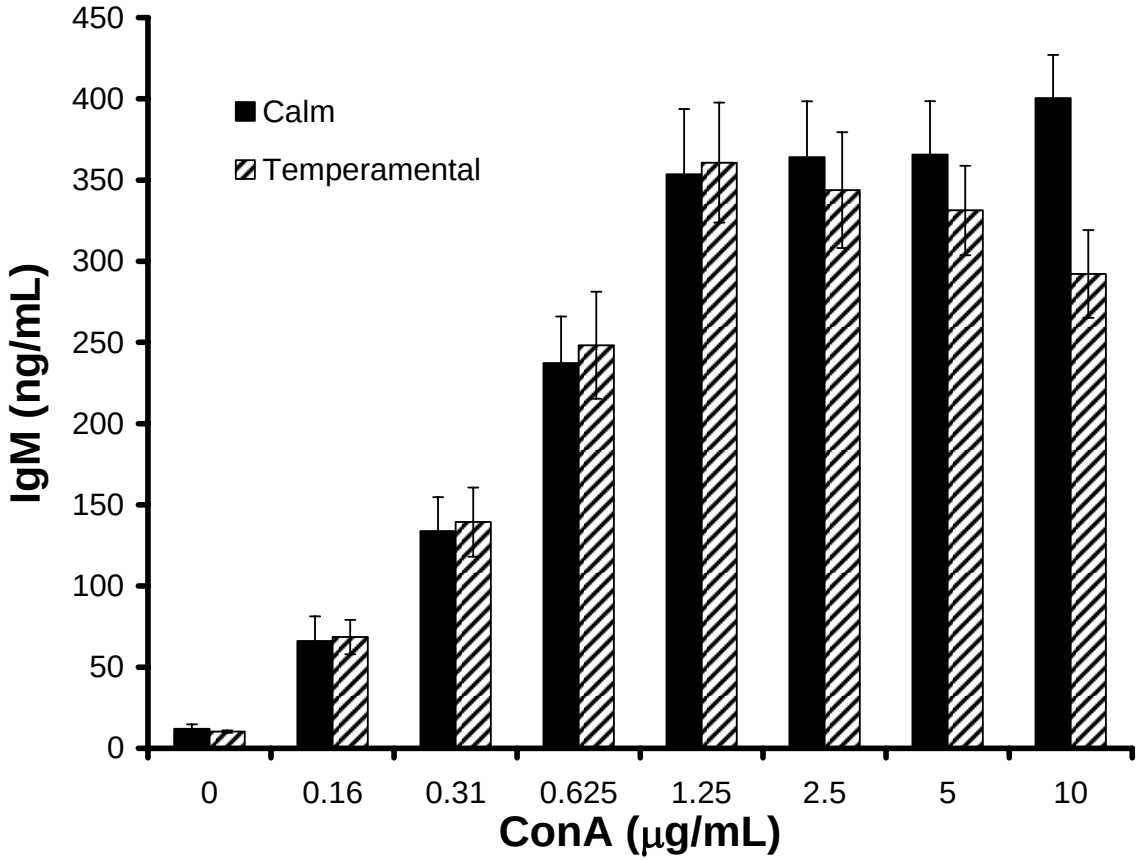

Figure 8.
Day 42 lymphocyte IgM production for calm (n = 10) and temperamental (n = 10) bulls with ConA as the stimulatory mitogen. (Interaction Pr > F = 0.328)

lymphocytes. This was not the case with the day 42 cultures where we see larger differences at higher concentrations on ConA. Fell et al. (1999) reported serum IgM concentrations for calm and temperamental steers upon entry to a feedlot and found IgM was significantly higher ($P < 0.05$) in nervous steers. One limiting factor in analyzing this IgM data is that it is difficult to determine if the plasma B cells were secreting more IgM, or if there was more activation of naïve B cells by antigen presenting cells (APC) leading to increased plasma cell numbers.

Cortisol Analysis. Serum CS concentrations (Figure 9) were significantly different ($P < 0.01$) throughout the study. There was a significant ($P < 0.01$) effect of temperament group with the C bulls averaging 5.32 ± 1.08 ng/mL vs. 10.20 ± 1.08 ng/mL for the T bulls. The interaction of treatment group and time tended to be significant ($P = 0.10$) showing differences in stress responsiveness over time between both groups. Put more clearly, all bulls were handled and managed in the same manner, allowing us to observe differences in the temperament groups CS profiles. Although there are differences in the CS profiles, similar, general trends can be seen between the two groups. Fell et al. (1999) reported similar results for nervous/temperamental cattle at weaning and at feedlot entry 6 months later. In the Fell et al. (1999) study, nervous cattle had significantly higher CS concentrations ($P < 0.01$) before and after weaning, and at feedlot entry ($P <0.05$). The data collected supports this previous research, stating that temperamental cattle have significantly higher serum CS concentrations at early stages of their production.

AUC analysis for CS determined that C bulls had significantly lower ($P < 0.01$) CS concentrations over the length of the study. AUC measurements averaged 401.83 ± 37.63 ng·d/mL and 799.36 ± 102.63 ng·d/mL for C and T bulls, respectively.

Direct IgG Analysis. Serum concentrations of IgG (Figure 10) specific to the Clostridium vaccine used in this study were significantly increased ($P < 0.01$) from day 0 across the length of the trial for both C and T bulls. There was no significant effect of temperament group ($P = 0.11$) with C bulls averaging a stimulation index (SI) of 7.12 ± 0.96 and 4.96 ± 1.01 for T bulls across the entire length of the study. There was no interaction of temperament and time ($P = 0.86$) meaning differences observed within days remained significantly constant throughout the study. Vaccine antibody (Ab) was first increased ($P < 0.01$) on day 6 post-vaccination for both the C and T bulls. Peak primary response was reached on day 13 with C bulls averaging a 6.61 ± 1.02 fold increase vs. a 4.53 ± 1.08 fold increase for the T bulls. By day 42, the primary responses tended to differ ($P < 0.10$) with the C bulls averaging a SI of 6.57 ± 1.13 vs. 3.76 ± 1.19 for the T bulls. On day 42, bulls were revaccinated. A significant ($P < 0.01$) secondary vaccination response was detected by day 49, where T bulls reached a peak response (SI = 9.43 ± 1.88 fold increase). By the end of the study, T bulls antibody levels had decreased ($P < 0.05$) to 6.30 ± 1.34 fold. Secondary responses for C bulls peaked on day 54 with a 11.49 ± 1.58 fold increase. By the end of the study, C bulls had not significantly decreased ($P = 0.22$) vs. day 42 SI, averaging a 9.84 ± 1.27 fold increase.

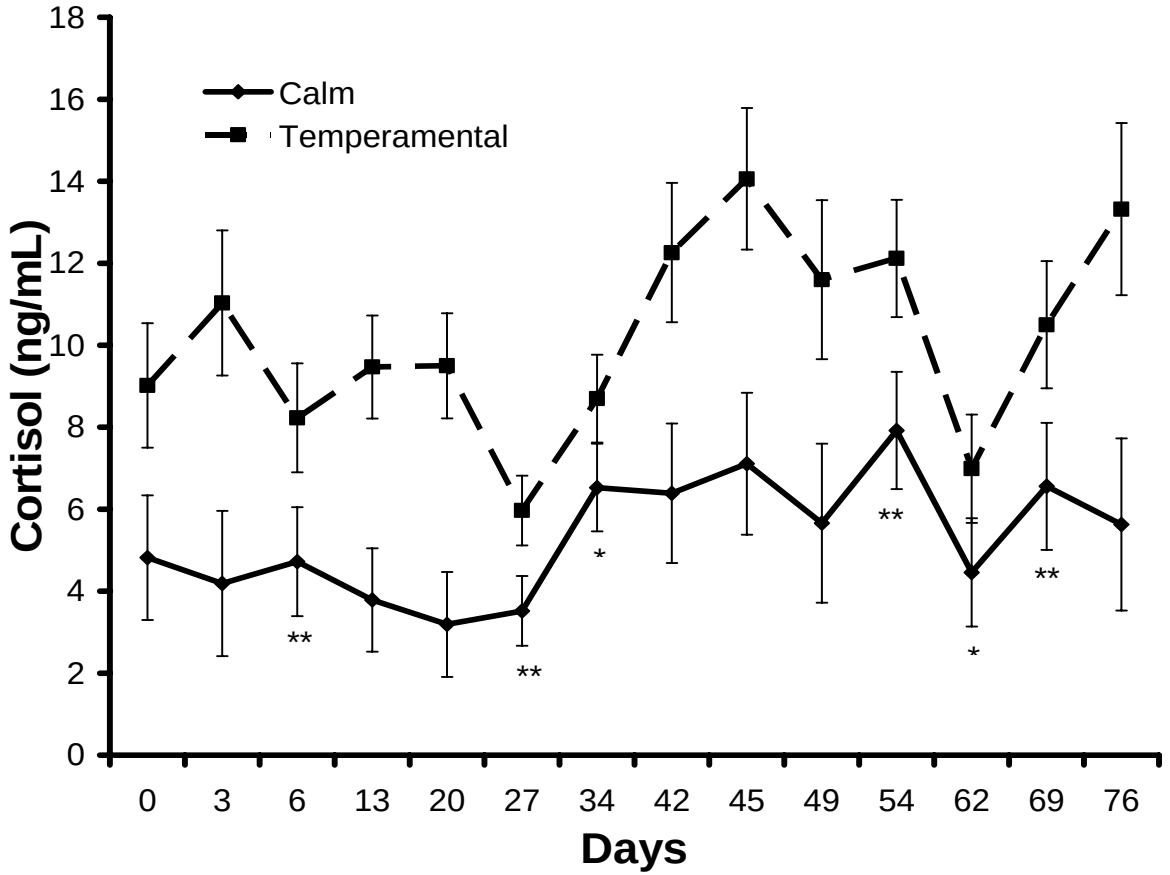

Figure 9.
Least-squares means for cortisol concentrations for entire length of vaccination trial in calm (n = 10) and temperamental (n = 10) bull calves. (Interaction Pr > F = 0.10) Values within day differ ($p < 0.05$)
*Values differ ($p < 0.10$) **Values not significantly different

Feng et al. (1991) reported that stress may not only effect antibody production, but the seroconversion from IgM to other isotypes. Delayed seroconversion during pathogenic challenges may increase likelihood of morbidity, even in the presence of sufficient immune system activation. In addition to delayed seroconversion, stress may also have a direct effect on primary immune responses. These include T-lymphocyte clonal expansion and maturation, initial B-lymphocyte clonal expansion and IgM production and production of memory lymphocytes and plasma secreting B cells (Burns et al., 2003). Perhaps one of the most important implications is that stress may have its principal effects on the rate of antibody deterioration (Burns et al., 2002). The Burns et al. (2002) study reported that students experiencing high levels of life stress events were 2.5 times more likely to have inadequate antibody titers than students with high levels of life stress events who were recently vaccinated. This study proposes a good model to test antibody deterioration in cattle with different levels of stress responsiveness.

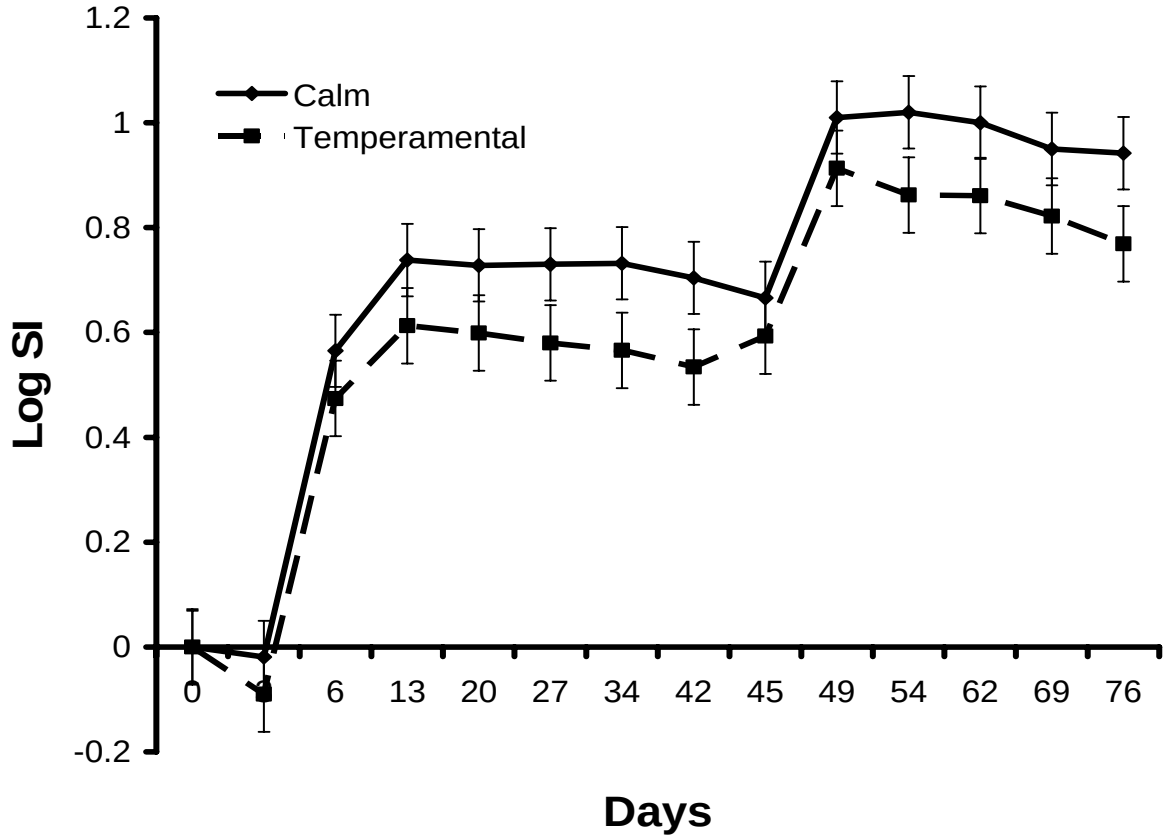

Figure 10.
Log transformed values of serum IgG concentrations after primary and secondary vaccinations in calm (n = 10) and temperamental (n = 9) bull calves. (Interaction Pr > F = 0.86) Vaccinations at day 0 and 42. Actual stimulation indices reported in text.

Table 4.
Pearson correlation coefficients for cortisol and serum concentrations of IgG at selected days post-vaccination. (n = 19)

		Serum IgG			
Variables	Day	0	13	49	76
Cortisol AUC	0	-0.125	-0.145	-0.248	-0.301
Pr > |r|		0.610	0.554	0.306	0.210
IgG	13	-	-	0.649	0.779
Pr > |r|		-	-	< 0.005	< 0.001

Cortisol AUC = Area under curve across entire study

Table 4 presents Pearson correlation coefficients for CS and serum IgG concentrations on selected days. The correlations between the AUC analysis and serum IgG concentrations were not significant, but did reflect a relationship of negative trends. Peak IgG response on day 13 was highly correlated to secondary IgG responses on day 49 ($r = 0.65$, $P < 0.01$) and day 76 ($r = 0.78$, $P < 0.01$). This relationship shows that primary antibody responses are predictive of the degree and direction of secondary responses. Adrenalectomized mice given corticosterone injections failed to have a suppressed humoral response following induced stress (Esterling and Rabin, 1987). This leads us to conclude that there are other mediators of stress induced immunosuppression. Due to the mixed results reporting CS effects on response to vaccination, there is likely a more complex nature of the relationship between stress, CS and antibody response (Feng et al., 1991).

EVALUATION OF THE INTER-RELATIONSHIPS OF TEMPERAMENT, STRESS RESPONSIVENESS AND IMMUNE FUNCTION IN FEEDLOT CATTLE

Introduction

Animal temperament has been implicated in negatively affecting animal and carcass performance (Gardner et al., 1998; Smith, 1998). The degree of excitability exhibited by cattle, when subjected to common handling procedures or novel situations, can be used as a measure of an animal's reaction to perceived stressful stimuli. This reaction can be can, in part, be measured by changes in physiological measures of stress responsiveness. This experiment was conducted to test the relationship of temperament and stress responsiveness to immunological characteristics measured *in vitro* in weaned and yearling feedlot steers.

Feedlot performance and carcass quality are directly affected by the health of cattle (Baker, 2002). It is important to be able to identify behavioral and physiological markers that influence the health of cattle to improve production efficiency. With current market competition with other meat livestock species, it is important to economically produce a high quality, retail product that stimulates consumer demand.

Trial 1 Materials and Methods

Animals. A contemporary group of spring born (2003), Brahman and Bonsmara sired crossbred calves (n=60) were calved and pastured with dams at the Texas Agricultural Experiment Station, Overton until weaning. Calves were weaned at seven to nine months of age (weighing 243.65 ± 9.93 kg) and stockered at Overton until approximately twelve months of age before being processed for shipment to the King Ranch South feedyard for finishing (700 km). Steers were fed to a fat thickness of 1.1 cm as determined by the feedyard manager.

Temperament Assessment. Exit velocity (EV), chute score (CHUTE) and pen score (PS) were taken prior to shipment to sort cattle into calm (C) and temperamental (T) groups (calm n = 7, temperamental n = 5). EV was measured by electronically timing (FarmTek Inc., North Wylie, TX) an animal's speed over a given distance (1.83 m) after exiting from restraint in a squeeze chute (Burrow et al., 1988). This measurement was taken at three time points: (1) pre-shipment to feedyard, (2) post-shipment and (3) mid-feeding. Chute scoring (Grandin, 1993) was conducted by observing the calf unrestrained in the chute. Scoring was based on a 1 to 5 scale, with 1 representing a very calm animal, to a 5 representing and extremely violent animal. Pen scoring (Hammond et al., 1996) is a subjective measure which describes an animal's calmness or aggressiveness in the presence of a human in the pen with them. A score of 1 would designate a steer that was calm and slow walking upon approach, and a 5 would be a steer running into fences and aggressively charging the scorer. All measurements were conducted by the same scorer to eliminate variation due to technician differences. Chute scores and pen scores were only conducted at pre-shipment in order to assign calves to a temperament group. Animals

(n=12) were selected based on these procedures from the larger group of 60 calves; 7 with the best temperament, and 5 with the worst temperament to maximize extremes in order to determine differences in adrenal and immune function between the two groups.

Blood Collection. Blood was collected at the three time points previously described to determine systemic cortisol and epinephrine concentrations. Blood was collected via caudal veni-puncture into a 10 mL additive free tube (BD, Franklin Lakes, NJ) and allowed to coagulate before being spun at 1984 x g, 4^{o} C for 30 min. Serum for cortisol was harvested and aliquoted into 12x75 mm, polypropylene tubes and frozen at -20^{o} C until assayed. An additional 10 mL EDTA coated tube was used to harvest plasma which was stored at -80^{o} C until assayed for epinephrine. At the expected midpoint of the feeding period, blood was drawn into two 10 mL EDTA treated tubes (BD, Franklin Lakes, NJ) and lymphocytes were isolated by density gradient centrifugation for 96-hr cell culture at Texas A&M University, Kingsville. Feeding midpoint was chosen to allow for acclimation to confined feeding, commingling, and to reduce the effects of shipping stress.

Cortisol RIA. Serum cortisol (CS) concentrations were determined from duplicate samples using a single antibody RIA procedure adapted from Curley et al. (2004) using: anti-cortisol rabbit serum (Pantex, Div. of Bio-Analysis Inc., Santa Monica, CA, Cat. #P44) diluted 1:1500; standard curve solutions that were made by serial dilution (of 8000 pg/100 µL to 3.9 pg/100 µL) of 4-pregnen-11β,17,21-triol-3,20-dione (Steraloids, Inc., Newport, RI, Cat #Q3); ^{3}H radio-labeled hydrocortisone (1,2-^{3}H, New England Nuclear, Boston, MA, Cat. #NET-185). Unknown CS concentrations were analyzed using Assay

Zap software (Biosoft, Cambridge, UK). The inter-assay CV was 11% and the intra-assay CV was 8.5%.

Catecholamine EIA. Plasma epinephrine concentrations were determined from duplicate samples using an enzyme immunoassay (Bi-CAT EIA, Alpco, Windham, NH). Standard curve dilutions ranged from 256 to 0 ng/mL serial diluted 1:4 for epinephrine. The sensitivity for plasma derived epinephrine was 11 pg/mL.

Lymphocyte Isolation. Blood collected from 2, 10 mL EDTA tubes were pooled into a 50 ml conical tube and the volume was increased to 25 mL with Hank's Balanced Salt Solution (HBSS) without $Ca2^{+}$ or $Mg2^{+}$ (Hyclone Inc., Logan, UT). The blood/HBSS solution was layered onto 15 mL of Histopaque-1077 (Sigma-Aldrich, St. Louis, MO; specific density 1.077 g/mL) and centrifuged at 800 x g for 30 min. The buffy coat layer was removed and placed in a sterile 50 mL tube and diluted to 40 mL with HBSS. Cells were pelleted by centrifugation (400 x g for 15 min) and the supernatant was aspirated and discarded. To remove any red blood cell contamination, the pellet was washed with 5 mL of 0.2% NaCl solution and titrated for 1 min. Five mL of 1.6% NaCl solution was added to normalize osmolarity and HBSS was added up to 40 mL total volume. The tube was centrifuged (400 x g for 10 min) to pellet the cells and the supernatant was aspirated. The pellet was re-suspended in 1 mL of media that consisted of: DMEM F-12 HAM medium (Sigma-Aldrich, St. Louis, MO) with 15mM HEPES, pyridoxine and $NaHCO_3$, 5% horse serum (Sigma-Aldrich, St. Louis, MO) that was heat inactivated at 56° for 1 hr and sterile-filtered (via 0.45 micron filter), 1 % Penicillin-Streptomycin, 1% L-Glutamine (Life Technologies, Grand Island, NY), 0.007% β-mercaptoethanol (BioRad Laboratories, Hercules, CA). Media was sterile-filtered

through a 0.22 micron filter. Suspended cells were kept on ice until concentrations could be determined and then plated into culture wells.

Cell Counts. Isolated cells were counted using a Coulter particle counter (Coulter Corporation, Miami, FL). Twenty µL of cell suspension was added to 19.6 mL of Isoton II diluent and 0.38 ml Zap-OGlobin II Lytic reagent (Coulter Corporation, Miami, FL). Each sample was read three times and a mean was calculated to determine cell concentration. The 1 mL suspension was diluted with culture media to yield a concentration of $2x10^6$ cells/mL. Cells were kept on ice until pipetted onto culture plates.

Cell Culture. Cell cultures were performed at the midpoint of the steers feeding period. Cultures were carried out on two (one for IgM and one for proliferation) 96 well plates allowing for: 4 animals on each plate, 8 rows of treatments (including zero control) and 3 repetitions of each treatment. Treatments consisted of the blastogenic mitogen Concanavlin (ConA, lot# 033K8936, Sigma-Aldrich, St. Louis, MO) beginning at 5 µg/mL, and serially diluted with culture media 1:1 to 0.08 µg/mL. Fifty µL of the treatments and cell suspension were added to the wells to yield a total volume of 100 µL and a final concentration of $1x10^5$ cells/well. Incubation conditions were kept at 37^o C, 5% CO_2, and 50% relative humidity for 96 hr.

Proliferation and IgM Analysis. ConA (Sigma-Aldrich Co. St. Louis, MO) induced lymphocyte proliferation was measured by the CellTiter 96 cell proliferation assay (lot 191851, Promega, Madison, WI) after 96 hrs of incubation. IgM plates were frozen (-80^o C) until assayed. ConA induced lymphocyte IgM production was measured by a bovine IgM ELISA quantication kit (Bethyl Inc., Montgomery, TX). A checkerboard ELISA was used to determine sample dilution (1:1). Proliferation and IgM culture plates

were read using a microplate spectrophotometer (Biotek Instruments, Inc., Winooski, VT) at 405 nm.

Statistical Analysis. The GLM procedure LSM of SAS (SAS, 1985) was used to analyze temperament measurements, ADG, *in vitro* proliferation and IgM productions, and ED_{50} calculations. The mixed procedure of SAS was used to analyze EV and hormone profiles. All correlations were analyzed by the CORR procedure of SAS.

Trial 2 Materials and Methods

Animals. Steers used in this trial were fall born, Angus-sired steers (n=49) that were weaned at approximately seven to nine months of age at Brown Loam Experiment Station in Raymond, MS. One month post-weaning, these steers (weighing 275.28 ± 12.99 kg) were shipped approximately 1,050 km to the King Ranch South feedyard for finishing. Steers were fed to a fat thickness of 1.1 cm as determined by the feedyard manager.

Temperament Assessment. Exit velocity, CHUTE and PS were taken prior to shipment to sort cattle into C and T treatment groups (calm n= 5, temperamental n = 5). EV was measured by electronically timing an animal's speed over a given distance (1.83 m) after exiting from restraint in a squeeze chute (Burrow et al., 1988). This measurement was taken at four time points: (1) pre-shipment to feedyard, (2) post-shipment and (3) mid-feeding. Chute scoring (Grandin, 1993) was conducted by observing the calf unrestrained in the chute. Scoring was based on a 1 to 5 scale, with 1 representing a very calm animal, to a 5 representing and extremely violent animal. Pen scoring (Hammond et al., 1996) is a subjective measure which describes an animal's calmness or aggressiveness in the presence of a human in the pen with them. A score of 1

would designate a steer that was calm and slow walking upon approach by the scorer and a 5 would be a steer running into fences and aggressively charging the scorer. All measurements were conducted by the same scorer to eliminate variation due to technician differences. Chute scores and pen scores were only conducted at pre-shipment in order to assign calves to a temperament group. Animals (n=10) were selected based on these procedures from the larger group of 49; 5 with the best temperament, and 5 with the worst temperament to maximize extremes in order to determine differences in adrenal and immune function between the two groups.

Blood Collection. In addition to the three time points previously described, blood was collected at feeding finish to determine systemic cortisol and epinephrine concentrations. Blood was collected via caudal veni-puncture into a 10 mL additive free tube and allowed to coagulate before being spun at 1984 x g, 4^{o} C for 30 min. Serum for cortisol was harvested and aliquoted into 12x75 mm, polypropylene tubes and frozen at -20^{o} C until assayed. An additional 10 mL EDTA coated tube was used to harvest plasma and stored at -80^{o} C until assayed for epinephrine. At the expected midpoint of the feeding period, blood was drawn into two 10 mL EDTA treated tubes and lymphocytes were isolated by density gradient centrifugation for 96-hr cell culture. Feeding midpoint was chosen to allow for acclimation to confined feeding, commingling, and to reduce the effects of shipping stress.

Cortisol RIA. Serum cortisol concentrations were determined from duplicate samples using a single antibody RIA procedure using: anti-cortisol rabbit serum diluted 1:1500; standard curve solutions that were made by serial dilution (of 8000 pg/100 μL to 3.9 pg/100 μL) of 4-pregnen-11β,17,21-triol-3,20-dione; ^{3}H radio-labeled hydrocortisone.

Unknown CS concentrations were analyzed using Assay Zap software. The inter-assay CV was 11% and the intra-assay CV was 8.5%.

Catecholamine EIA. Plasma epinephrine concentrations were determined from duplicate samples using an enzyme immunoassay. Standard curve dilutions ranged from 256 to 0 ng/mL serial diluted 1:4 for epinephrine. The sensitivity for plasma derived epinephrine is 11 pg/mL.

Lymphocyte Isolation. Blood collected from 2, 10 mL EDTA tubes were pooled into a 50 ml conical tube and the volume was increased to 25 mL with HBSS without $Ca2^{+}$ or $Mg2^{+}$. The blood/HBSS solution was layered onto 15 mL of Histopaque-1077 and centrifuged at 800 x g for 30 min. The buffy coat layer was removed and placed in a sterile 50 mL tube and diluted to 40 mL with HBSS. Cells were pelleted by centrifugation (400 x g for 15 min) and the supernatant was aspirated and discarded. To remove any red blood cell contamination, the pellet was washed with 5 mL of 0.2% NaCl solution and titrated for 1 min. Five mL of 1.6% NaCl solution was added to normalize osmolarity and HBSS was added up to 40 mL total volume. The tube was centrifuged (400 x g for 10 min) to pellet the cells and the supernatant was aspirated. The pellet was resuspended in 1 mL of media that consisted of: DMEM F-12 HAM medium with 15mM HEPES, pyridoxine and $NaHCO_3$, 5% horse serum that was heat inactivated at 56° for 1 hr and sterile-filtered (via a 0.45 micron filter), 1 % Penicillin-Streptomycin, 1% L-Glutamine, 0.007% β-mercaptoethanol. Media was sterile-filtered through a 0.22 micron filter. Suspended cells were kept on ice until concentrations could be determined.

Cell Counts. Isolated cells were counted using a Coulter particle counter. Twenty μL of cell suspension was added to 19.6 mL of Isoton II diluent and 0.38 ml Zap-

OGlobin II Lytic reagent. Each sample was read three times and a mean was calculated to determine cell concentration. The 1 mL suspension was diluted with culture media to yield a concentration of $2x10^6$ cells/mL. Cells were kept on ice until pipetted onto culture plates.

Cell Culture. Cell cultures were performed at the midpoint of the steers feeding period. Cultures were carried out on two (one for IgM and one for proliferation) 96 well plates allowing for: 4 animals on each plate, 8 rows of treatments (including zero control) and 3 repetitions of each treatment. Treatments consisted of the blastogenic mitogen Concanavlin A beginning at 10 μg/mL, and serially diluted with culture media 1:1 to 0.16 μg/mL. Fifty μL of the treatments and cell suspension were added to the wells to yield a total volume of 100 μL and a final concentration of $1x10^5$ cells/well. Incubation conditions were kept at 37^o C, 5% CO_2, and 50% relative humidity for 96 hr.

Proliferation and IgM Analysis. ConA induced lymphocyte proliferation was measured by the CellTiter 96 cell proliferation assay after 96 hr of incubation. IgM plates were frozen (-80^o C) until assayed. ConA induced lymphocyte IgM production was measured by a bovine IgM ELISA quantication kit. A checkerboard ELISA was used to determine sample dilution (1:1). Both proliferation and IgM Plates were read using a microplate spectrophotometer at 405 nm.

Statistical Analysis. The GLM procedure LSM of SAS was used to analyze temperament measurements, ADG, *In vitro* proliferation and IgM productions, and ED_{50} calculations. The mixed procedure of SAS was used to analyze EV and hormone profiles. All correlations were analyzed by the CORR procedure of SAS.

Trial 1 Results and Discussion

Temperament and Growth. Prior to shipment to the feedyard, PS, CHUTE and EV were taken to assign steers into temperament groups (n = 12). CHUTE did not differ (P = 0.78) between treatment groups. However, PS differed ($p < 0.01$) with calm (C) steers scoring an average of 1.14 ± 0.14 vs. 3.2 ± 0.20 for temperamental (T) steers. The EV model (Figure 11) tended to be different (P = 0.06) across the three evaluation points. There was a significant treatment effect ($P < 0.01$) of temperament with the C steers averaging 1.45 ± 0.14 m/s and 2.34 ± 0.16 m/s for the T steers. There was not a significant interaction (P = 0.35) between treatment group and time meaning that differences observed between the treatment groups remained similar across the evaluation period. Pre-shipment and post-shipment velocities were significantly different ($P < 0.01$), although midpoint velocities were not significantly different (P = 0.27) due to large standard errors (SE) observed at this time. Temperamental steers showed a downward trend across the three evaluation times. Calm steers EVs decreased at post-shipment, but showed a slight increase (0.35 ± 0.22 m/s) at feeding midpoint. The overall decrease could be attributed to acclimation to handling and the absence of shipping stress.

Figure 12 shows the serum CS concentrations over the evaluation period. The effect of treatment group (P = 0.13), time (P = 0.61) and their interaction (P = 0.21) were not significant. Calm and Temperamental steers averaged 10.23 ± 1.52 ng/mL and 15.35 ± 2.01 ng/mL respectively over the evaluation period.

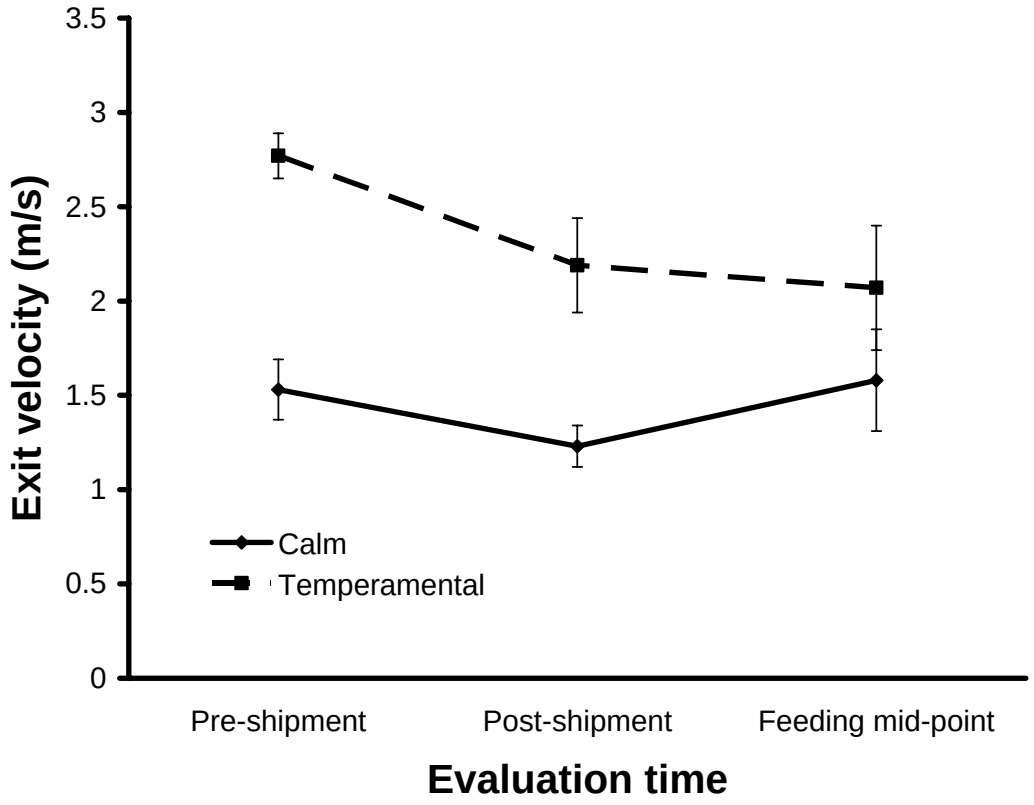

Figure 11.
Least-squares means for exit velocity in trial 1, for calm (n = 7) and temperamental (n = 5) steers at three measurement points. (Interaction Pr > F = 0.35)

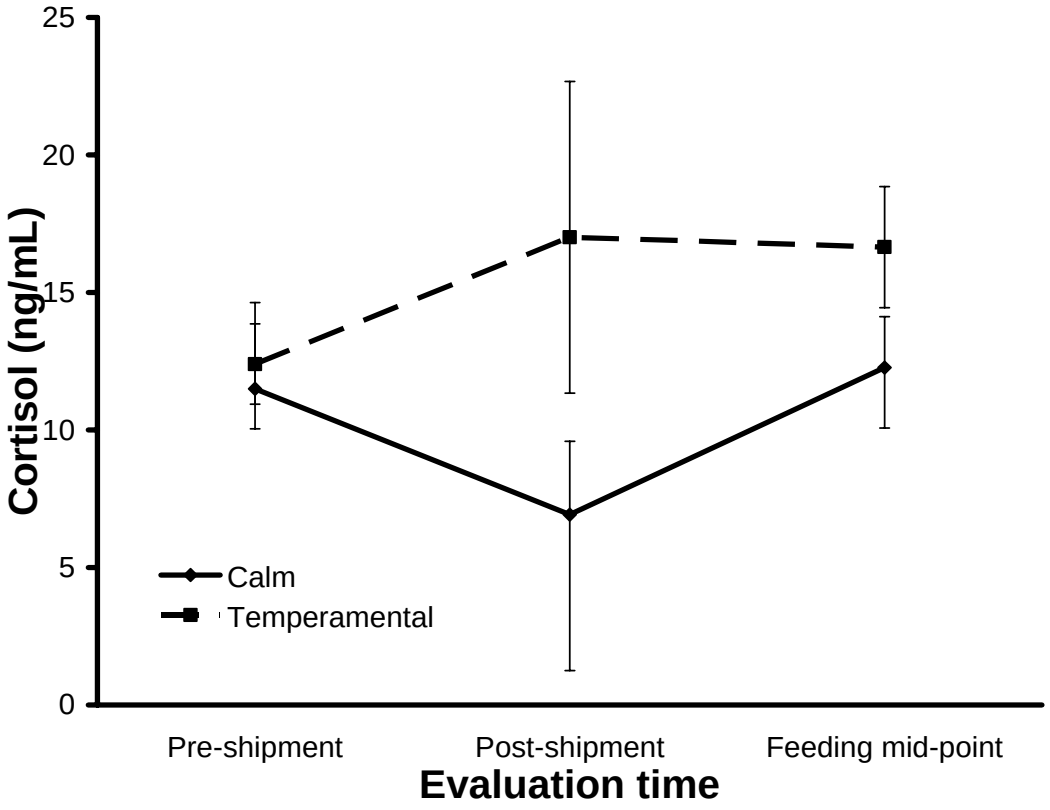

Figure 12.
Least-squares means for cortisol concentrations in trial 1, for calm (n = 7) and temperamental (n = 5) steers at three measurement points. (Interaction Pr > F = 0.21)

ADG did not significantly differ (P = 0.25) between treatment groups with C steers gaining 1.98 ± 0.07 kg/day compared with 1.81 ± 0.13 kg/day for T steers. The difference is similar to findings that calm cattle have higher ADG than temperamental cattle (Fordyce and Goddard, 1984; Burrow and Dillon, 1997; Voisinet et al., 1997a). However, a significant difference was not detectable due to the low number of subjects used in this trial. Table 5 presents the Pearson correlation coefficients of temperament, CS and ADG. PS was highly correlated with pre EV (r = 0.83, P < 0.01) and post EV (r = 0.85, P < 0.01), and somewhat correlated to midpoint EV (p = 0.46, P 0.13). Post-shipment CS concentrations showed high correlations to post EV (r = 0.68, P = 0.01) and midpoint EV (r = 0.77, P = < 0.01). Other observations of temperament were less revealing as to their relationships with CS and temperament measures. ADG correlations were not significant with temperament measures and CS (not shown), but did show negative trends showing that ADG was negatively influenced by temperament and CS.

Plasma epinephrine (Epi) concentrations (Figure 13) were significant (P < 0.01) within the model. However, the effect of treatment group (P = 0.17) and the interaction of treatment group and time (P = 0.19) were not significant. The effect of time tended to be significant (P < 0.10) showing changes in adrenal medullary responsiveness at different evaluation points. At post-shipment, there was a significant increase (P < 0.05) in Epi concentrations in T steers (1020.02 ± 407.51 pg/mL). Calm steers showed a slight physiological, but statistically insignificant increase (370.97 ± 407.51 pg/mL, P = 0.39). The large SE in addition to the low degrees of freedom attribute to the wide range of values observed.

Table 5.
Pearson correlation coefficients (and associated P values) for temperament measures, cortisol and average daily gain in trial 1. (n = 12)

			CS			
	PS	CHUTE	Pre	Post	Midpoint	ADG
PS	-	0.12	-0.03	0.51	0.54	-0.25
P = |r|	-	0.70	0.93	0.09	0.07	0.43
CHUTE	-	-	-0.18	-0.27	0.13	-0.14
	-	-	0.57	0.40	0.68	0.66
Pre exit velocity	0.83	0.25	-0.19	0.34	0.37	-0.29
	<.001	0.43	0.55	0.28	0.23	0.36
Post exit velocity	0.85	-0.07	0.05	0.68	0.57	-0.33
	<.001	0.83	0.89	0.01	0.05	0.30
Midpoint exit velocity	0.46	-0.09	0.56	0.77	0.29	-0.25
	0.13	0.79	0.06	0.00	0.37	0.43

PS = Pen score, CHUTE = Chute score, Pre = Pre-shipment to feed yard
Post = Post-shipment to feed yard, Midpoint = Feeding midpoint, CS = Cortisol
ADG = Average daily gain

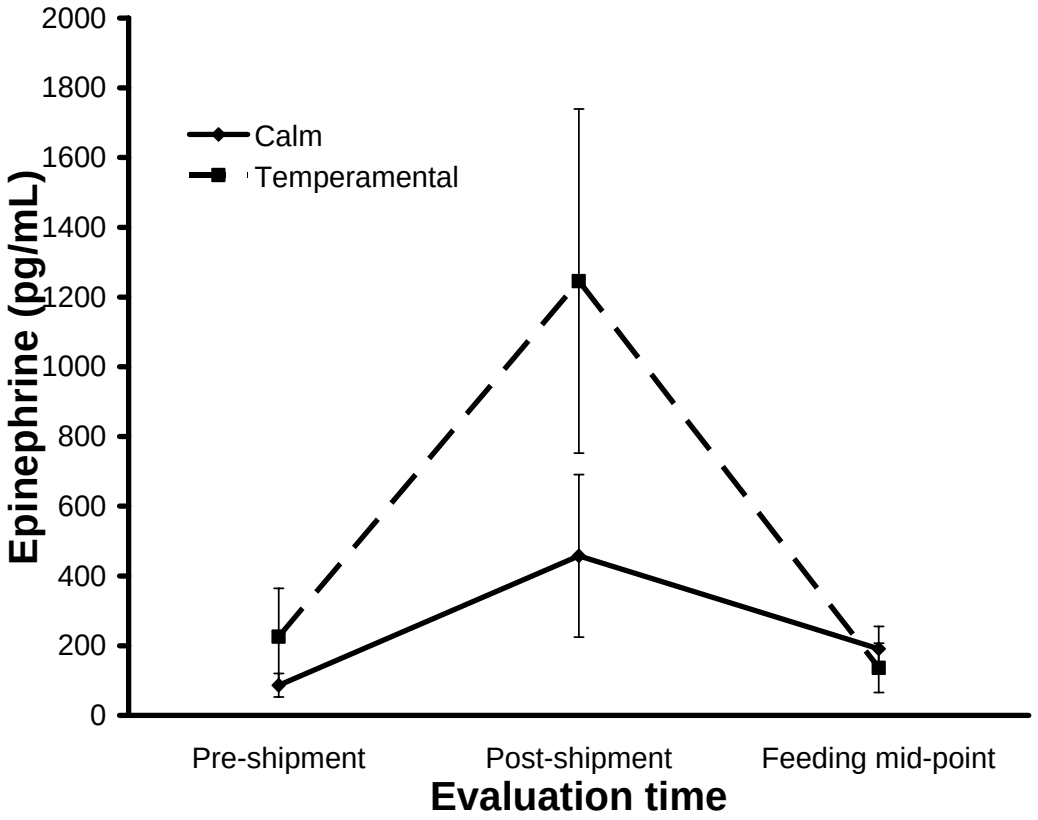

Figure 13.
Least-squares means for plasma epinephrine concentrations in trial 1, for calm and temperamental steers at three measurement points. (Calm n = 5, temperamental n = 5; Interaction Pr > F = 0.19)

In Vitro Lymphocyte Proliferation. The *in vitro* lymphocyte culture conducted at the feeding midpoint (Figure 14) in trial 1 produced a ConA dose dependent increase ($P < 0.01$) in blastogenesis in both C and T steers. There was a significant treatment effect ($P < 0.01$) with C steers averaging a SI of 1.96 ± 0.10 and T steers 2.52 ± 0.21 across all concentrations of ConA. As expected, the highest stimulatory effects were seen at 5 μg/mL of ConA (SI of 4.27 ± 0.31 and 5.46 ± 0.53 for C and T steers respectively). There was a significant interaction ($P < 0.01$) of treatment group and ConA showing that the differences between groups differed among increasing ConA concentrations. Significant lymphocyte proliferation was not seen until the 0.63 ng/mL concentration of ConA. Subsequent increasing doses of ConA showed a significant ($P < 0.01$) increase in proliferation. Temperamental steers had 1.1, 1.34 and 1.19 fold increases in proliferation at 1.25, 2.5 and 5 μg/mL of ConA, respectively. Serum concentrations of CS at the time of lymphocyte harvest and culture were not different ($P = 0.16$). The C steers averaged 12.27 ± 1.86 ng/mL when the T steers averaged 16.65 ± 2.20 ng/mL. It does not seem that the CS or Epi concentrations (C = 191.02 ± 67.59 pg/mL, T = 136.25 ± 67.59 pg/mL) affected the proliferative response. Contrary to our prior hypothesis, T steers proliferated significantly greater than C steers in this trial. Fell et al. (1999) reported no differences in ConA proliferative responses in post-shipment feedlot steers sorted by temperament, but no studies have been published showing increased proliferation in temperamental cattle.

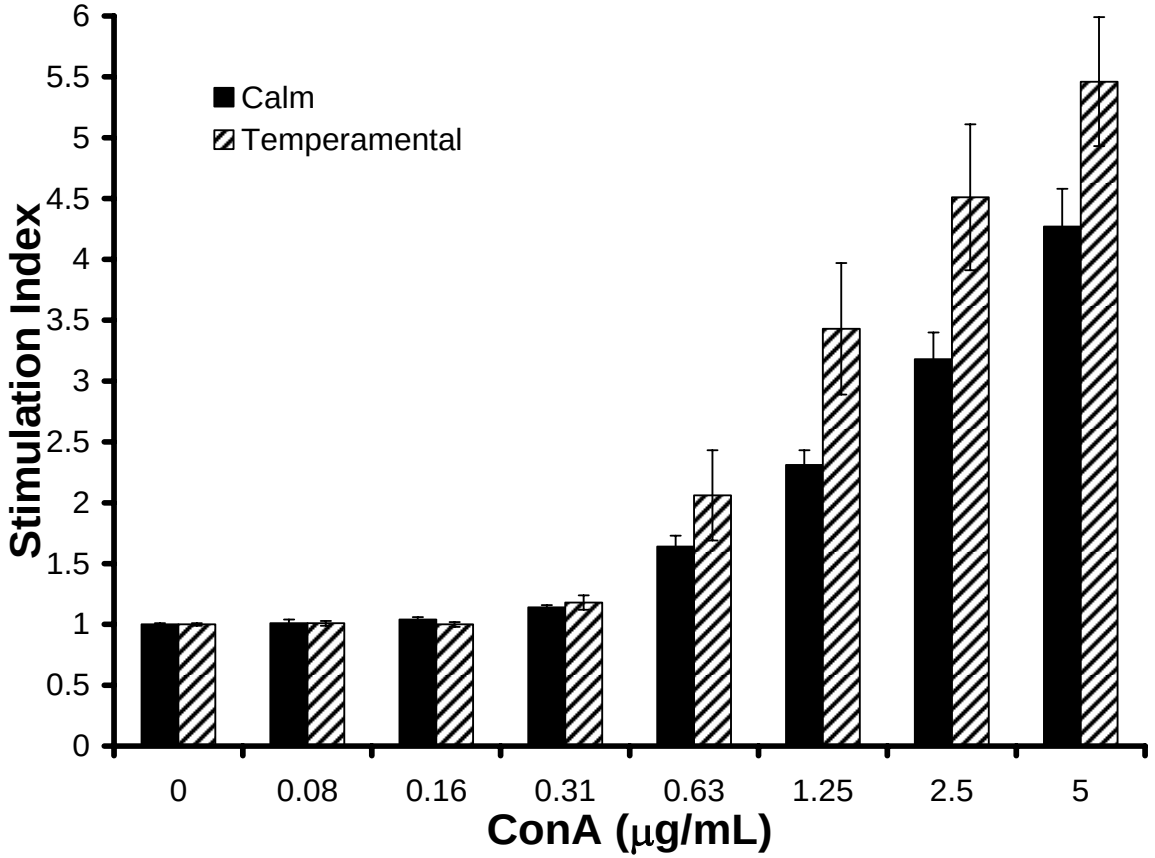

Figure 14.
Feeding midpoint, *in vitro* lymphocyte proliferation in trial 1, for calm (n = 7) and temperamental (n = 5) steers with ConA as the stimulatory mitogen. (Interaction Pr > F = 0.007)

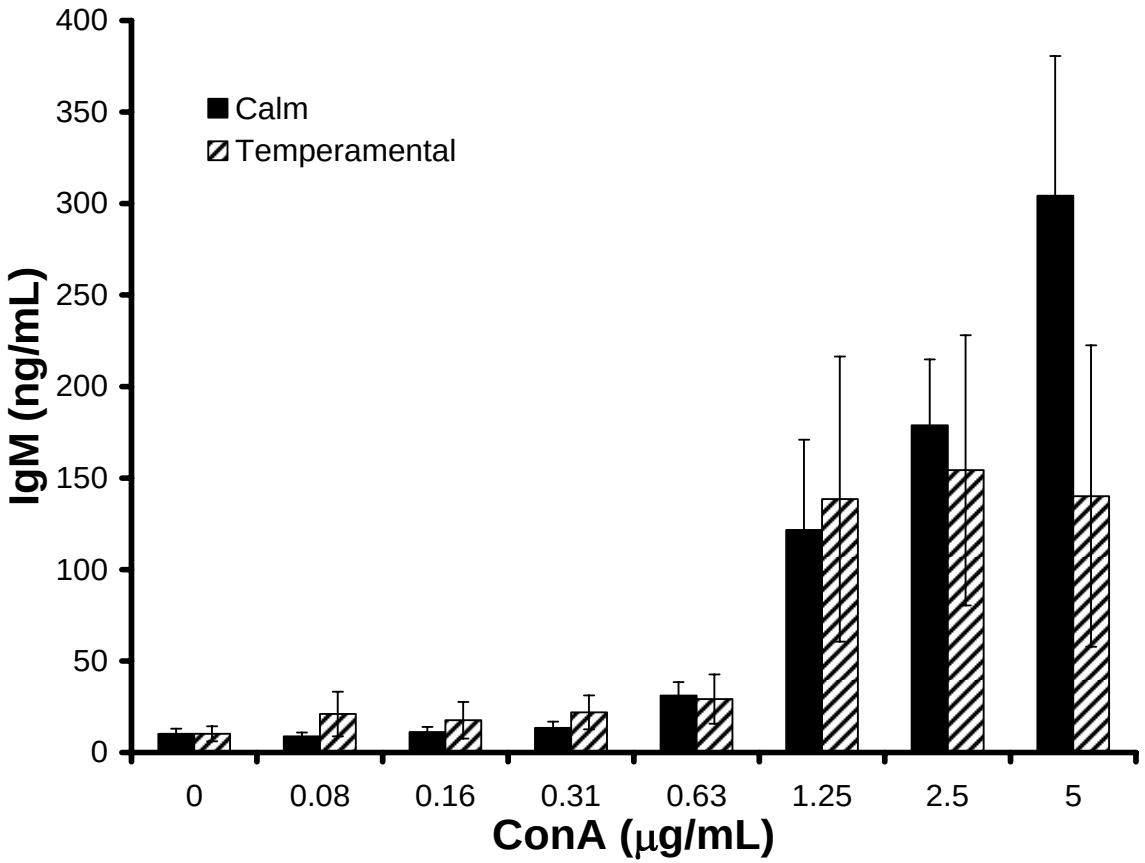

Figure 15.
Feeding midpoint, *in vitro* lymphocyte IgM production in trial 1, for calm (n = 5) and temperamental (n = 2) steers with ConA as the stimulatory mitogen. (Interaction Pr > F = 0.57)

Analysis of the ED_{50} value for the proliferation curve was not significantly different ($P = 0.97$) with C steers averaging 1.61 ± 0.24 ng/mL of ConA and 1.62 ± 0.36 ng/mL for T steers. Pearson correlation coefficients were calculated for ED_{50} with pre-shipment EV, pre-shipment CS and midpoint CS. None of the values showed strong relationships again probably due to the low number of observations used in the analysis.

In Vitro Lymphocyte IgM Production. The *in vitro* lymphocyte culture conducted at the feeding midpoint (Figure 15) in trial 1, produced a ConA dose-dependent increase in ($P < 0.01$) lymphocyte IgM production for both C and T steers. The effect of treatment ($P = 0.42$) and the interaction of treatment and ConA ($P = 0.57$) were not significant with C steers averaging 85.58 ± 15.31 ng/mL and 68.39 ± 18.63 ng/mL for T steers across all concentrations of ConA. The highest IgM production was seen at 5µg/mL of ConA in the C steers, and at 2.5 ng/mL in T steers. Large SE were observed due to technical difficulties in the assay, leading to the low numbers of subjects (calm n = 5, temperamental n = 2) used in the analysis.

Trial 2 Results and Discussion

Temperament and Growth. Prior to shipment to the feedyard, PS, CHUTE and EV were taken to assign steers into temperament groups (n = 10). CHUTE did not differ (P = 0.26) between treatment groups. However, PS differed (P < 0.01) with C steers scoring an average of 1.40 ± 0.25 and 3.8 ± 0.58 for T steers. The EV model was not significantly different (P = 0.438) across the three evaluation points (Figure 16). However, there was a significant temperament effect (P < 0.01) with C steers averaging 2.07 ± 0.20 m/s and 3.28 ± 0.21 m/s for T steers. There was a significant treatment by time interaction (P < 0.05) noting that the large differences (C = 1.65 ± 0.19 m/s and T = 4.01 ± 0.32 m/s) seen at the pre-shipment evaluation (basis for sorting for culture) were significantly reduced at subsequent observations. T steers showed a decrease (P < 0.01) from pre-shipment to feeding midpoint, while C steers remained statistically similar (P = 0.19) except for an expected slight increase (P = 0.18) at post shipment due to shipping stressors.

Figure 17 depicts the serum concentrations of CS over the evaluation period. The CS model was significantly different (P = 0.05) across the four evaluation points. There was a significant treatment (P < 0.05) and time (P < 0.05) effect with C steers averaging 10.32 ± 1.58 ng/mL and 18.70 ± 1.63 ng/mL for T steers. There was not a difference (P = 0.58) in the temperament by time interaction meaning treatment differences did not differ across all evaluation times. However, individual evaluations did differ at pre-shipment (P < 0.05) and at feeding midpoint (P < 0.05). Both groups had characteristic increases in circulating CS concentrations at post-shipment with C steers averaging an increase of 5.97 ± 2.66 ng/mL and 5.39 ± 2.66 ng/mL increase in T steers. Subsequent

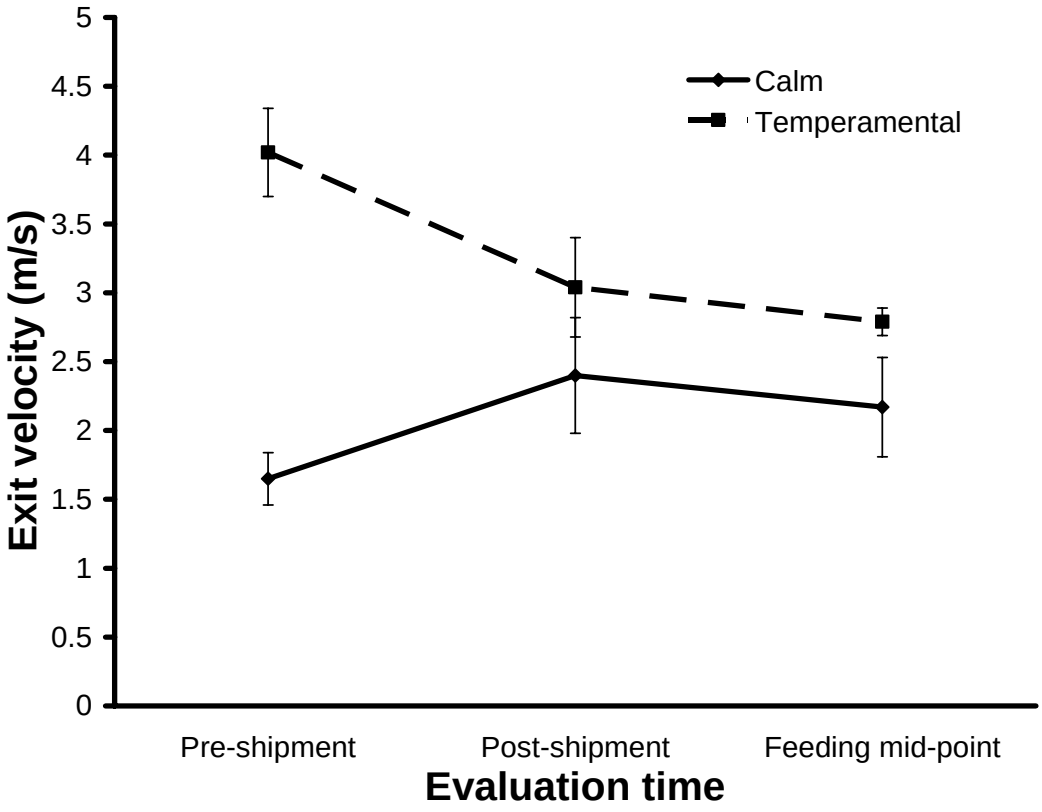

Figure 16.
Least-squares means for exit velocity in trial 2, for calm (n = 5) and temperamental steers (n = 5) at four measurement points. (Interaction Pr > F = 0.03)

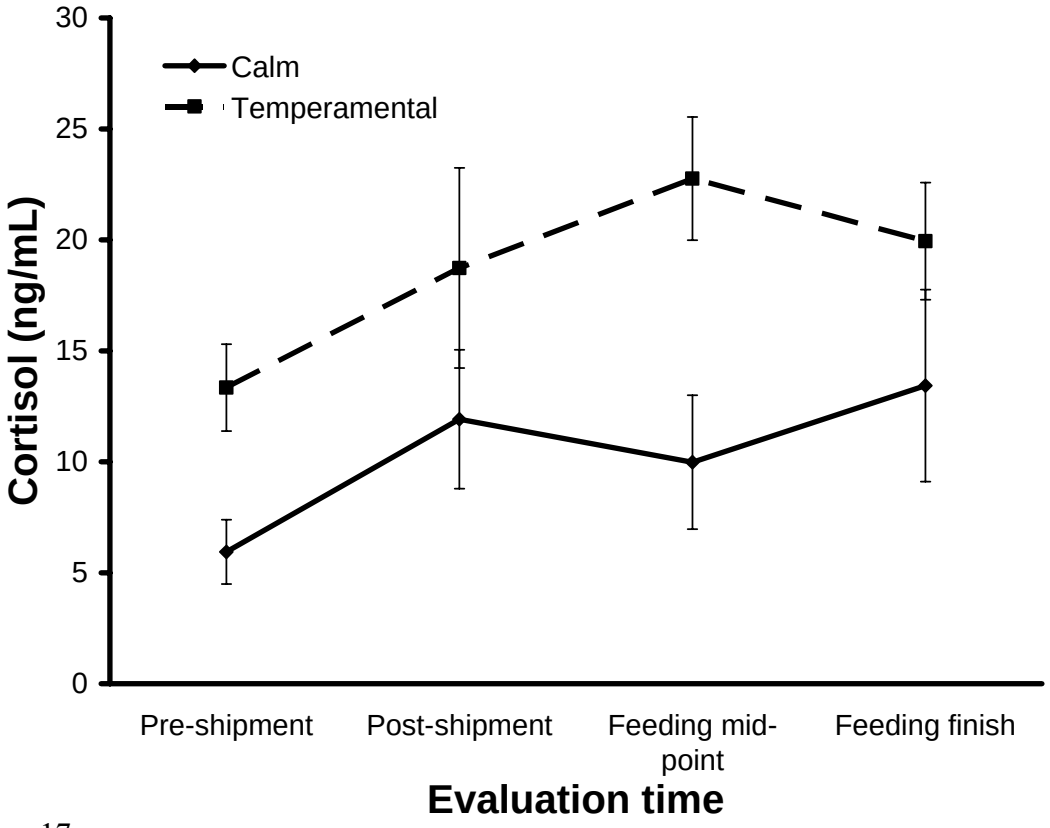

Figure 17.
Least-squares means for serum concentrations of cortisol in trial 2, for calm (n = 5) and temperamental (n = 5) steers at four measurement points. (Interaction Pr > F = 0.58)

observations plateaued for C and T steers alike. Overall, the C steers had less of a CS response than the T steers.

Average daily gain did not differ ($P = 0.68$) between treatment groups with C steers gaining 1.69 ± 0.11 kg/day and 1.63 ± 0.08 kg/day for T steers. Table 6 presents the Pearson correlation coefficients of temperament, CS and ADG. PS was highly correlated ($r = 0.72$, $P < 0.05$) to pre-shipment EV, but not to post-shipment or feeding midpoint EV ($r = 0.48$, $P = 0.16$; $r = 0.36$, $P = 0.30$ respectively). Chute score was correlated to pre-shipment CS ($r = 0.69$, $P < 0.05$) and tended to be correlated to post-shipment CS ($r = 0.60$, $P < 0.10$), but not to any measurement of EV. Pre-shipment EV was highly correlated to pre-shipment CS ($r = 0.72$, $P < 0.05$) and feeding midpoint CS ($r = 0.74$, $P < 0.05$) but not to any other measures. Post-shipment EV was only correlated to feeding finish CS ($r = 0.76$, $P < 0.05$), but not to any other measure of CS. Feeding midpoint EV was highly correlated to feeding midpoint CS ($r = 0.78$, $P < 0.01$) and tended to be correlated to post-shipment CS ($r = 0.58$, $P < 0.10$) and feeding finish CS ($r = 0.56$, $P < 0.10$). Average daily gain was not significantly correlated to any measure of temperament, which is consistent with the measured similarities of ADG throughout the feeding period. It is difficult to infer general trends in the data due to inconsistency of many of the variables measured in this trial.

Plasma concentrations of Epi for the four evaluation points is illustrated in Figure 18. The Epi model was significantly different ($P < 0.05$) across the four evaluation points.

There were significant effects of temperament group ($P < 0.01$) and time ($P < 0.05$) with C steers averaging 288.64 ± 92.55 pg/mL, whereas the T steers averaged 1305.92 ± 188.84 pg/mL. There tended to be a significant interaction ($P < 0.10$) between temperament and time with the T steers Epi concentrations approaching the C steers at feeding midpoint and feeding finish. The C steers Epi concentrations did not differ ($P = 0.25$) across the four evaluation times. Temperamental steers Epi concentrations increased by 871.78 ± 429.43 pg/mL from pre-shipment to post-shipment, but only showed a tendency ($P < 0.10$) to increase due to the large SE. Subsequent observations of the T steers showed a 486.90 ± 371.44 pg/mL decrease ($P = 0.23$) at feeding midpoint and a 887.54 ± 304.09 pg/mL decrease ($P < 0.05$) at the feeding endpoint. Cattle have been shown to become accustomed to routine handling (Hearnshaw et al., 1979). Therefore, acclimation to commingling and novel surroundings could explain these large decreases in plasma Epi concentrations.

Table 6.
Pearson correlation coefficients (and associated P values) for temperament measures, cortisol and average daily gain in trial 2. (n = 10)

			CS				
	PS	CHUTE	Pre	Post	Midpoint	Finish	ADG
PS	-	0.55	0.49	0.19	0.42	0.44	0.05
P =	-	0.10	0.15	0.59	0.23	0.21	0.89
CHUTE	-	-	0.69	0.60	0.33	0.12	0.00
	-	-	0.03	0.07	0.35	0.74	1.00
Pre exit velocity	0.72	0.39	0.72	0.32	0.74	0.46	0.18
	0.02	0.27	0.02	0.37	0.02	0.18	0.63
Post exit velocity	0.48	0.31	0.34	0.34	0.27	0.76	0.08
	0.16	0.38	0.34	0.34	0.46	0.01	0.83
Midpoint exit velocity	0.36	0.25	0.42	0.58	0.78	0.56	0.34
	0.30	0.48	0.22	0.08	0.01	0.09	0.34

PS = Pen score, CHUTE = Chute score, Pre = Pre-shipment to feed yard
Post = Post-shipment to feed yard, Midpoint = Feeding midpoint, CS = Cortisol
Finish = End of feeding period, ADG = Average daily gain

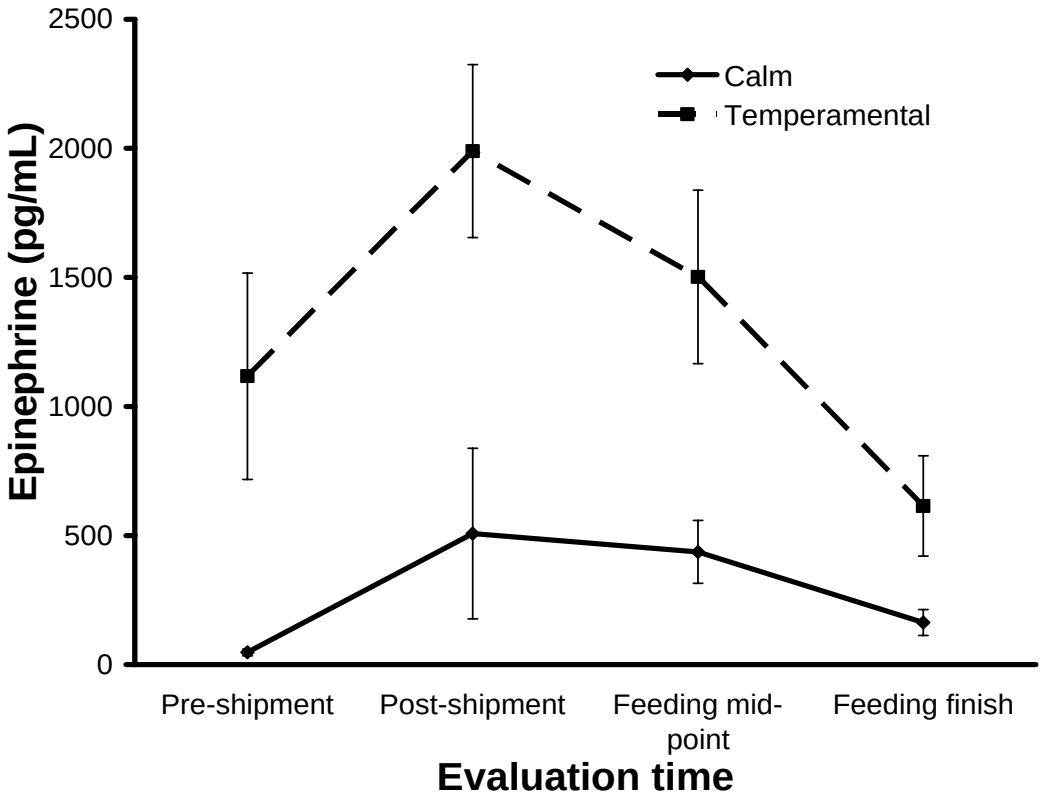

Figure 18.
Least-squares means for plasma epinephrine concentrations in trial 2, for calm (n = 5) and temperamental (n = 5) steers at four measurement points. (Interaction Pr > F = 0.08)

In Vitro Lymphocyte Proliferation. The *in vitro* lymphocyte culture conducted at the feeding midpoint (Figure 19) in trial 2, produced a ConA dose dependent increase ($P < 0.01$) in blastogenesis in both the C and T steers. There was a significant temperament effect ($P < 0.01$) with the C steers averaging a SI of 1.45 ± 0.05 and 1.60 ± 0.06 for the T steers across all concentrations of ConA. The highest stimulatory effects were seen 10μg/mL for the C steers (SI of 2.05 ± 0.07) and at 5 μg/mL (SI of 2.10 ± 0.13) for T steers. There was no significant interaction ($P = 0.18$) of treatment group and ConA showing that the differences between groups did not change at subsequent concentrations of ConA. Significant proliferation was not seen in the C steers ($P < 0.01$) until 1.25 μg/mL; however, the T lymphocytes significantly ($P < 0.05$) proliferated at 0.31 μg/mL of ConA. The sensitivity of the T steers lymphocytes could be in part to the high levels of catecholamines observed at time of culture. Catecholamines have been shown to activate different lymphocyte subsets and cytokines needed for proliferation in times of acute stress (Dhabhar, 2000). Cortisol was significant ($P < 0.01$) as a covariate in this model but its usefulness in explaining the differences seen in this culture is in question.

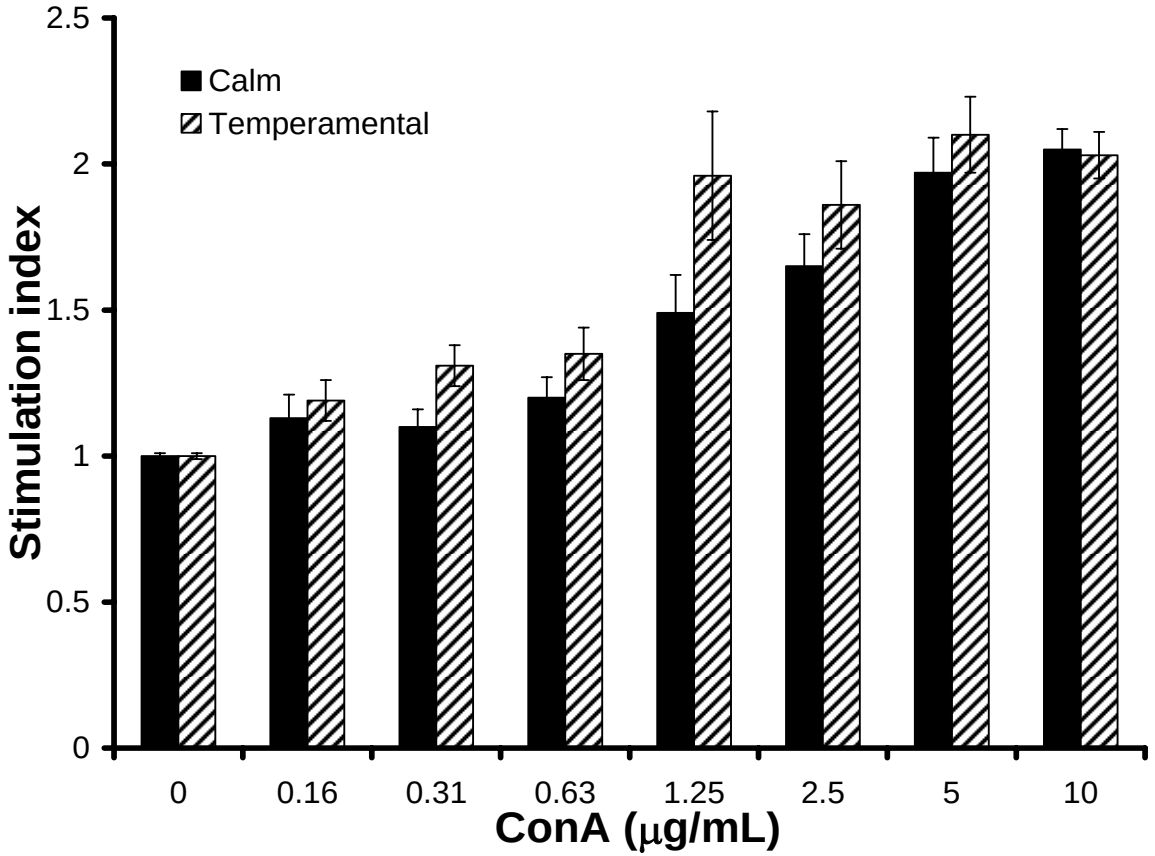

Figure 19.
Feeding midpoint, *in vitro* lymphocyte proliferation in trial 2, for calm (n = 5) and temperamental (n = 5) steers with ConA as the stimulatory mitogen. (Interaction Pr > F = 0.18)

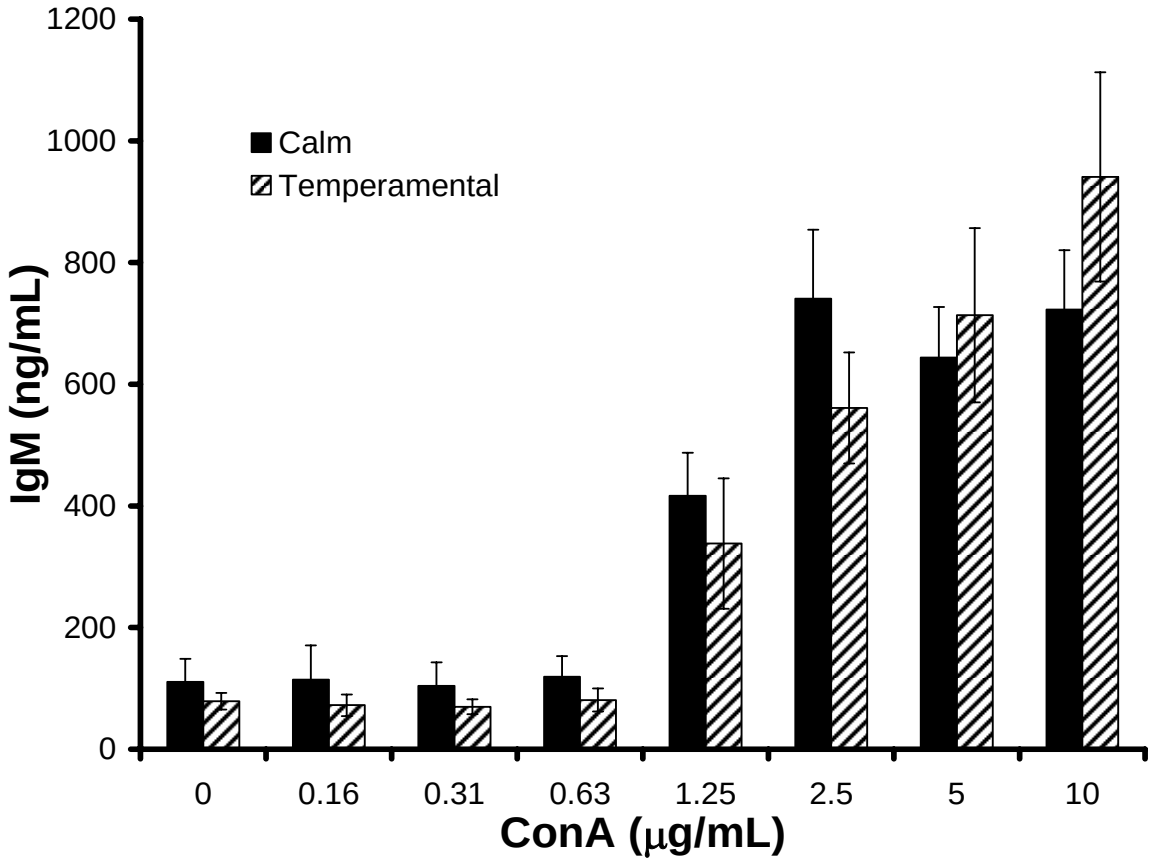

Figure 20.
Feeding midpoint, *in vitro* lymphocyte IgM production in trial 2, for calm (n = 5) and temperamental (n = 5) steers with ConA as the stimulatory mitogen. (Interaction Pr > F = 0.44).

Analysis of the ED_{50} for the proliferation curve was not significantly different (P = 0.50) with C steers averaging 2.41 ± 0.79 ng/mL of ConA and 1.68 ± 0.67 ng/mL for T steers. Pearson correlation coefficients were conducted for ED_{50} with pre-shipment EV, CS and feeding midpoint. None of these values showed strong relationships possibly due to the low number of observations used in the analysis.

In Vitro Lymphocyte IgM Production. The *In vitro* lymphocyte culture conducted at the feeding midpoint (Figure 20) in trial 2, produced a ConA dose dependent increase (P < 0.01) in lymphocyte IgM production for both C and T steers. The effect of treatment (P = 0.72) and the interaction of treatment and ConA (P = 0.44) was not significant with C steers averaging 367.62 ± 35.11 ng/mL and 351.79 ± 43.85 ng/mL of IgM for T steers across all concentrations of ConA. The highest IgM production was seen at 10 µg/mL of ConA in the C steers, and at 5 µg/mL in T steers. Significant increases (P < 0.01) in IgM production were not seen until 1.25 µg/mL of ConA in both C and T steers. C steers showed a significant increase (P < 0.01) in IgM from 1.25 to 2.5 µg/mL of ConA. C steers increased 323.29 ng/mL in the period and then plateaued. Conversely, T steers increased by 602.41 ng of IgM stepwise between 1.25 and 10 µg/mL of ConA. However, due to large SEs observed at higher concentrations of ConA, treatment differences only tended to be different (P = 0.06).

GENERAL CONCLUSIONS AND IMPLICATIONS

There are observed behavioral differences in cattle that can be characterized by measures of temperament (exit velocity, chute and pen score). Temperament differences identified at weaning were still evident through different commercial cattle production phases. Differences in stress responsiveness, and their effects on physiological systems, can be ascertained by these measurements of temperament. While the scope of the effects of stress responsiveness can not be fully characterized by examining hormone concentrations or *in vitro* effects alone, they do give helpful insight into the complexities of their effects on body systems. Animals identified as temperamental were generally associated with higher serum cortisol and epinephrine concentrations. Increased serum cortisol concentrations were also associated with lower average daily gains. During sub-clinical infections, immune system activation can lead to inflammatory conditions (mediated by cytokines and acute phase proteins) that affect appetite and nutrient utilization. Cortisol plays a complex role in containing immune responses and has been implicated in immunosuppression. These effects of stress responsiveness can decrease efficiency of gain and carcass quality leading to profit loss.

Weaning vaccinations are critical in conferring immunity to calves at times in their production when they are more susceptible to disease. Calm cattle had a higher Clostridial antibody concentration at the end of the study than temperamental cattle. The effects of glucocorticoids on antibody population maintenance are beginning to be studied, but this research substantiates previous research in human medicine that stress events can alter antibody numbers over time. In an integrated immune response, the long term maintenance of serum antibody levels is dependent on the survival of a memory B

lymphocyte pool and germinal center follicle production of antigen specific plasma cells. These memory lymphocytes, as well as immature thymocytes, can be induced into pre-programmed cell death cycle, apoptosis, by glucocorticoids. In addition, genes that protect against apoptosis, can be suppressed by glucocorticoids in immature B lymphocytes. These mechanisms are involved in sustaining a sufficient vaccination response, which is needed to confer immunity in cattle to common pathogens which are encountered in beef production.

If groups of cattle can be identified as having a higher risk of infection or altered immune responses, management practices can be altered to reduce risk associated with cattle morbidity and mortality. Additional research is needed to further investigate vaccination responses in cattle with different temperaments to more effectively manage weaning practices and pre-conditioning programs.

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