EVALUATION OF PERFORMANCE TRAITS IN BRAHMAN CATTLE: BLOOD PARAMETERS, CALF TEMPERAMENT, RESIDUAL FEED INTAKE, AND BULL REPRODUCTIVE DEVELOPMENT

A Thesis

by

KARA J. MATHENEY

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

August 2009

Major Subject: Physiology of Reproduction

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Approved by:

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ABSTRACT

Evaluation of Performance Traits in Brahman Cattle:

Blood Parameters, Calf Temperament, Residual Feed Intake,

and Bull Reproductive Development.

(August 2009)

Kara J. Matheney, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Ronald D. Randel

The objectives of these studies were (1) evaluate the relationship between temperament, blood parameters, and performance in Brahman calves (n = 300); (2) evaluate the relationship between residual feed intake (RFI) and reproductive development in Brahman bulls (n = 41). Serum was collected at 24 h and d 21 to 24, and analyzed for total protein (TP) immunoglobulin G (IgG), and cortisol (CS). Calves were weighed at 24 h, weighed and evaluated for temperament using exit velocity (EV) at d 21 to 24, and at 28 d intervals thereafter. Beginning 28 d prior to weaning, and at 28 d intervals through 56 d post-weaning calves were evaluated for pen score (PS) used to calculate temperament score (TS = (EV+PS)/2). The average TS from 28 d prior to weaning and weaning was used to generate temperament groups; calves 1 SD below the mean being calm, those 1 SD above the mean being temperamental and all remaining classified as intermediate. Calf TS influenced WW ($P = 0.04$) and ADG from birth to weaning ($P = 0.03$). Serum TP at 24 h affected ($P < 0.05$) WW and ADG from birth to

weaning. Serum IgG at 24 h affected ($P = 0.03$) WW. Brahman bulls (n = 41) were evaluated for RFI, insulin-like growth factor I (IGF-I), temperament, reproductive development, and ultrasound carcass traits. Serum was collected at d 0 and d 70 of the feeding trial and analyzed for IGF-I. Bulls were classified as efficient, intermediate, or inefficient (RFI classification method I) and as efficient or inefficient (RFI classification method II). Bulls were evaluated for temperament at weaning using TS. Temperament influenced ($P < 0.05$) IGF-I concentrations at d 0. Reproductive development was not affected ($P > 0.05$) by TS. Residual feed intake classification did not influence ($P > 0.05$) age at reproductive milestones. Ultrasound carcass traits were not affected by TS or RFI. Serum TP at 24 h was a viable indicator of future growth performance. Temperamental animals had lower growth rates in both studies. Reproductive development was not affected by RFI. BW at reproductive milestones was lower in temperamental bulls.

DEDICATION

In loving memory of my wonderful Pepaw, Shirley Lee "Matt" Matheney (1929-2007), you were the rock in my life and the anchor of our family. Your silence was strong, your heart was warm, and your wisdom was endless. You gave me the courage to reach for the stars, but the common sense to keep my feet planted firmly on the ground.

To my mom for all of her encouragement and support, no matter the obstacle you gave me the courage to overcome it.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Efficient production is a primary concern of the cattle industry. As the number of beef producers continues to fall and land available for production dwindles, efficient and productive cattle are of primary importance for sustained food production. Research related to growth and performance in *Bos indicus* (Brahman) cattle has been limited, as compared to *Bos taurus* breeds. The adaptive nature of Brahman cattle to hot, harsh environments, and the high heterosis for reproductive and maternal traits expressed by Brahman crossbred cows, account for the widespread use of the breed in cow-calf production (Riley et al., 2004). Evaluation of growth and performance of *Bos indicus* influenced cattle is an important aspect to be evaluated given the diverse cattle population and environments in which beef cattle are produced.

Calf morbidity and mortality result in significant losses to the beef cattle industry. Understanding the parameters surrounding pre-weaning performance of calves is essential for improving beef cattle performance. In the past decade the effect of calf temperament on health, and subsequent growth, has been the subject of several reviews (Fell et al., 1999; Mondal et al., 2006). Passive transfer of immunity is traditionally measured using circulating concentrations of immunoglobulins, but recent research suggests that plasma or serum total protein may be a reliable predictor of preweaning performance (Wittum and Perino, 1995). Determination of the role which certain blood

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

parameters and temperament play on calf growth and performance from birth to weaning, and post-weaning, is vital in developing management strategies to improve performance for all segments of the beef industry.

The largest expense associated with livestock production is feed, accounting for approximately 60-70% of total production costs (Sainz and Paulino, 2004). Improving production efficiency as it relates to feed consumption, and subsequent weight gain, is of primary concern to the beef industry. Identification of animals that are more efficient in utilization of available nutrients is an area of interest for beef producers, with the objective of reducing production costs related to feed expenses. Methods such as feed conversion ratio (FCR) have been the primary tools used to evaluate and describe efficiency of beef cattle. Selection of cattle based primarily on FCR leads to increased size at maturity (Arthur et al., 2001a; Herd and Bishop, 2000)due to the high correlations of FCR with growth rate and body size (Arthur et al., 2001a). An alternative method for evaluating efficiency, residual feed intake (RFI) was first proposed by Koch et al. (1963). Residual feed intake calculates the difference between feed consumed and expected feed intake, as predicted by body weight and growth rate of an animal. This allows RFI to be a selection tool that should not result in increased mature size of beef cattle (Arthur et al., 2001b; Nkrumah et al., 2004). Methods for evaluating feed efficiency and their application in *Bos indicus* cattle are areas in need of further research.

Furthermore, research pertaining to the relationship between RFI and reproductive development in bulls has been limited. Evaluating the well documented relationship between nutrition and reproduction in RFI tested pre-pubertal and pubertal bulls is of interest. It is important to understand how selection for efficiency based on RFI could potentially impact reproductive performance in bulls. Evaluation of growth and performance in *Bos indicus* influenced cattle is important in understanding overall performance and production of the beef cattle industry. As a result of these interests, the following studies were designed to evaluate:

1. the relationship between temperament and subsequent growth and performance in Brahman calves, pre- and post-weaning
2. the relationship between serum blood parameters including total protein (TP), cortisol (CS), and immunoglobulin G (IgG) with subsequent growth and performance in Brahman calves, pre- and post-weaning
3. and the relationship between RFI and reproductive development in Brahman bulls.

Passive Transfer of Immunity in the Calf

The bovine neonate is born hypogammaglobulinemic, lacking a fully functional immune system and rendering the calf susceptible to invading pathogens. Bovine fetuses have a epitheliochorial type of placentation, which does not allow passive transfer of immunoglobulins (Ig) from maternal to fetal circulation (Dewell et al., 2006). Studies conducted to evaluate the affects of passive immunity on calf survival and performance have focused primarily on dairy calves, due to the intensive management of dairy programs. The paramount importance of passive immunity on calf survival is important in both the diary and beef cattle industries, and failures in passive transfer of immunity contribute significantly to death and economic losses each year.

Passive transfer of immunity can be defined as the transfer of Ig from the dam to the calf through colostrum. Calf survival depends largely on the success of passive transfer of immunity during the initial h after birth, as absorptive ability decreases greatly within the first d of life (Weaver et al., 2000). Traditionally, the measure of passive transfer of immunity has been the evaluation of Ig concentrations at and around birth. The primary source of Ig is found in colostrum, provided by the dam. Evaluation of factors influencing and affecting passive transfer of immunity in the neonate offers further insight into potential management strategies to decrease incidences of calf morbidity or mortality.

Immunoglobulins. The term immunoglobulins is general and refers to a family of high molecular weight proteins that share common physio-chemical characteristics and antigenic determinants (Butler, 1969). Immunoglobulins are high molecular weight

glycoprotiens synthesized by cells of the reticuloendothelial system (Butler, 1974). According to Butler (1969) all immunoglobulins appear to be either monomers or polymers of a four-chain molecule. These molecules consist of two light polypeptide chains and two heavy polypeptide chains, with molecular weights ranging from 50,000 to 70,000 depending on classification. Several classes of Ig exist; however, the primary classes produced by cattle are IgG, IgG_1, IgG_2, IgM, and IgA. The predominant class present in bovine serum and lacteal secretions is IgG, which is comprised of the subclasses of IgG_1 and IgG_2. In cattle IgG_1 comprises 60% of total serum IgG, but makes up more than 95% of colostral IgG (Butler, 1969).

Total Protein. Protein present in plasma, or serum, is made up of albumin and globulin. The subclasses within the globulin classification include α1, α2, β, and γ globulins. Serum total protein concentration, as determined by refractometer, has been proposed as a method to estimate serum Ig concentration and passive transfer in the calf. In a study utilizing 185 calves evaluated for passive transfer of immunity, serum total protein concentration was correlated with serum Ig concentration ($r = 0.72$; McBeath et al., 1971).

Colostrogenesis. Colostrum is the first lacteal secretion produced by the mammary gland. Colostrum is comprised of high concentrations of Ig, fats, proteins, peptides, fat-soluble vitamins, minerals, various enzymes, hormones, growth factors, nucleotides, polyamines, and cytokines (Campana and Baumrucker, 1995). Colostrogenesis is also defined as the prepartum transfer of Ig from maternal circulation

into mammary secretions; which begins several weeks prior to parturition and ceases abruptly just prior to parturition (Brandon et al., 1971).

During late gestation colostrogenesis is regulated by changes in hormonal activity. Hormonal changes observed include: increased estrogen concentrations approximately 1 month prior to parturition, increased serum corticosteroids, growth hormone, and prolactin 1 week prior to parturition, and a significant decrease in serum progesterone 1 to 2 d prior to parturition (Tucker, 1985). Studies have demonstrated that the activity of estrogen, alone or in combination with progesterone, influences or initiates the activity of Ig receptors in mammary tissue (Hunter et al., 1970; Smith, 1971; Smith and Schanbacher, 1973). Progesterone concentrations begin to decrease 2 to 3 weeks prior to parturition and drop significantly to <1 ng/ml at 2.5 d prior to parturition, coinciding with the onset of colostrogenesis. While the mechanisms are still unclear, growth hormone appears to have a homeorhetic effect on the partitioning of blood flow to the mammary gland, increasing the transfer and availability of Ig for uptake (Barrington et al., 2000). Prolactin appears to modulate receptor expression on the glandular epithelial cells as its concentrations begin to rise at the onset of lactation (Barrington et al., 2000).

Colostrum Delivery. Several studies have been conducted to evaluate different methods of colostrum delivery and the effectiveness therein; however, these studies were inconclusive. One study reported higher Ig absorption by Holstein calves allowed to suckle over a 24 h period as compared to those fed with a bottle. One limiting factor to be considered is that the greatest amount fed by bottle was 4 L, suggesting the

differences observed may not have been strictly a result of method of colostrum delivery but total volume (Stott et al., 1979c). A later study reported that Holstein calves fed 5.45 L of colostrum in 3 bottle feedings during the first 12 h after birth had higher serum protein and Ig concentrations than did calves that remained with the dam for 24 h and were allowed to suckle (Nocek et al., 1984). Contradictory to the previous study, Jersey calves that suckled had higher serum Ig concentration when compared to calves which were bottle fed 2 L of colostrum within the first 12 h (Quigley et al., 1995). More important than the method of colostrum delivery is the volume, quality, and timing of colostrum consumption by the neonate.

Colostrum Quality-Breed of Dam. Successful passive transfer of immunity relies on several factors including method of delivery and volume as previously discussed, as well as quality of colostrum. Timing of lactogenesis, or the onset of copious milk secretion, may be important in determining colostral Ig concentration at calving. A study conducted using Holstein cows found a negative correlation between total volume and Ig concentration in colostrum (Pritchett et al., 1991). This data would suggest that the increase in milk volume produced by dairy cows, when compared to beef cows, may dilute colostral Ig concentrations. While dairy cows transport more Ig into secretion than do beef cows, total colostral Ig concentrations were lower; again suggesting that dilution effects are greater in magnitude for dairy cows as they approach parturition (Guy et al., 1994). When comparing dairy and beef breeds, volume produced and the subsequent dilution of colostral Ig may account for the breed differences observed.

Differences in a variety of production related traits have been observed between *Bos indicus* and *Bos taurus* beef breeds. Colostrum production is among these traits where differences have been observed. Vann et al., (1995) conducted a study to evaluate the effect of calf breed type on colostrum production, Ig concentration in colostrum and calf serum, and availability and absorption efficiency of Ig. Brahman (B) and Angus (A) cows were bred to Brahman and Angus sires to produce A x A, B x B, A x B, and B x A calves. Brahman cows produced more colostrum than did Angus cows ($P < 0.001$). Additional results from this study suggest that calf breed type influenced colostral Ig in cattle. Cows producing crossbred calves had higher Ig concentrations than did those producing purebred calves ($P < 0.001$). Serum Ig concentrations and efficiency of absorption were not effected by breed type or calf sex.

Presence of the Dam. Dairy cattle operations commonly utilize the practice of removing newborn calves from the dam. While this is not a common practice for beef cattle operations, the presence of the dam appears to have an affect on calf Ig absorption and subsequent serum Ig concentrations. Calves that were housed in close proximity to the dam had greater Ig absorption than calves housed separately from their dam (Selman et al., 1971). A comparison study, between calves left with the dam and calves removed from the dam immediately postpartum and fed by nipple bottle, found that calves left with the dam had consistently higher Ig absorption rates and higher serum Ig concentrations (Stott et al., 1979c). While all calves had adequate passive transfer with restricted feeding of 2 L of colostrum (Stott et al., 1979c), it is apparent that presence of the dam has an affect on Ig absorption in the neonate. Calves which remain with the

dam are still at risk for failure of passive transfer due to possible inadequate colostrum consumption, potential differences in colostral Ig concentration, and delayed suckling resulting in decreased Ig absorption.

Neonatal Immunoglobulin Absorption. A critical factor for colostral Ig absorption is the period of intestinal permeability to macromolecules. The ability of the neonatal ruminant gut to allow unrestricted passage of the large Ig molecules provides the young animal with passive immunization, via colostral Ig. These Ig are ingested by the calf and absorbed through the gastrointestinal enterocyte via pinocytosis. Both the concentration of colostral Ig and permeability of the gut decrease rapidly and progressively over the first 48 h following parturition (Bush and Staley, 1980; Moore et al., 2005; Vann et al., 1995). As the calf ages the gastrointestinal enterocyte becomes less capable of absorbing large proteins, including Ig. Timing of colostrum ingestion is crucial to the success of passive transfer of immunity in the calf, which subsequently plays a role in future performance.

Reports in the dairy industry suggest gut closure occurs at approximately 24 h postpartum but with delayed feeding can be extended to 36 h (Stott et al., 1979a). Due to the continued transfer of Ig across the enterocyte, peak Ig concentrations are not seen until approximately 32 h after birth (Stott et al., 1979a). Despite the length of time to gut closure, optimal Ig transfer occurs within the first 4 h following parturition and begins to rapidly decline 12 h postpartum (Bush et al., 1971; Matte et al., 1982; Stott et al., 1979b). Greater Ig concentrations were seen in calves fed earlier following parturition as compared to those fed later, given equal amounts and qualities of

colostrum (Stott et al., 1979b). While the calf is capable of producing endogenous Ig at a rate of 1 g IgG_1 per d (Devery et al., 1979), they are still unable to respond to certain antigens including lipopolysaccaride until 30 d of age (Osburn et al., 1982). Timely consumption of colostrum by the calf is vital to the absorption of Ig and acquisition of passive immunity, all of which affect mortality, morbidity, and overall performance.

Failure of Passive Transfer of Immunity. Failure of passive transfer (FPT) is a well accepted cause of neonatal mortality. Low serum Ig concentrations is indicative of FPT (McGuire et al., 1976). Long term effects of FPT have largely been over looked, (Weaver et al., 2000) encouraging further investigation. The effects of FPT related to mortality and morbidity within the initial d and weeks of life have been thoroughly studied. In a study utilizing dairy heifers those suffering from FPT had significantly lower mature equivalent milk production and had a greater tendency to be culled in the first lactation (DeNise et al., 1989). Beef calves with FPT had an increased risk of neonatal and preweaning mortality as well as preweaning morbidity when compared to calves with adequate passive transfer. Additionally, the calves with FTP had decreased performance in the feedlot and an increased risk of mortality and respiratory tract morbidity as compared to calves with adequate passive transfer (Wittum and Perino, 1995).

A multitude of factors can affect passive transfer of immunity in the suckling calf, including traumatic events during parturition, environmental conditions, and differences in calf vigor within the initial h of life among different breeds. Prolonged parturition can lead to the development of respiratory and metabolic acidosis in calves.

One study reported decreased serum Ig concentrations at 12 h after birth in calves with respiratory acidosis (Besser et al., 1990). In another study, hypoxic calves had delayed Ig absorption in the initial 18 h after birth but had appreciable Ig absorption after subsequent colostrum feedings (Tyler and Ramsey, 1991). The increase in FPT among calves with traumatic events during parturition can partially be explained by the decrease in calf vigor and not simply a lack of Ig absorptive capacity.

Extreme environmental conditions have been shown to influence colostral Ig absorption by the calf. Extreme cold stress of newborn calves under range conditions has been indicated as a potential cause in the delayed onset and decreased rates of absorption of colostral Ig (Olson et al., 1980). Research conducted by Blecha and Kelley (1981) indicated that cold stress at birth reduces gamma globulin concentrations in the serum of piglets. Furthermore, cold stress may also cause a reduction in colostrum consumption, impair absorptive capacity of Ig, or increase the catabolic rate of Ig upon entering the systemic circulation (Blecha and Kelley, 1981; Kelley et al., 1982).

FPT, Morbidity, and Mortality. Calves which do not achieve adequate passive transfer of immunity have greater risks related to preweaning morbidity and mortality. Several studies have been conducted to evaluate the relationship between FPT and incidences of morbidity during the neonatal and preweaning phases of the production cycle. A study conducted by Wittum and Perino (1995) utilized crossbred calves representing a variety of dairy and beef breeds to evaluate serum Ig concentration using a commercial radial immunodiffusion kit. The results from this study found that 25% of calves classified as having inadequate Ig concentration (< 800 mg/dL) at 24 h became

morbidity cases during the neonatal period. In the same study, only 5% of calves with adequate Ig concentrations (> 1600 mg/dL) were observed to be ill during the same period. In all cases of morbidity and mortality during the neonatal, preweaning, and weaning phases of production, both serum Ig and plasma protein concentrations were lower for cases of morbidity and mortality than noncases. In a similar study, calves classified as having FPT (serum Ig concentration ≤ 800 mg/dL) were 2.24 times more likely to have a preweaning morbidity event as compared to calves classified as having adequate passive transfer (serum Ig concentration > 1,600 mg/dL; Dewell et al., 2006). Additionally, calves in the same study classified as having FPT were 4.9 times more likely to die before weaning than calves classified as having adequate passive transfer (Dewell et al., 2006). In a study conducted by Braun and Tennant (1983) calves with serum total protein concentrations of < 5.0 g/dL were more likely to become sick than calves with higher concentrations of total protein. Similarly, Donovan et al., (1998) reported calf mortality within the initial 6 months of life was increased 3-to-6 times with total protein concentrations below 6.0 g/dL. These data emphasize the importance of passive transfer of immunity in the calf and the potential production losses associated with FPT.

Previous studies suggested that 5.5 g/dL of total serum solid concentration or Ig concentration of 1,500 mg/dL should be used as goals to assess adequate passive transfer (Boyd et al., 1974; Irwin, 1974). Further studies in dairy calves suggest that these goals are not consistently achievable, also showing no decrease in mortality for levels exceeding the recommended goals (Tyler et al., 1998). Research suggests monitoring

failure as a percent of calves <5.0 g/dL, rather than mean serum Ig or protein concentrations, would prove a better indicator when evaluating calves for passive immunity (Weaver et al., 2000). Tyler et al. (1998) reported that 77% of calves with complete FPT (<4.0 g/dL serum protein) would survive. Many factors influence the absorption of Ig and subsequent serum Ig concentration in the calf. Overall, low serum Ig or total serum solid concentrations do not translate into definitive incidences of morbidity or mortality. Similarly, adequate passive transfer as measured by serum Ig concentration does not ensure survival. These measures of passive transfer of immunity can be utilized by management when evaluating early-life calf survival and potential influences on future performance.

Calf Performance. Successful passive transfer of immunity early in life provides increased protection for the calf against infectious challenges. This passive transfer ultimately plays a role in overall calf performance, through the establishment of a functional and competent immune system. Several studies have reported significant correlations between serum Ig concentration and subsequent weight gains (Dewell et al., 2006; Odde, 1988; Robison et al., 1988). Results from the study conducted by Wittum and Perino (1995) indicate that the lowest calf weaning weights were observed among calves classified as having inadequate Ig or total protein concentrations 24 h after birth. However, the effect of passive transfer on weaning weight was indirect through its effect on neonatal morbidity. Similarly, this study found no direct effect of passive immune status at 24 h on feedlot growth rate. The association reported was an indirect relationship through the effect of total protein at 24 h on morbidity in the feedlot

(Wittum and Perino, 1995). Similar to the previous study, Dewell et al. (2006) found that serum Ig concentrations were not significantly associated with ADG from birth to weaning. Postweaning performance data from the same study found no significant effect of serum Ig concentrations on feedlot health or performance.

Banta et al. (2007) evaluated the utility of several blood parameters including serum protein and Ig at 24 h after birth to predict future performance of beef calves. Calves were classified based on blood parameters into high, medium, and low based on 1 standard deviation above or below the mean, respectively. Calves classified as having high concentrations of total protein were heavier at weaning than the medium group, which were heavier than the low group. Results from these studies suggest serum protein may be a potential indicator of future performance based on the blood parameters evaluated.

In an earlier study by Vann and Baker (2001) beef calves were evaluated for the effects of serum Ig concentration measured 24 h after birth, on growth and performance through weaning. Calves were classified as having superior Ig concentrations (>1600 mg IgG/100 ml), average Ig concentration (between 400 and 1600 mg IgG/100 ml), and inferior Ig concentration (below 400 mg Ig/100 ml). Calves classified as superior were heavier at all weigh d than the remaining calves classified as average or inadequate. Additionally, the superior calves were 14 kg heavier than the average group and 29 kg heavier than the inferior group at weaning. The results from this study reiterate that passive transfer of immunity during the initial d of life impacts future performance of the calf.

Assay Methods. In order to accurately evaluate passive immunity researchers have developed assays capable of providing objective measures of Ig concentration in both serum and colostrum. Multiple assays have been developed to evaluate passive transfer of immunity in domestic animals. Among these are assays capable of directly quantifying serum Ig concentrations, such as radial immunodiffusion and enzyme-linked immunosorbent assay (ELISA). Other assays estimate serum Ig concentration based on total protein or total globulin concentration, which are statistically associated with serum Ig concentrations. Examples of assays using the statistical association as an estimation of serum Ig concentration include serum solids by refraction, sodium sulfite turbidity test, zinc sulfate turbidity test, serum GGT activity, and whole blood glutaraldehyde gelation (Weaver et al., 2000).

Radial immunodiffusion (sRID) is a quantitative gel diffusion technique historically used for the detection of serum Ig concentrations (Fahey and McKelvey, 1965; Mancini et al., 1964). An agar plate is prepared incorporating antibody throughout the agar. The test sample is put into a small antigen well and on diffusion into the agar forms a ring of antigen-antibody precipitate around the well. The diameter of the precipitate ring reflects the concentration of antigen (Vann, 1993). Radial immunodiffusion has historically been the most widely used method for measuring Ig concentrations in serum and colostrum (Besser et al., 1990; Blecha and Kelley, 1981; Fahey and McKelvey, 1965; Kelley et al., 1982; McGuire et al., 1976; Stott et al., 1976).

Enzyme-linked immunosorbent assay (ELISA) has become readily available for application in the evaluation of passive transfer status in calves. Improvements of the

ELISA assay to monitor serum protein concentration in neonatal serum have aided in the increased application of this assay for practitioners (Kiriyama et al., 1989). The ELISA assay performs similarly to that of the radial immunodiffusion assay, with an increase in sensitivity over the sRID, especially when determining low concentrations of IgG (Vann, 1993). Serum and colostral Ig concentrations are determined by employing a double antibody sandwich, resulting in a color change. The intensity of the color is proportional to the concentration of Ig present in the sample. Due in large part to the ease of use and economic feasibility this assay is now a common method in evaluating concentrations of proteins such as Ig in biological fluids.

Serum total protein concentration as measured by refractometry is one of the oldest, simplest, and most commonly used operations performed in biological research. This methodology was initially proposed to estimate serum Ig concentration, in a study utilizing 185 calves evaluated for passive transfer of immunity. In this study serum total solid concentration had a strong positive correlation ($r = 0.72$) with serum Ig concentration (McBeath et al., 1971).

In an additional study comparing the utility of several commonly used assays for passive transfer, serum total solid concentration by refractometer produced comparative values. Tyler et al. (1996) demonstrated that a serum total protein concentration of 5.2 g/dL was equivalent to 1,000 mg/dL Ig concentration. Depending on the endpoint chosen this assay correctly classified >80% of calves evaluated. The use of this assay to evaluate passive transfer status in calves is recommended by several studies given the ease of application by practitioners, accuracy in classifying, and minimal cost as compared to other methods (Tyler et al., 1996; Weaver et al., 2000).

Most of the studies conducted to evaluate the relationship between passive transfer of immunity and future performance have been limited to dairy calves. As the research continues to expand into the beef cattle industry more evaluation is needed to fully understand which indicators of passive immunity might best predict future performance. Further evaluation of the differences seen between *Bos indicus* and *Bos taurus* calves within the beef industry is important, due to the variation in cattle populations dependent on suitability of cattle to a wide range of climates.

Temperament in Relation to the Cattle Industry

Temperament is a measure of an animal's reactivity to human interactions (Fordyce et al., 1988). Domestication of livestock has allowed for observations of behavioral differences among species, and within herds. Cattle in particular, are frequently subjected to interactions with humans for a variety of management practices, including weaning, vaccinations, and health related events. Temperament in cattle can

be characterized as a fear response to interactions with humans. This fear response is often termed a stress response, and its induction is not limited to human-animal interactions (Burrow, 1997). The animal's responses to these interactions have a significant impact on performance. Animals with a poor temperament will be easily excitable and in turn exhibit a greater fear response to the human interaction. Conversely, an animal with a calmer temperament will be less likely to exhibit such a response to these human interactions. There are a variety of factors that contribute to temperament including breed, age, handling history, and genetics (Burrow, 1997; Curley, 2004). Understanding the role of temperament in beef cattle production is vital for more efficient production and management of cattle.

Temperament Assessment. Evaluation of temperament has traditionally been conducted at weaning; however, determination earlier in the production cycle could prove beneficial for producers. Several methods of evaluating temperament have been used to quantify the reactivity of livestock to human interaction. Subjective and objective measures have been designed to better quantify animal temperament. Additionally, calculations including both a subjective and objective measure can be assigned to individual animals for a composite representation of their temperament.

Exit velocity (EV) or flight speed, is an objective measure of temperament introduced by Burrow et al. (1988). Exit velocity is calculated as the rate in meters per second at which an animal transverses a fixed distance (1.83 m) upon exiting a squeeze chute (Curley et al., 2006). Exit velocity is determined using two infrared sensors (FarmTek Inc., North Wylie, TX) in a system which records elapsed time between the

two sensors, used in the calculation of velocity [velocity = distance (m) / time (s)] (Figure 1.1). Burrow et al. (1988) demonstrated that flight speed was correlated to animal temperament. Cattle which exit the chute faster were more excitable than those having a slower exit velocity. This method of temperament evaluation is objective and is positively correlated with cortisol concentrations (Curley et al., 2006). Exit velocity has also been reported to be an effective measure of temperament in Brahman bulls after weaning (Curley et al., 2006).

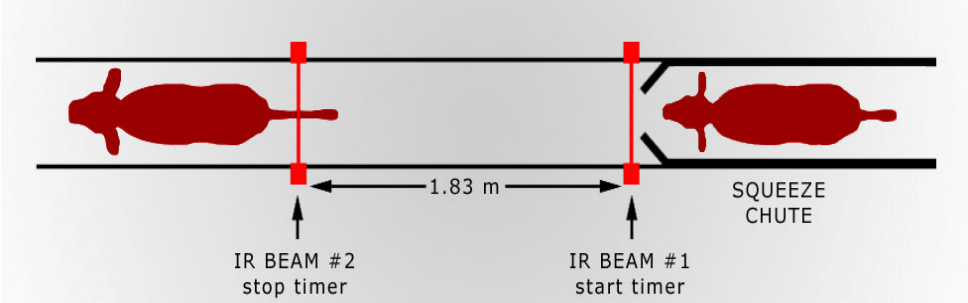

Figure 1.1. Exit velocity, m/s

Subjectively, cattle temperament can be evaluated with the use of pen scoring (PS). Pen scoring places a small group of animals (n = 3 to 5) in a pen or corral and assesses the animal's reactivity to a human handler. Measured on a scale from 1 to 5, PS identifies an animal with a score of 1 as nonaggressive and unafraid. Where as a score of 5 equates to an animal being extremely aggressive and excitable (Table 1.1; Hammond et al., 1996). The calculation of a composite score combining both the objective measure of EV and the subjective measure of PS results in a temperament

score assessed for each animal (TS = ((EV+PS)/2)); King et al., 2006). For the studies described in this thesis all PS assessments were conducted by a single handler at weaning and 28-d after weaning, EV data were also collected at these times and several additional time points to be discussed.

Table 1.1. Behavioral observations associated with Pen Scores to evaluate temperament

Pen Score	Description
1	Nonaggressive, walks slowly, can be approached slowly, not excited by humans
2	Slightly aggressive, may pace fence, stands in corner if humans stay away
3	Moderately aggressive, head up and will run if humans come closes, avoids humans
4	Aggressive, stays in back of group, head high and very aware of humans, may run into fences and gates
5	Extremely aggressive, excited, runs into fences, runs over anything in its path

[a]Adapted from Hammond et al. (1996).

Cortisol. Temperament, while affected by breed, age, and previous handling, is also correlated with cortisol concentrations, a major component of the stress response. In conjunction with catecholamines, cortisol can modulate immune function and is believed to affect the transfer and absorption of immunoglobulins from dam to calf in the early stages of life (Burdick, 2007). In recent studies temperament has been reported to be positively correlated with cortisol concentration (Curley et al., 2006; King et al.,

2006). Increased basal concentrations of glucocorticoids have been shown in cattle with a more excitable temperament (Curley, 2004). Greater cortisol concentrations have been associated with decreases in antibody titers in calves and their dams (Yorty et al., 2004). After birth and through the first week of life plasma concentrations of cortisol decrease (Blum and Hammon, 2000). Positive correlations exist between cortisol and catecholamine concentration with temperament score (Burdick, 2007). Serum IgG concentrations and temperament have been negatively correlated (Bauer et al., 2001) suggesting that as those more temperamental animals secrete more adrenal hormones they will have decreased Ig concentrations. Additionally, more excitable calves have a reduced response to vaccination (Fell et al., 1999; Oliphint, 2006).

Temperament and Cattle Production. The effects of temperament on production efficiency are numerous, impacting weight gains and vaccination response as well as carcass characteristics. Temperament plays an important role in beef cattle production. Temperament has been reported to impact reproduction, immune function, and growth in cattle (Fordyce et al., 1988). Cattle classified as temperamental have been reported to exhibit lower weight gain (Burrow and Dillon, 1997; Voisinet et al., 1997b), produce tougher meat (King et al., 2006; Voisinet et al., 1997a), and yield an increased amount of bruise trim (Fordyce et al., 1988). Studies also indicate that calmer cattle had a higher ADG than did more temperamental cattle (Müller and von Keyserlingk, 2006; Voisinet et al., 1997b).

Several studies have been conducted to evaluate the effects of temperament on meat quality. Steers with an excitable temperament exhibited greater Warner-Bratzler

shear force value when evaluating the effects of temperament on meat tenderness (Voisinet et al., 1997a). Carcass yield has also been shown to be influenced by temperament, excitable cattle yield less meat due to increased bruise trim from injuries acquired during transport (Fordyce et al., 1988). Additionally, excitable cattle have also been shown to exhibit greater percentages of borderline dark cutters when compared to calmer cattle (Voisinet et al., 1997a). Results from these studies suggest that cattle temperament impacts multiple facets of beef production, with the excitable temperament producing undesirable results in several carcass characteristics.

Frequent handling of cattle influences the responses derived from these human interaction. Studies have indicated that intensive handling of young animals will improve their temperament (Fordyce et al., 1985). Additionally, intensive handling could improve or prevent negative effects of temperament on carcass quality in beef cattle (Burrow and Dillon, 1997). Variations in temperament among animal populations emphasizes the need for further investigation into the effects of temperament on growth and performance in beef cattle, particularly of interest in this thesis are those of *Bos indicus* breeding.

Feed Efficiency

The largest expense associated with livestock production is feed, accounting for approximately 60-70% of total production costs (Sainz and Paulino, 2004). Improving production efficiency as it relates to feed consumption and subsequent weight gain is of primary concern to the beef industry. Identification of animals that are more efficient in

utilizing available nutrients is an area of interest for beef producers, with the objective of reducing production costs related to feed expenses. Methods for identifying animals which perform better than the general population have been used in the industry for decades. However, a common method for selecting animals that consume less feed than expected while maintaining the predicted gains has not been implemented throughout the beef industry. A review of methods used for evaluation of feed efficiency is presented in the table and descriptive format below (Table 1.2).

Table 1.2. Definition of feed efficiency traits[a]

Feed Efficiency Trait	Definition	Calculation	Desired Phenotype
Feed conversion ratio (FCR)	Feed intake required to produce one unit of weight gain	DMI ÷ ADG	Low
Partial efficiency of growth (PEG)	Efficiency of BW gain after maintenance requirements have been accounted for	ADG ÷ (Actual DMI – DMI required for maintenance) when maintenance is calculated as $0.077 \times BW^{0.75} \div NE_m$ concentration of the diet (NRC, 2000)	High
Residual feed intake (RFI)	Difference in expected DMI for maintenance and growth at a given level of production and actual DMI	DMI – Expected DMI, where expected DMI is obtained by the regression of DMI on mid-test metabolic BW and ADG	Low

[a]Adapted from Arthur et al. (2001b), Hennessy and Arthur (2004), Brown (2005), and Dittmar (2007).

Traditionally, the measure of feed efficiency used has been feed conversion ratio (FCR). Feed conversion ratio can be defined as the amount of feed it takes to produce one unit of gain (Brody, 1945). Alternatively, this measure can be described as the ratio of dry matter intake to live-weight gain (Arthur et al., 2001a; Arthur et al., 1996). A low FCR is the desired phenotype, indicating increased feed efficiency. A low FCR results from less feed input to produce a single unit of gain where a high FCR is indicative of increased feed inputs to produce the same single unit of gain. Several studies have shown that FCR is negatively correlated with average daily gain (ADG; Arthur et al., 2001a; Hennessy and Arthur, 2004; Nkrumah et al., 2004). Selection of animals using FCR has led to an increase in mature size, resulting in increased feed requirements of the mature cow herd (Herd and Bishop, 2000). Due to the failure of FCR to take into consideration the requirements for maintenance alternative methods have been suggested. For a true expression of feed efficiency, calculations need to include requirements for both maintenance and growth (Arthur et al., 1996). Continued use of selection based primarily on FCR leads to an increase in mature cow size and feed requirements for maintenance and growth, and therefore may decrease overall production efficiency of the cattle industry.

One alternative method for evaluating feed efficiency is partial efficiency of growth (PEG; Kellner, 1909). While similar to FCR, PEG adds the incorporation of maintenance requirements for the animal in the evaluation of efficiency. Partial efficiency of growth is calculated as the ratio of ADG to DMI available for growth after accounting for maintenance requirements. The expected DMI available for growth is

calculated as the difference in actual DMI and expected DMI for maintenance (Arthur et al., 2001a). A higher PEG indicates that an animal had a greater increase in body weight per unit of available energy, taking into account both maintenance and gain (Hennessy and Arthur, 2004). Although PEG accounts for maintenance requirements these estimates are not entirely reliable, due in part to assumptions made when predicting the DMI necessary for maintenance among different animals (Archer et al., 1999). Correlations between ADG and PEG range from nearly zero (Hennessy and Arthur, 2004) to 0.24 (Nkrumah et al., 2004). Nkrumah et al. (2004) suggests that the partial correlation between ADG and PEG may be explained by the feeding standards used in the calculation of expected feed intake for maintenance.

Residual Feed Intake. Residual feed intake (RFI) was first introduced by Koch et al. (1963)(Koch et al., 1963), as an alternative to traditional measures of feed efficiency. Koch et al. (1963) explained that efficient use of feed was not a directly measurable trait, rather a function of feed consumed, BW gain, and the time required to reach a given point in production. Residual feed intake can be defined as the residual, or difference between the amount of feed actually consumed and predicted feed consumption. Residual feed intake is calculated using the residual between the actual and expected intakes for each animal in a cohort (Arthur et al., 1996). This measure of efficiency describes net feed conversion efficiency (Arthur et al., 1996). Based on this definition, an animal that consumes less feed than expected has a negative RFI value, while an animal that consumes more feed than expected has a positive RFI value. Residual feed intake makes use of linear regression to estimate the feed intake from BW

and ADG (Koch et al., 1963). Residual feed intake is an alternative efficiency measure which has been reported to be phenotypically independent of BW and ADG (Archer et al., 1999). Several studies have presented findings showing that RFI was independent of the production traits used in the calculation (Arthur et al., 2001a; Arthur et al., 2001b; Carstens et al., 2002; Nkrumah et al., 2004). Nkrumah et al. (2004) also reported that RFI was independent of age. Comparing the traditional method of FCR with RFI suggests that selection for efficiency using RFI should not lead to increased mature size. Additionally, reports have suggested that selection based on RFI can improve feed efficiency due to its moderate heritability, with estimates of 0.28 (Koch et al., 1963), 0.14 (Fan et al., 1995) and 0.16 (Herd and Bishop, 2000).

A significant disadvantage of RFI is the laborious nature, and expense associated with individually feeding animals. Each animal must be individually fed using facilities such as the GrowSafe™ or Calan gate feeder systems. Currently there are a limited number of facilities capable of evaluating cattle for RFI. Originally the testing period was set to 112-d, as this was the amount of time required to accurately evaluate the growth rate of cattle being tested (Brown et al., 1991). Subsequent research conducted using *Bos taurus* heifers and bulls evaluated the effect of test period duration ranging from 7 to 119-d, finding minimal variation in RFI after d 70 (Archer et al., 1997). Differences in feeding behavior between the *Bos taurus* and *Bos indicus* breeds suggested a need for the investigation into test duration for the different breed types. Subsequently, a study was conducted which determined that a 70-d testing period was appropriate for either breed type (Archer and Bergh, 2000). In addition to determining

the length of the testing period, several studies evaluated the frequency of BW collection to be used in the calculation of ADG. In order to accurately calculate ADG Archer et al. (1997) determined that a maximum of two week intervals was ideal for BW collection on cattle being tested; measurements beyond this window introduce increased variability.

The regression calculation used to determine RFI includes metabolic BW to aid in accounting for the variation in maintenance. The variation in RFI for a given cohort of animals is reflective of differences in the animal's use of energy for maintenance and growth (Kennedy et al., 1993). Identifying those animals with a negative RFI, or more efficient in nutrient utilization, in a feedlot setting should translate into efficient animals in a grazing setting (Arthur et al., 2001a). Furthermore, the identification of animals more efficient in nutrient utilization should translate into cattle with a lower maintenance requirement being retained in the breeding herd.

Insulin-like Growth Factor-I (IGF-I). Given the costly nature of evaluating cattle for RFI there has been limited application in the industry, with most of the available facilities being used for research. In order to overcome these obstacles for implementation of RFI as a selection tool, finding an inexpensive and noninvasive indicator of RFI has been of interest. Studies have focused on the utility and repeatability of serum IGF-I as a predictor, or indicator, of feed efficiency.

Insulin-like growth factor-I is a polypeptide growth hormone which is structurally related to insulin. Secreted primarily from the liver under stimulation by growth hormone (GH), IGF-I has been shown to play a role in promoting cell

proliferation and inhibition of cell death. Responsible for regulating cellular processes and acting as a signaling molecule between cells, IGF-I targets bone, muscle, adipose, and a variety of organ systems. Insulin-like growth factor I is a modulator of the growth axis, responsible for the stimulation of glucose uptake and protein synthesis in a variety of species (Baxter, 1986). Serum and plasma concentrations of IGF-I have been linked to a variety of economically important traits in several species. Successful implementation of IGF-I as a selection tool has been accomplished in both pigs (Buntner et al., 2002) and sheep (Blair et al., 2002). Recent research in *Bos taurus* cattle has proposed a link between RFI and IGF-I (Moore et al., 2008; Wood et al., 2004). Additionally, it has been suggested that IGF-I may be predictive of RFI in *Bos taurus* cattle (Johnston et al., 2002). However, when evaluating Brangus heifers no significant correlation was found between IGF-I and RFI (Lancaster et al., 2007). Furthermore, data from recent studies suggest that tropical adaptation may influence circulating concentrations of IGF-I (Caldwell, 2009). These findings suggest that the correlation between RFI and IGF-I may vary by breed type, indicating a need for further investigation into the application of IGF-I as a physiological indicator for RFI.

Selecting for a negative RFI value, or an efficient animal, results in a decrease in adipose tissue (Herd and Arthur, 2008). The decreases in fat deposition in efficient animals could potentially impact reproductive performance. Testing and selection based on RFI must be accompanied with puberty evaluation until conclusive data can be presented showing the full impact of this selection tool. Evaluation of the effect of selection, based on RFI on reproductive development and performance later in life has

not been specifically investigated. Further research is needed to elucidate the impact of selection for feed efficiency, based on RFI, and its relationship to future development and reproductive performance. A selection tool such as RFI should be evaluated in conjunction with factors such as carcass traits, reproductive performance, and longevity.

Bull Reproductive Development

Understanding the effect of selection for RFI on reproductive performance is important to multiple segments of the beef industry. Growth and development can be evaluated with measures of body mass and the attainment of milestone steps, including first sperm, puberty, and sexual maturity, leading to maturity. Reproductive development in bulls is dependent on multiple factors including season of birth (Tatman et al., 2004), hormonal presence (Lunstra et al., 1978), age (Lacroix and Pelletier, 1979), nutrition (Van Demark and Mauger, 1964), breed (Lunstra and Echternkamp, 1982), and genetics (Steffen, 1997).

Early Development. Hormones secreted by the male gonad early in fetal life induce regression of potentially female structures and development of the male reproductive tract. Compartmentalization of the testis and formation of the blood testis barrier during prepubertal development permit the steriodogenic and gametogenic activities of the adult testes to proceed normally (Amann and Schanbacher, 1983). Early initiation of testicular function is essential for the development of the reproductive track in the male. The Leydig cells of the fetal male gonad produce testosterone, which stimulates development of the mesonephric tubules and duct into the efferent ducts,

ductus epididymides, ductus deferens, and vesicular glands (Amann and Schanbacher, 1983; Senger, 2003). The indifferent fetal supporting cells produce a Mullerian duct inhibiting hormone which causes degeneration of potentially female structures (Josso et al., 1979; Josso et al., 1977). Induction of the enzyme 5α-reductase allows metabolism of testosterone to dihydrotestosterone, which induces differentiation of the urogenital sinus into the prostate and bulbourethral glands and male type urethra and phallus (Wilson and Siiteri, 1973). The urogenital folds develop into the scrotum. Final development of the mammalian testis involves testicular decent which occurs long before birth in cattle, sheep, and swine but within 2 weeks before or after birth in horses (Amann and Schanbacher, 1983).

Endocrinology. The hypothalamic-pituitary-testicular axis is an endocrine network that allows for maturation and development of reproductive tissues and systems. Established during gestation, the male endocrine system is one of the primary systems responsible for reproductive development, relying on several key hormones for maturation. Produced by the same gonadotroph cells of the anterior pituitary, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are crucial for development of the male reproductive systems. Prepubertal bulls lack the capacity to secrete adequate GnRH, limiting the dependent secretions of LH, FSH, and testosterone (Courot, 1978)

Episodic LH secretion from the anterior pituitary is triggered by the release of gonadotropin releasing hormone (GnRH) from the hypothalamus into the hypophyseal portal system (Hafez, 2000). The pulsatile release of GnRH is responsible for the similarly pulsatile release of LH. In turn, LH targets the Leydig cells of the testis

stimulating release of testosterone. Testosterone then diffuses into the seminiferous tubules where it is converted into estradiol and aids in the support of spermatogenesis. Increased concentrations of testosterone appear to be crucial for normal spermatogenesis (Amann, 1983). In the blood, testosterone acts to increase libido and inhibit GnRH secretions from the hypothalamus (Malven, 1993). The onset of puberty is brought about because of decreased hypothalamic sensitivity to the negative feedback of testosterone on the GnRH neurons (Senger, 2003).

Follicle-stimulating hormone is released in a semi-continuous pattern targeting the Sertoli cells, which induces the secretion of androgen binding protein (ABP) and promoting the conversion of testosterone to estradiol (van der Molen et al., 1981). Sertoli cells act in the conversion of testosterone to estradiol utilizing a mechanism identical to granulosal cells of the antral follicle in the female (Senger, 2003). Regulation of FSH is accomplished through the hormone inhibin, which is secreted by the Sertoli cells following FSH stimulation. This secretion causes a negative feedback on the anterior pituitary to suppress FSH production (Amann and Schanbacher, 1983).

The increase in frequency of LH and GnRH are the primary mechanisms responsible for testicular maturation (Amann et al., 1986; Rodriguez and Wise, 1989). Endocrine changes associated with the functional hypothalamic-pituitary-testicular axis are age dependent, and the transition from prepubertal to pubertal status in Holstein bulls is reported to begin at approximately 4 months of age (Rodriguez and Wise, 1989). Some research indicates that testosterone release occurs at 1-2 months of age (Schams et al., 1981); however, the majority of researchers suggest that testosterone secretion does

not occur until after 4 months of age (Lacroix and Pelletier, 1979; Pelletier et al., 1981; Toelle and Robison, 1985). Testosterone secretion increases with age, particularly around 6 to 12 months (McCarthy et al., 1979; Pelletier et al., 1981) but reaching adult concentrations at approximately 10 months of age in *Bos taurus* bulls (Amann and Schanbacher, 1983; Amann and Walker, 1983; Lunstra et al., 1978).

Endocrine changes in the bull are responsible for the establishment and maintenance of spermatogenesis. Establishment of spermatogenesis is progressive and associated with the rapid increase in testicular size. Holstein bulls establish spermatogenesis and increased testicular size from 16 to 32 weeks of age (Curtis and Amann, 1981). In the same study, the establishment of spermatogenesis was complete upon attainment of testis weights of 80 g at approximately 32 weeks of age. Although complete establishment was not achieved until 32 weeks of age, initiation began earlier at approximately 16 weeks; as evident by the presence of A-spermatogonia in the testis and subsequently in the Sertoli cells at 24 weeks of age. Thus, suggesting that spermatogenesis begins between the two reported ages (Curtis and Amann, 1981).

Spermatogenesis. Spermatogenesis is the process of transforming spermatogonia into spermatozoa while maintaining spermatogonial numbers (Amann and Schanbacher, 1983). Primary goals of spermatogenesis are to (1) provide a male with a continual supply of male gametes, (2) provide genetic diversity, (3) provide billions of sperm per d to maximize reproduction, (4) provide an immunologically privileged site where germ cells are not destroyed by the immune system (Senger, 2003). This process occurs in the

seminiferous tubules and can be divided into three phases: proliferation, meiotic, and differentiation (Johnson et al., 2000).

The initial proliferation phase occurs primarily in the basement membrane and consists of all mitotic divisions of spermatogonia. A_1 spermatogonia undergo mitosis producing increasingly differentiated spermatogonia which continue mitotic division into A_2, A_3, A_4, intermediate (I) and eventually B spermatogonia. B spermatogonia continue mitotic division forming the primary spermatocyte, which then yield secondary spermatocyte and finally resulting in haploid, spherical spermatids (Senger, 2003). The process of meiosis allows for exchange of genetic material between homologous chromosomes of primary spermatocytes and the production of spermatids (Johnson et al., 2000).

The differentiation phase of spermatogenesis is the stage in which the spherical undifferentiated spermatid becomes a fully differentiated highly specialized spermatozoon containing a head, flagellum, and principal piece (Senger, 2003). Differentiation is also divided into distinct phases including, the Golgi, cap, acrosomal, and maturation phases. The Golgi phase is characterized by the development of the acrosome. In this phase the highly-developed Golgi apparatus within the spermatid gives rise to the acrosome. The acrosome is a double walled structure that will eventually cover the anterior 2/3rd of the nucleus, and contains hydrolytic enzymes (acrosin, hyaluronidase, zona lysine, esterase, and acid hydrolase) required for penetration of the zona pellucida and subsequent fertilization. During acrosomal formation centrioles migrate from the cytoplasm to the base of the nucleus, where the

proximal centriole will give rise to the flagellar anchor and the distal centriole gives rise to the axonome (Senger, 2003).

Subsequently, during the cap phase the acrosome spreads over the anterior portion of the nucleus. At this point the Golgi apparatus has completed its function and migrates away from the nucleus to the caudal end of the spermatid where it eventually disappears. During the acrosomal phase the nucleus and cytoplasm begin to elongate. The spermatids are deeply embedded in Sertoli cells, projecting the flagellum into the lumen of the seminiferous tubule. Microtubules known as the manchette develop near the posterior nucleus, and eventually give rise to the postnuclear cap. The final stage of spermatozoa development is the maturation phase. Mitochondria migrate toward and cluster from the base of the flagellum to the anterior 1/3rd of the tail. Dense outer fibers of the flagellum and the fibrous sheath are produced and final assembly is complete (Senger, 2003).

Spermiation is the release of spermatozoa from the Sertoli cells into the lumen of the seminiferous tubules. Spermiation occurs continuously throughout the testis (Senger, 2003). The three phases of spermatogenesis (proliferation, meiosis, and differentiation) take approximately 21, 23, and 17 d respectively in the bull, resulting in a 61 d duration (Johnson et al., 2000). Given the report of spermatogenesis establishment at 32 weeks of age in Holstein bulls by Curtis and Amann (1981), spermatogenesis likely begins around 23 weeks of age.

Milestones of Reproductive Development. Puberty, as related to many species, can be defined as the age at which gonad function reaches maturity relative to endocrine

and gametogenic capacity. Many definitions for puberty have been proposed. A general definition of puberty in males incorporates both behavioral and spermatogenic components. It includes age when behavioral traits are expressed, age at first ejaculation, age when spermatozoa first appear in the ejaculate, age when spermatozoa first appear in the urine, and the age when the ejaculate contains a threshold number of spermatozoa (Senger, 2003). The pubertal period is associated with rapid testicular growth, changes in hormonal patterns, and the initiation of spermatogenesis. Puberty is not synonymous with sexual maturity or adult status which occurs months or years later (Amann and Schanbacher, 1983). The occurrence of both behavioral and spermatogenic development rarely occur at the same time, creating a challenge in combining the two components for a single definition. Puberty is considered a process and not an event (Senger, 2003). Assessing puberty in bulls related to behavioral traits is difficult. In the female, these behavioral measures can be assessed with the detection of estrus, service of estrus, libido, and sexual aggression. Spermatogenic components are easier to measure, and provide accuracy and reliability for the evaluation of reproductive development in the male. Spermatogenic development is characterized by two main factors: the presence of first motile spermatozoa, or the total concentration of spermatozoa with a minimum motility. Studies evaluating peripubertal behaviors, seminal, and endocrine characteristics in *Bos tarus* bulls defined puberty as the age at which a bull can produce an ejaculate containing 5×10^6 spermatozoa with 10% motility (Wolf et al., 1965). To date, the definition of puberty proposed by Wolf et al. (1965) has become generally accepted and used for *Bos indicus*, *Bos taurus*, and crossbred bulls.

After the onset of puberty, reproductive development continues as a gradual process to sexual maturity. The primary changes observed between the two stages of development are changes in testicular growth, body weight, and spermatogenic development. Testicular size in young bulls is positively correlated with overall spermatozoa production; with increased testicular size there is increased sperm production. Again, there is a challenge in accurately and effectively evaluating bulls for maturity related to reproductive development when considering both behavioral and spermatogenic components. The Society of Theriogenology developed the Breeding Soundness Exam (BSE), which is used to evaluate bulls using scrotal circumference, sperm motility and morphology, and reproductive tract soundness. None of these factors have direct effects on libido, but all influence fertility (Chenoweth et al., 1988; Mwansa and Makarechian, 1991). The accepted definition for sexual maturity in bulls encompasses the spermatogenic component with an ejaculate with sperm concentration of 50 X 10^7 spermatozoa with a minimum motility of 50% and normal morphology greater than 80% (Chenoweth et al., 1992).

Scrotal Circumference. Assessment of reproductive development of bulls is not easily obtained. In the animal science industry female reproductive traits often receive significantly more attention than do their male counterparts, due in part to the challenges in assessing male reproductive development. Several different techniques have been used to access reproductive development in bulls; the most widely used tool is scrotal circumference (SC). Scrotal circumference has been linked to both male and female reproduction characteristics. The high degree of association between SC and several

female reproductive traits is important due to the low heritability of female reproductive traits (Bourdon and Brinks, 1982; Dearborn et al., 1973)

Scrotal circumference has been negatively correlated with age at puberty in bulls (Godfrey et al., 1990; Lunstra et al., 1978). When evaluating SC at puberty previous studies have repeatedly reported a SC of approximately 28.1 cm (Barber and Almquist, 1975; Godfrey et al., 1990; Lunstra et al., 1978). This consistency suggests that SC is an accurate indicator of puberty over the previously used body weight, age, or weight per d of age. Scrotal circumference at puberty in Brahman bulls has been reported to be less than 28 cm, (Fields et al., 1982; Neuendorff et al., 1985), however several studies have reported conflicting results. A two year study conducted in the same Brahman herd reported an average SC at puberty of 31 cm in year one and 28.2 in year two (Chase et al., 1997). As the percent of Brahman influence increased in crossbred calves a linear decrease in testicular size at puberty was observed (Browning et al., 1997). Furthermore, this relationship continues beyond puberty, with Brahman influenced bulls requiring more time to achieve testicular size similar to that of *Bos taurus* bulls (Chase et al., 2001; Lunstra et al., 1978). At approximately 3 years of age; SC has been found to be similar between Brahman and *Bos taurus* bulls (Fields et al., 1982; Godfrey et al., 1990). Moreover, SC has been strongly correlated with testis weight ($r = 0.95$; Coulter and Foote, 1976) which is correlated with daily sperm production, and semen quality characteristics.

Measurement of yearling testis size is a reliable indicator and simple method to predict age at pubertal onset among many divergent breeds of bulls. Regardless of

differences in age, weight, and breed, scrotal circumference is a useful tool in the selection of early maturing bulls (Lunstra and Echternkamp, 1982). Yearling bulls with larger scrotal circumferences produce higher quality semen, (Cates, 1975; Fields et al., 1982) with more sperm, (Almquist and Amann, 1976; Hahn et al., 1969), at a younger age (Lunstra and Echternkamp, 1982) suggesting an important relationship between testicular development and subsequent reproductive function.

Nutrition-Reproduction Interaction. Nutrition is a key mediator of reproductive performance. Energy intake influences growth and reproductive development in all animals. Dietary energy affects reproductive development by directly affecting circulating testosterone and testicular development (Nolan et al., 1990). Reproductive development characteristics such as age at first sperm (Chase et al., 1994) and puberty (Van Demark and Mauger, 1964) are also influenced by dietary energy. Limiting the availability of energy in pre-pubertal bulls delays the onset of puberty and may even result in permanent reduction in semen yield (Van Demark et al., 1964). In the male, undernutrition is accompanied by hypogonadism and infertility (Brown, 1994).

Nolan et al., (1990) examined the effects of nutrient intake on hypothalamic-hypophyseal-testicular function in pre-pubertal Brahman bulls and explored the relationship between metabolism and onset of puberty. The results from this study indicated that feeding Brahman bulls to achieve 1.0 kg per d growth rates hastened the onset of puberty compared with a lower rate of growth (0.1 to 0.25 kg per d). The earlier onset of puberty was achieved by indirectly enhancing testicular function. Serum LH

was unaffected by nutrient intake while testosterone increased with age in all bulls, but a more rapid increase was observed in higher gaining bulls.

It has been proposed that excessive energy intake by post-weaning British breed bulls may have detrimental effects on reproductive potential. Increased fat deposits in the neck of the scrotum, and or scrotal tissues may cause the testis to be insulated. Increasing the temperature of the testis has detrimental effects on sperm production and quality of semen (Senger, 2003). It is obvious that dietary factors such as energy intake play a role in reproductive development; potentially by influencing steroidogenesis and thus the maturation of gonads and production of spermatozoa.

Reproductive development is directly related to body composition, specifically adipose deposition. In the female, a threshold body size must be attained prior to the onset of puberty. Nutrient intake influences critical BW and age required for the onset of puberty (Short and Bellows, 1971). Heifers with increased nutrient intake reach puberty at a younger age and are heavier than nutritionally restricted heifers. Similarly, body composition, which is closely related to age and weight, is important in the attainment of puberty. Rats fed a high-fat diet were heavier and reached puberty at an earlier age than rats fed a low-fat diet (Frisch, 1972). Adipocytes produce the hormonal peptide leptin which has been linked to regulation of food intake, energy expenditure, and whole-body energy balance in rodents and humans. Leptin has also been thought to be a metabolic signal that regulates nutritional status effects on reproductive function (Houseknecht et al., 1998).

Limited research has been conducted regarding the influence of metabolic status on the onset of puberty in the male. It is well understood that compromised nutrition retards the pubertal onset of high frequency pulsatile LH (Senger, 2003). Recent research suggests that selection for RFI has an impact on body composition, specifically adipose deposition. Nkrumah et al., (2004) reported a positive correlation between RFI and back fat. As RFI becomes more positive, or as an animal becomes more inefficient, back fat increases. Selection for a negative RFI value, or an animal that is efficient, resulted in a decrease in back fat deposition. It is important to fully investigate the relationship between RFI and body composition as it could potentially have adverse effects on reproductive development.

It can be concluded that a variety of factors can alter or effect reproductive development of bulls, including nutrition. In order to fully grasp the repercussions of RFI based selection on reproductive development, assessment of puberty and of the milestones in development must accompany any testing for RFI until conclusive data can be presented showing the full impact of this selection tool.

CHAPTER II

EVALUATION OF GROWTH, PERFORMANCE, AND TEMPERAMENT IN BRAHMAN CALVES

Introduction

Beef cattle production represents a large proportion of the agriculture economy in the United States and Texas. A majority of cattle operations in the United States utilize *Bos taurus* cattle; however, for Texas and other Gulf Coast states *Bos indicus*, specifically Brahman, cattle are a common, versatile, and functional breed. Brahman cattle are a tropically-adapted breed known for their heat tolerance and resistance to internal and external parasites. Additionally, these cattle are capable of more efficiently utilizing poor quality forages and add value in terms of heterosis to beef crossbreeding systems (Koger, 1980; Turner, 1980). Maximizing profits in the beef cattle industry requires minimizing inputs while improving performance and reducing death loss. Beginning with the earliest phase of cattle production, calf survival and performance translates into profit potential for the producer.

Calf mortality and morbidity account for significant losses in the beef cattle industry. Passive transfer of immunity in the neonate is essential for calf survival and subsequent performance. Early establishment of a functional and competent immune system enables the calf to resist invading pathogens and infectious diseases. Colostral Ig serves as the primary source for passive immunity in the neonate, requiring almost immediate and adequate ingestion of colostrum following birth. Passive transfer of immunity has traditionally been assessed with the evaluation of serum Ig concentrations

in the calf. However, recent data suggests that plasma or serum total protein may be a useful tool, for both the evaluation of passive transfer and as a possible predictor of future performance.

In the past decade, the effect of calf temperament on health, immune function, growth, and reproduction has been the subject of several experiments and reviews (Fell et al., 1999; Mondal et al., 2006). The effects of excitable temperament on performance are numerous, including reduced response to vaccination (Fell et al., 1999; Oliphint, 2006), lower weight gain (Burrow and Dillon, 1997; Voisinet et al., 1997b), tougher meat (King et al., 2006; Voisinet et al., 1997a), and increased yield of bruise trim (Fordyce et al., 1988). Fully understanding the role of temperament in calf growth and performance is crucial in maximizing herd efficiency. Temperament evaluations are commonly conducted at weaning; however, identifying calves with excitable temperament earlier in the production cycle through the use of EV and PS may prove valuable for the producer.

Materials and Methods

Animals & Experimental Design. This study utilized Brahman calves born in the springs of 2006 (n = 111), 2007 (n = 108), and 2008 (n = 81). Calves were pastured with their dams at the Texas AgriLife Research Center in Overton until weaning. Calves were 173 ± 2 d, 173 ± 2 d, and 163 ± 2 d of age at weaning for the 2006, 2007, and 2008 calf crops respectively. Blood samples (15 mL) were collected from each calf 24 h after birth and at 21 to 24 d of age via jugular venipuncture. Blood samples were centrifuged at 1700rcf at 4°C for 30 m (3200 rpm) 24 h after collection; serum was harvested and stored at -20°C until analysis for serum TP, CS, and IgG. Calves were weighed approximately 24 h after birth, at 21 to 24 d of age and at 28 d intervals until weaning for calculation of ADG. Beginning at 21 to 24 d of age and at 28 d intervals thereafter calves were evaluated for temperament using EV (Burrow et al., 1988; Curley et al., 2006). Beginning 28 d prior to weaning and at 28 d intervals through 56 d after weaning calves were evaluated for PS (Hammond et al., 1996); a subjective measure of temperament used in the calculation of temperament score (TS = (EV+PS)/2); King et al., 2006).

As mentioned above, calves in this study were born in 3 different years, and each year consisted of a 3–month calving season with variable temperatures and environmental conditions. Management practices varied after weaning for each year. In 2006 the heifers were placed on Calan gates immediately following weaning and fed at a rate of 2.25% of BW (as-fed basis; gross BW). Bull calves from 2006 were maintained on coastal bermudagrass pasture and supplemented with 3:1 corn and soybean meal at a

rate of 1.5% of BW. Calves from the 2007 calf crop were managed similar to the 2006 calves, with heifers being placed on Calan gates and bull calves maintained on pasture. Management after weaning for the 2008 calf crop was the same between heifer and bull calves, each being maintained on coastal bermudagrass with 3:1 corn and soybean meal supplementation.

Total Protein. Serum TP concentrations were determined from duplicate aliquots using a colorimetric assay. Total Protein Reagent (Sigma Chemical, St. Louis, MO, Cat. #T1949), was used for the determination of unknown protein concentrations. Deionized water was used for the blank in all assays. Standards (Sigma P5369) were developed by using a 1:1 dilution with deionized water from a concentration of 200 mg/ml to 100 mg/ml, equivalent to 10 g/dl. The addition of the alkaline copper salt solution to the serum sample allowed complex formation between copper ions and protein, resulting in copper-protein complexes with purple color. Color intensity is proportional to the total protein concentration which can be measured spectrophotometrically in the 540 nm region. Final concentrations were calculated and are presented as g/dL. The intraassy coefficient of variation was 2.30% for all assays.

Serum IgG Concentrations. Serum concentrations of IgG were determined using a commercially available double antibody sandwich enzyme-linked immunosorbant assay, (ELISA) specific for bovine immunoglobulins (Bethyl Laboratories, Montgomery, TX, Cat # E10-118). Each 96-well plate was coated with primary antibody (100 μL/well; affinity purified, sheep anti-bovine Ig), allowed to incubate for 1 h, and then stored at 4°C overnight. Plates were washed with TRIS containing 0.05%

Tween20 (wash buffer) 3 times. After this wash 200 μL of wash buffer was added to each well and allowed to incubate for 1 h. Plates were then washed 3 more times with buffer and 100 μL of serum diluted in wash buffer (1:250,000) were added in duplicate. Plates were incubated for 2 h, and then washed 4 times prior to the addition of sheep anti-bovine Ig conjugated to horseradish peroxidase, diluted 1:1600. Plates were then incubated for 1 h and washed 4 times. Next 100 μL/well enzyme substrate (2,2'-azino-bis[3-thylbensthiazoline-6-sulfonic acid] + 0.05% H_2O_2; pH 4.5; Sigma Chemical Co; St. Louis, MO) was added to each plate (Burdick, 2007). Plates were incubated for 10 m away from direct sunlight and read at 405 nm using a spectrophotometer (Bio-Rad Model 680 Microplate Reader). Serum concentrations were determined by comparison to a standard curve generated with known concentrations of bovine IgG. Intraassay coefficients of variation for 2006, 2007, and 2008 were 8.58%, 9.99%, and 9.58% respectively.

Cortisol. Serum concentrations of CS for the 2006 calf crop were determined using a commercially available single antibody radioimmunoassay kit (RIA; cat # DSL-2100-5; DSL, Webster, TX) utilizing rabbit cortisol antiserum coated tubes. Serum concentration of CS for the 2007 and 2008 calf crops were determined in duplicate aliquots using a single antibody radioimmunoassay procedure utilizing rabbit anti-cortisol antiserum (Pantex, Div. of Bio-Analysis Inc., Santa Monica, CA, Cat. #P44) diluted 1:2500; standards were made by serial dilution (7.8 pg/100μL to 8000 pg/100 μL) of 4-pregnen-11β,17,21-triol-3,20-dione (Steraloids Inc., Newport, RI, Cat. #Q3880-000) and radio-labeled cortisol: ^{3}H-Hydrocortisone (1,2-^{3}H, NEN, Boston, MA, Cat.

#NET-185) (Curley, 2004). Unknown cortisol concentrations were calculated using Assay Zap software (Biosoft, Cambridge, UK) and counts per minute obtained from a liquid scintillation spectrophotometric beta-counter (Beckman Coulter LS 6500). Final concentrations were calculated and are presented as ng/mL. Intraassay coefficients of variation for 2006, 2007, and 2008 were 5.80%, 6.89%, and 7.45%, respectively.

Temperament. Calves were evaluated for temperament beginning at 21 to 24 d of age and at 28 d intervals through 56 d after weaning with the use of EV. Exit velocity (Burrow et al., 1988; Curley, 2004; King et al., 2006) is a measure of the rate of travel over a distance of 1.83 m when an animal exits a squeeze chute, determined using two infrared sensors (FarmTek Inc., North Wylie, TX) and calculated as velocity [velocity = distance (m) / time (s)] (Figure 1). At 28 d prior to weaning and at 28 d intervals through 56 d after weaning calves were subjectively evaluated for temperament using PS. Calves were separated into a pen (5x10 m) in small groups (n = 3 to 5). Scores were assigned on the basis of reactivity to an observer, 1 being completely calm and 5 being extremely excitable and reactive (Table 1; Hammond et al., 1996). Temperament score (TS) was determined using the average of PS and EV; (EV+PS/2); King et al., 2006). An average TS from data collected at 28 d prior to weaning and at weaning was utilized in the statistical analysis.

Ultrasound Carcass Traits. Ultrasound measures were collected at weaning and at 56 d post-weaning. Measures of REA, REA per 45.36 kg of BW (REA:CWT), intramuscular fat (IMF), fat over the 12th rib (Back fat), and rump fat were all collected by the same Ultrasound Guidelines Council certified technician. The Aloka 500V real-

time ultrasound machine (Corometrics Medical Systems, Wallingford, CT) was equipped with a 17.2 cm, 3.5 MHz linear transducer which was fitted with a Superflab (Designer Genes Technologies, Inc., Harrison, Arkansas) guide for image capture. Animals were brushed with a curry comb prior to the application of 100% vegetable oil to obtain proper acoustic contact. Images were interpreted using Beef Image Analysis Pro Plus Software 2.0.3 (Designer Genes Technologies, Inc., Harrison, AR.).

Calf Classification. To determine the relationship between calf serum concentrations of TP, IgG, and CS measured at 24 h and 21 to 24 d post-calving and future growth, calves were assigned classifications for each parameter. Calves were classified based on each blood parameter evaluated, with calves 1 SD above the mean classified as high, those 1 SD below the mean classified as low, and all remaining classified as intermediate (Table 2.4, 2.5, and 2.6). Classifications were conducted within each year and then the data was pooled for the final analysis.

In order to evaluate the effect of calf temperament early in life and at weaning on measures of performance calves were classified based on temperament assessed at both time points. Calves were also classified based on temperament using EV at d 21 to 24. Calves 1 SD above the mean classified as temperamental, calves 1 SD below the mean classified as calm, and all remaining classified as intermediate. Additionally, calves were classified based on TS; calves 1 SD above the mean classified as temperamental, calves 1 SD below the means classified as calm, and all remaining classified as intermediate.

Cow Classification. Dams were classified based on both cow age and temperament to determine the effect of cow age and cow temperament on calf blood parameters measured at 24 h and 21 to 24 d post-calving. Dams were classified based on temperament as calm, intermediate, or temperamental, prior to calving by an experienced herdsman; the same herdsman was used all 3 years. Dams were also classified based on age, with those 2 to 4 yr of age being classified as young, those 5 to 9 yr being classifies as mature, and all dams 10 yr and older classified as aged.

Statistical Analysis. All data were analyzed using MIXED MODEL procedures of SAS 9.2 (SAS Inst. Inc., Cary, NC) and the Satterthwaite approximation for df.

To evaluate the effect of calf temperament early in life on measure of performance the models for weaning weight and ADG included calf temperament classification as assessed by EV, calf sex, and the blood parameter being evaluated as fixed effects; year and calf sire were included as random effects. The models for weaning weight and ADG also included cow age and calf age as covariates.

To evaluate the effect calf temperament at weaning as assessed with TS the models for weaning weight and ADG included calf temperament score classification, calf sex, and the blood parameter being evaluated as fixed effects; year and calf sire were included as random effects. The models for weaning weight and ADG also included cow age and calf age as covariates.

The models used to determine the effects of cow age and cow temperament on calf serum concentrations of TP, IgG, and CS included cow age classification, cow

temperament classification, calf sex, and their interactions as fixed effects; year and calf sire were included as random effects.

To evaluate the effect of calf temperament on serum concentrations of TP, IgG, and CS statistical models that included calf temperament classification, cow temperament classification, calf sex, and their interactions as fixed effects were used. These models also included calf sire and year as random effects and cow age as a covariate.

To evaluate the effect of calf temperament on ultrasound carcass traits models included calf temperament classification, cow temperament classification, calf sex, and their interactions as fixed effects; calf age and cow age were included as covariates. The models also included calf sire and year as random effects.

Least square means were separated using the LSD procedures of SAS ($\alpha = 0.05$). Pearson's correlation coefficients were calculated to evaluate the relationship between measurements of temperament, blood parameters, and performance measures.

Results

Calf Performance and Temperament. As expected, bull calves (196.20 ± 8.76 kg) were heavier than heifer calves (180.16 ± 8.78 kg) at weaning ($P < 0.001$). Additionally, bull calves (0.95 ± 0.02 kg/d) had greater ADG from birth to weaning than did heifer calves (0.88 ± 0.02 kg/d; $P < 0.001$). For post-weaning performance bull calves (0.58 ± 0.15 kg/d) continued to gain more weight per d than heifer calves (0.42 ± 0.15 kg/d; $P < 0.001$).

Calves were classified based on temperament as assessed by EV at d 21 to 24 of age. Mean EVs were 0.33 ± 0.13 m/s, 1.14 ± 0.42 m/s, 2.65 ± 0.52 m/s for calm, intermediate, and temperamental classifications respectively. Calf temperament as assessed by EV at d21 to 24 had no affect on weaning weight ($P = 0.45$), ADG from birth to weaning ($P = 0.44$), or ADG from weaning to 56 d post-weaning ($P = 0.52$; Table 2.1).

However, there was an affect of calf temperament as assessed by TS on weaning weight ($P = 0.04$). Intermediate (191.69 ± 2.80 kg) calves were heavier than temperamental (184.41 ± 3.51 kg) calves but there was no difference between calm (191.44 ± 4.66 kg) and intermediate or temperamental. Similarly, TS had an affect ($P = 0.03$) on ADG from birth to weaning with intermediate calves (0.92 ± 0.02 kg/d) gaining more than temperamental calves (0.88 ± 0.02 kg/d). There was no difference in ADG from birth to weaning between calm and intermediate ($P = 0.88$) or calm and temperamental calves ($P = 0.08$). Additionally, calf TS had no affect ($P = 0.11$) on ADG from weaning to 56 d post-weaning (Table 2.2).

Table 2.1. The relationship between calf temperament classification at 21 to 24 d of age (EV) and weaning weight, ADG from birth to weaning, and ADG from weaning to 56 d post-weaning in Brahman calves[a]

	Calf Temperament Classification[b]						
	Calm	n	Intermediate	n	Temperamental	n	*P* =
Weaning weight, kg	187.4 ± 4.0	43	191.7 ± 3.0	203	191.4 ± 3.7	54	0.45
ADG, pre-weaning, kg/d[c]	0.90 ± 0.02	43	0.92 ± 0.02	203	0.92 ± 0.02	54	0.44
ADG, post-weaning, kg/d[d]	0.45 ± 0.15	43	0.50 ± 0.15	203	0.50 ± 0.15	54	0.52

[a]Data are pooled for 2006, 2007, 2008 calf crops

[b]Calm = EV was < 1 SD below the mean; Intermediate = EV was ± 1 SD above or below the mean; Temperamental = EV was > 1 SD above the mean.

[c]ADG, pre-weaning = ADG from birth to weaning.

[d]ADG, post-weaning = ADG from weaning to 56 d post-weaning

Table 2.2. The relationship between calf temperament classification (TS) and weaning weight, ADG from birth to weaning, and ADG from weaning to 56 d post-weaning in Brahman calves[a]

	Calf Temperament Classification[b]						
	Calm	n	Intermediate	n	Temperamental	n	*P* =
Weaning weight, kg	191.44 ± 4.66[e,f]	45	191.69 ± 2.80[e]	196	184.41 ± 3.51[f]	59	0.04
ADG, pre-weaning, kg/d[c]	0.92 ± 0.03[e,f]	45	0.92 ± 0.02[e]	196	0.88 ± 0.02[f]	59	0.03
ADG, post-weaning, kg/d[d]	0.49 ± 0.15	45	0.52 ± 0.15	196	0.44 ± 0.15	59	0.11

[a]Data are pooled for 2006, 2007, 2008 calf crops
[b]Temperament classification = TS average from 28 d prior to weaning and at weaning; Calm = TS was < 1 SD below the mean; Intermediate = TS was ± 1 SD above and below the mean; Temperamental = TS was > 1 SD above the mean.
[c]ADG, pre-weaning = ADG from birth to weaning.
[d]ADG, post-weaning = ADG from weaning to 56 d post-weaning
[e,f]Means within a row without a common superscript differ ($P < 0.05$).

Partial correlations between measures of performance and temperament assessments are presented in Table 2.3. Weaning weight was strongly correlated (r = 0.72; $P < 0.001$) with ADG from birth to weaning but only moderately correlated (r = 0.21; $P < 0.001$) with ADG from weaning to 56 d post-weaning. A moderate correlation (r = 0.23; $P < 0.001$) existed between ADG from birth to weaning and ADG from weaning to 56 d post-weaning. Additionally, WW had a weak negative correlation (r = -0.12; $P = 0.04$) with TS. Average daily gain from birth to weaning had a moderate negative correlation (r = -0.21; $P < 0.001$) with TS. Exit velocity at d 21 to 24 was moderately correlated (r = 0.48; $P < 0.001$) with TS. This correlation between the measures of temperament suggests that EV can be used early in life for temperament evaluation, but may not be reliable enough to implement as the sole selection tool.

Table 2.3. Partial correlations between measures of performance and temperament assessments in Brahman calves

Trait[a]	WW	ADG, pre-weaning	ADG, post-weaning	EV d 21 to 24	TS
WW	1.00	0.72[b]	0.21[b]	-0.03	-0.12[c]
ADG, pre-weaning[b]		1.00	0.23[b]	-0.04	-0.21[b]
ADG, post-weaning[c]			1.00	0.07	-0.00
EV d 21 to 24				1.00	0.48[b]
TS					1.00

[a]WW = weaning weight; ADG, preweaning = ADG from birth to weaning; ADG, post-weaning = ADG from weaning to 56 d post-weaning; EV = exit velocity; TS = temperament score.
[b]Correlations are different than zero ($P < 0.01$).
[c]Correlations are different than zero ($P < 0.05$).

Calf Performance and Blood Parameters. Blood samples were collected from Brahman calves at approximately 24 h after birth and at d 21 to 24 of age; serum was harvested and analyzed for TP, IgG, and CS to evaluate the effects of these parameters on performance. Calves were classified based on each parameter; calves 1 SD above the mean classified as high, those 1 SD below the mean were classified as low and all remaining were classified as intermediate. Classifications were conducted within year and then the data was pooled for the final analysis. Summary statistics for blood parameters measured at 24 h and d 21 to 24 for the 2006, 2007, and 2008 calf crops are presented in Tables 2.4, 2.5, 2.6 respectively.

Weaning weight was affected ($P = 0.046$) by TP at 24 h. Calves classified as high (195.57 ± 3.68 kg) were numerically heavier than intermediates (189.89 ± 2.81 kg) and significantly heavier than calves classified as low (186.62 ± 3.56). Additionally, IgG classification at 24 h had an affect ($P = 0.03$) on weaning weight, with calves classified as high (197.15 ± 3.79 kg) being heavier than intermediate (189.03 ± 2.85 kg) and low (188.31 ± 4.24 kg) calves. There was no difference ($P = 0.84$) in weaning weight between the intermediate and low IgG calves.

Cortisol classification at 24 h had no affect ($P = 0.64$) on weaning weight. Calf weaning weight was not affected by blood parameter classifications at d 21 to 24 for TP ($P = 0.35$), IgG ($P = 0.18$), or CS ($P = 0.99$; Table 2.7).

Table 2.4. Summary statistics for blood parameter classifications in Brahman calves born in the Spring of 2006[a]

	Blood Parameter Classification[b]								
	High	Range	n	Intermediate	Range	n	Low	Range	n
24 h									
Total protein, g/dL	8.79 ± 0.42	8.37 – 9.76	20	7.16 ± 0.61	5.77 – 8.23	69	5.03 ± 0.37	4.59 – 5.74	22
IgG, mg/mL	58.95 ± 11.68	46.00 – 82.00	11	30.17 ± 7.00	14.66 – 45.15	80	4.09 ± 4.08	0.14 – 11.56	19
Cortisol, ng/mL	13.46 ± 3.54	10.35 – 22.64	15	5.75 ± 1.98	2.95 – 10.00	82	2.26 ± 0.55	1.30 – 2.88	14
d 21 to 24									
Total protein, g/dl	6.84 ± 0.24	6.54 – 7.12	13	5.94 ± 0.33	5.35 – 6.50	80	5.01 ± 0.29	4.25 – 5.33	18
IgG, mg/mL	44.49 ± 7.64	35.23 – 62.86	15	22.34 ± 5.88	11.78 – 34.15	72	8.25 ± 2.66	1.51 – 11.36	24
Cortisol, ng/dL	8.05 ± 2.75	6.17 – 15.93	13	3.52 ± 1.23	1.68 – 6.15	82	1.19 ± 0.37	0.60 – 1.65	16

[a]Data are presented as mean ± SD.
[b]High = concentration was > 1 SD above the mean; Intermediate = concentration was ± 1 SD above and below the mean; Low = concentration was < 1 SD below the mean.

Table 2.5. Summary statistics for blood parameter classification in Brahman calves born in the Spring of 2007[a]

	Blood Parameter Classification[b]								
	High	Range	n	Intermediate	Range	n	Low	Range	n
24 h									
Total protein, g/dL	9.31 ± 0.45	8.71 – 10.41	17	7.44 ± 0.72	6.06 – 8.69	73	5.32 ± 0.59	4.25 – 6.02	18
IgG, mg/mL	60.59 ± 15.15	43.84 – 98.02	17	19.41 ± 9.22	6.66 – 42.20	85	4.98 ± 1.63	2.34 – 6.31	6
Cortisol, ng/mL	22.19 ± 5.96	17.14 – 33.55	13	10.88 ± 2.83	6.05 – 16.87	83	4.08 ± 1.19	1.46 – 5.76	12
d 21 to 24									
Total protein, g/dl	7.05 ± 0.48	6.68 – 8.30	16	5.99 ± 0.38	5.31 – 6.56	74	4.92 ± 0.37	3.78 – 5.21	18
IgG, mg/mL	41.25 ± 12.76	27.62 – 71.50	12	13.19 ± 6.29	4.06 – 26.29	91	3.38 ± 0.28	3.07 – 3.77	5
Cortisol, ng/dL	6.99 ± 0.84	6.06 – 9.27	22	3.75 ± 1.00	2.01 – 5.96	66	1.24 ± 0.40	0.33 – 1.85	20

[a]Data are presented as mean ± SD.

[b]High = concentration was > 1 SD above the mean; Intermediate = concentration was ± 1 SD above and below the mean; Low = concentration was < 1 SD below the mean.

Table 2.6. Summary statistics for blood parameter classification in Brahman calves born in the Spring of 2008[a]

	Blood Parameter Classification[b]								
	High	Range	n	Intermediate	Range	n	Low	Range	n
24 h									
Total protein, g/dL	9.09 ± 0.47	8.53 – 9.94	10	7.34 ± 0.65	6.00 – 8.31	56	5.38 ± 0.40	4.82 – 5.95	15
IgG, mg/mL	66.97 ± 10.71	51.68 – 83.83	14	24.00 ± 11.42	9.71 – 48.80	60	6.11 ± 2.43	2.75 – 8.86	7
Cortisol, ng/mL	19.30 ± 1.17	17.68 – 23.35	11	14.42 ± 1.71	10.68 – 17.28	55	9.12 ± 1.20	6.16 – 10.24	15
d 21 to 24									
Total protein, g/dl	7.67 ± 1.35	6.89 – 10.67	7	6.09 ± 0.48	5.23 – 6.86	62	5.00 ± 0.19	4.67 – 5.18	12
IgG, mg/mL	57.71 ± 15.36	33.50 – 78.81	7	15.30 ± 7.62	4.23 – 32.31	72	2.74 ± 0.02	2.46 – 2.49	2
Cortisol, ng/dL	4.75 ± 0.65	4.11 – 6.36	16	2.63 ± 0.65	1.66 – 3.95	53	1.02 ± 0.28	0.62 – 1.53	12

[a]Data are presented as mean ± SD.

[b]High = concentration was > 1 SD above the mean; Intermediate = concentration was ± 1 SD above and below the mean; Low = concentration was < 1 SD below the mean.

Table 2.7. The relationship between blood parameter classification and weaning weight in Brahman calves (kg)[a]

	Blood Parameter Classification[b]						
	High	n	Intermediate	n	Low	n	*P* =
24 h							
Total protein	195.57 ± 3.68[c]	47	189.89 ± 2.81[c,d]	198	186.62 ± 3.56[d]	55	0.046
IgG	197.15 ± 3.79[c]	42	189.03 ± 2.85[d]	225	188.31 ± 4.24[d]	32	0.03
Cortisol	190.49 ± 4.03	39	189.62 ± 2.95	220	192.59 ± 3.95	41	0.64
d 21 to 24							
Total protein	191.32 ± 4.03	36	190.65 ± 2.90	216	186.59 ± 3.77	48	0.35
IgG	190.37 ± 4.20	34	190.46 ± 3.00	235	183.89 ± 4.44	31	0.18
Cortisol	190.25 ± 3.70	51	190.06 ± 3.02	201	190.15 ± 3.78	48	0.99

[a]Data are pooled for 2006, 2007, 2008 calf crops.
[b]High = concentration was > 1 SD above the mean; Intermediate = concentration was ± 1 SD above and below the mean; Low = concentration was < 1 SD below the mean.
[c,d]Means within a row without a common superscript differ ($P < 0.05$).

Similar to weaning weight data, ADG from birth to weaning was affected (P = 0.047) by TP at 24 h. Calves classified as high (0.94 ± 0.02 kg/d) gained significantly more than intermediate (0.91 ± 0.02 kg/d) or low (0.90 ± 0.02 kg/d) calves. There was no difference in ADG between the intermediate and low classifications. There was a trend (P = 0.09) for IgG classification at 24 h to have an effect on calf ADG from birth to weaning. Calves classified as high (0.94 ± 0.02 kg/d) tended to gain more than intermediates (0.91 ± 0.02 kg/d) and low (0.90 ± 0.02 kg/d) calves. Calf ADG was not affected by CS (P = 0.77) classification at 24 h. Average daily gain from birth to weaning was not affected by TP (P = 0.57), IgG (P = 0.21), or CS (P = 0.87) measured at d 21 to 24 of age (Table 2.8).

Table 2.8. The relationship between blood parameter classification and ADG from birth to weaning in Brahman calves (kg/d)[a]

	Blood Parameter Classification[b]						
	High	n	Intermediate	n	Low	n	P =
24 h							
Total protein	0.94 ± 0.02[c]	47	0.91 ± 0.02[d]	198	0.90 ± 0.02[d]	55	0.047
IgG	0.94 ± 0.02	42	0.91 ± 0.02	225	0.90 ± 0.02	32	0.09
Cortisol	0.91 ± 0.02	39	0.91 ± 0.02	220	0.92 ± 0.02	41	0.77
d 21 to 24							
Total protein	0.92 ± 0.02	36	0.91 ± 0.02	216	0.90 ± 0.02	48	0.57
IgG	0.93 ± 0.02	34	0.91 ± 0.02	235	0.88 ± 0.02	31	0.21
Cortisol	0.92 ± 0.02	51	0.91 ± 0.02	201	0.91 ± 0.02	48	0.87

[a]Data are pooled for 2006, 2007, 2008 calf crops.
[b]High = concentration was > 1 SD above the mean; Intermediate = concentration was ± 1 SD above or below the mean; Low = concentration was < 1 SD below the mean.
[c,d]Means within a row without a common superscript differ (P < 0.05).

Post-weaning performance was assessed using ADG from weaning to 56 d post-weaning. There was no affect of TP ($P = 0.20$), IgG ($P = 0.13$), or CS ($P = 0.62$) measured at 24 h on post-weaning ADG.

Additionally, post-weaning ADG was not affected by TP ($P = 0.95$) or CS ($P = 0.20$) measured at d 21 to 24 of age. However, there was a tendency ($P = 0.06$) for IgG at d 21 to 24 to have an affect on ADG with calves classified as high (0.55 ± 0.15 kg/d) tending to gain more weight/d than intermediates (0.47 ± 0.14 kg/d), and low (0.56 ± 0.15 kg/d) calves tending to gain more than intermediate calves (Table 2.9).

Table 2.9. The relationship between blood parameter classification and ADG from weaning to 56 d post-weaning in Brahman calves (kg/d)[a]

	Blood Parameter Classification[b]						
	High	n	Intermediate	n	Low	n	*P* =
24 h							
Total protein	0.52 ± 0.15	47	0.47 ± 0.15	198	0.53 ± 0.15	55	0.20
IgG	0.48 ± 0.15	42	0.48 ± 0.14	225	0.57 ± 0.15	32	0.13
Cortisol	0.49 ± 0.15	39	0.48 ± 0.15	220	0.52 ± 0.15	41	0.62
d 21 to 24							
Total protein	0.49 ± 0.15	36	0.49 ± 0.15	216	0.48 ± 0.15	48	0.95
IgG	0.55 ± 0.15	34	0.47 ± 0.14	235	0.56 ± 0.15	31	0.06
Cortisol	0.53 ± 0.15	51	0.49 ± 0.15	201	0.45 ± 0.15	48	0.20

[a]Data are pooled for 2006, 2007, 2008 calf crops
[b]High = concentration was > 1 SD above the mean; Intermediate = concentration was ± 1 SD above or below the mean; Low = concentration was < 1 SD below the mean.

Blood Parameters. Interactions between cow temperament and calf sex as well as cow temperament and calf temperament are reported below. In addition to the appropriate interaction means, main effect means are also reported for calf sex, cow temperament, and calf temperament. There was an interaction of cow temperament by calf sex observed for several of the blood parameters measured at both time points. Serum TP concentrations at 24 h were affected ($P = 0.01$; Figure 2.1) by this interaction. Bull calves born to calm cows had significantly higher concentrations of TP than did heifer calves born to calm cows ($P = 0.02$), bull calves born to intermediate cows ($P = 0.03$), and bull calves born to temperamental cows ($P = 0.05$). Additionally, heifer calves born to calm cows had significantly ($P = 0.04$) lower concentrations of TP than did heifer calves born to intermediate cows. Bull calves had numerically lower concentrations of TP than did heifer calves born to intermediate and temperamental cows.

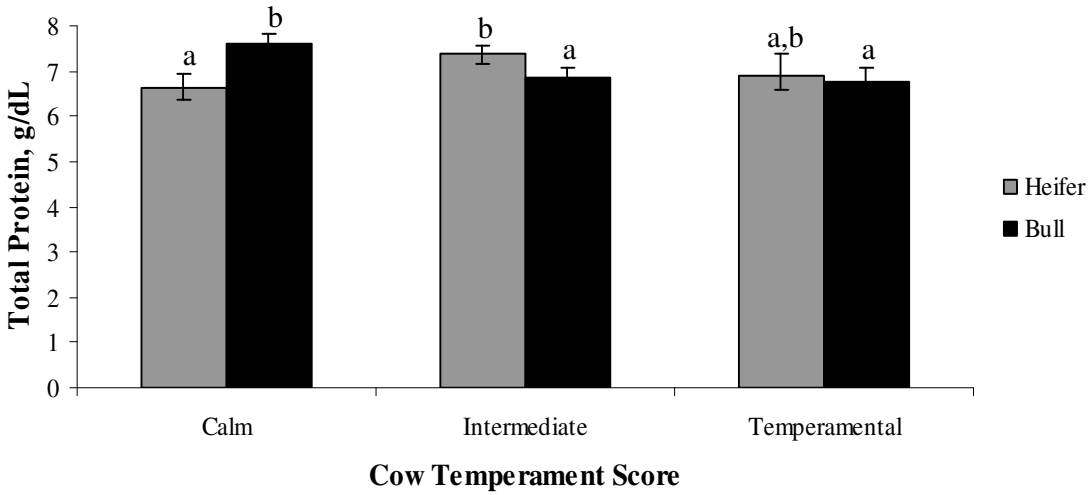

Figure 2.1. The effect of cow temperament by calf sex on serum TP concentrations at 24 h after birth in Brahman calves. Concentrations of serum TP were not affected by calf sex or cow temperament. However, TP concentrations were affected (P = 0.01) by the interaction with bull calves born to calm cows having greater concentrations of TP than heifer calves.
[a,b]Means without a common superscript differ ($P < 0.05$).

Serum IgG concentrations at 24 h after birth were also affected (P = 0.04; Figure 2.2) by the interaction of cow temperament and calf sex. Heifer calves born to intermediate cows had significantly (P = 0.02) greater concentrations of IgG than did bull calves born to intermediate cows. Additionally, bull calves born to calm cows had significantly (P = 0.04) higher concentrations of IgG than did bull calves born to intermediate cows. There was no effect of the interaction of cow temperament by calf sex on TP (P = 0.) or CS (P = 0.70) at 24 h after birth.

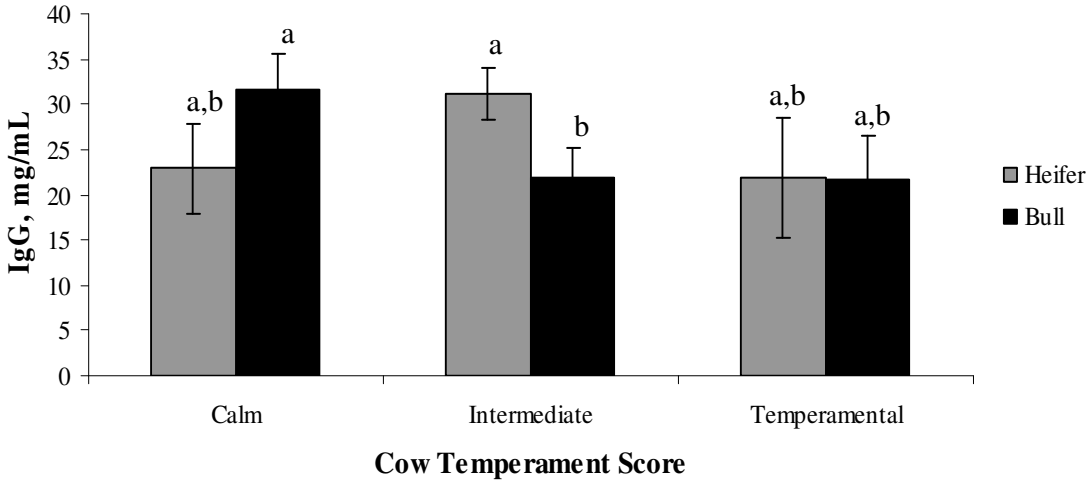

Figure 2.2. The effect of cow temperament by calf sex on serum IgG concentrations at 24 h after birth in Brahman calves. Concentrations of serum IgG were not affected by calf sex or cow temperament. However, IgG concentrations were affected ($P = 0.04$) by the interaction, with heifer calves born to intermediate cows having greater concentrations of IgG than bulls calves. [a,b]Means without a common superscript differ ($P < 0.05$).

Of the parameters evaluated at d 21 to 24 of age, serum TP concentrations also were affected ($P = 0.05$; Figure 2.3) by the interaction of cow temperament by calf sex. Bull calves born to calm cows had significantly ($P = 0.01$) greater concentrations of TP than did heifer calves born to calm cows. Heifer calves born to calm cows had significantly lower concentrations of TP than heifer calves born to intermediate cows ($P < 0.01$) and male calves born to intermediate cows ($P = 0.02$). There was no effect of the interaction of cow temperament by calf sex on IgG ($P = 0.37$) or CS ($P = 0.56$).

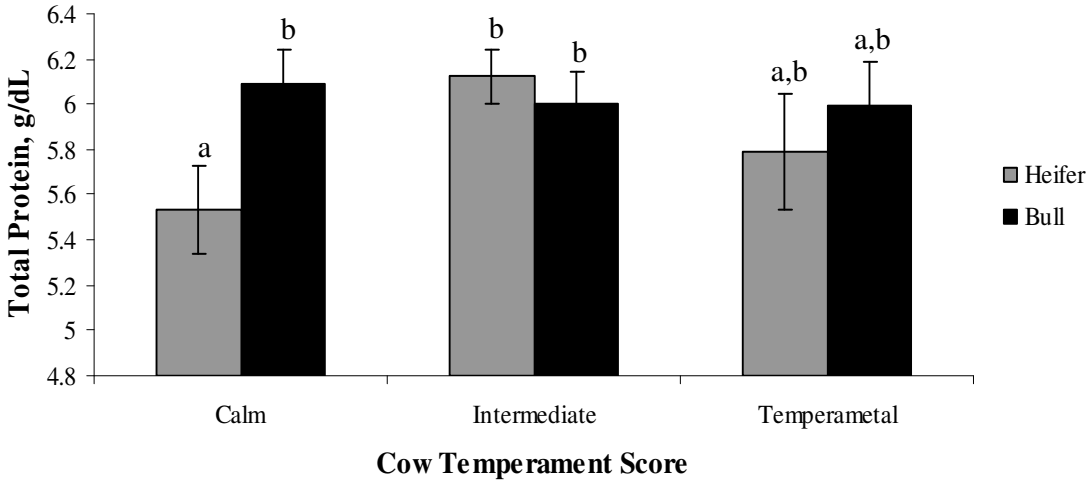

Figure 2.3. The effect of cow temperament by calf sex on serum TP concentrations at d 21 to 24 of age in Brahman calves. Concentrations of serum TP were no affected by calf sex or cow temperament. However, TP concentrations were affected ($P = 0.049$) by the interaction, with bull calves born to calm cows having greater concentrations of TP than heifer calves.
[a,b]Means without a common superscript differ ($P < 0.05$).

Cow temperament had no affect on serum TP ($P = 0.65$), IgG ($P = 0.50$), or CS ($P = 0.27$) 24 h after birth in the calf (Table 2.10). Similarly, there was no affect of cow temperament on serum TP ($P = 0.16$), IgG ($P = 0.37$), or CS ($P = 0.71$) at d 21 to 24 in the calf (Table 2.10).

In addition to cow temperament, cow age was also included in the model for blood parameter analysis. Cow age had a significant affect ($P = 0.04$) on calf serum IgG concentration 24 h after birth. Calves born to young (30.25 ± 2.03 mg/mL) cows had greater IgG concentrations than calves from aged cows (19.44 ± 5.06 mg/mL). Calves from mature cows were not different from young or aged cows. There was no effect of cow age on TP ($P = 0.18$) or CS ($P = 0.57$) 24 h after birth in the calf. Additionally, cow age had no effect on TP ($P = 0.14$), IgG ($P = 0.60$), or CS ($P = 0.93$) evaluated 21 to 24 d of age in the calf (Table 2.11).

There were no differences in serum TP ($P = 0.71$), IgG ($P = 0.93$), or CS ($P = 0.47$) concentrations 24 h after birth between bull and heifer calves. Interestingly, the only difference observed between calf sexes among the blood parameters at d 21 to 24 of age was CS. Heifer calves had significantly ($P = 0.04$) greater CS concentrations than did bull calves. Serum concentrations of TP ($P = 0.12$) and IgG ($P = 0.31$) at d 21 to 24 of age were not affected by calf sex (Table 2.12).

Table 2.10. The effect of cow temperament on serum blood parameters in Brahman calves[a]

	Cow Temperament Score[b]						
	Calm	n	Intermediate	n	Temperamental	n	*P* =
24 h							
Total protein, g/dL	7.11 ± 0.21	80	7.12 ± 0.14	151	6.84 ± 0.28	69	0.65
IgG, mg/mL	27.31 ± 3.26	80	26.50 ± 2.40	150	21.73 ± 4.22	69	0.50
Cortisol, ng/mL	9.61 ± 2.43	80	10.97 ± 2.37	151	11.12 ± 2.51	69	0.27
d 21 to 24							
Total protein, g/dL	5.81 ± 0.13	80	6.06 ± 0.10	151	5.89 ± 0.17	69	0.16
IgG, mg/mL	18.81 ± 2.08	80	19.30 ± 2.34	151	14.88 ± 3.38	69	0.37
Cortisol, ng/mL	3.44 ± 0.37	80	3.69 ± 0.37	151	3.38 ± 0.52	69	0.71

[a]Data are pooled for 2006, 2007, 2008 calf crops.

Table 2.11. The effect of cow age classification on serum blood parameters in Brahman calves[a]

	Cow Age Classification[b]						
	Young	n	Mature	n	Aged	n	*P* =
24 h							
Total protein, g/dL	7.27 ± 0.11	153	7.19 ± 0.12	125	6.61 ± 0.34	22	0.18
IgG, mg/mL	30.25 ± 2.03[c]	153	25.85 ± 2.15[c,d]	124	19.44 ± 5.06[d]	22	0.04
Cortisol, ng/mL	10.46 ± 2.35	153	11.00 ± 2.36	125	10.14 ± 2.61	22	0.57
d 21 to 24							
Total protein, g/dL	5.92 ± 0.09	153	6.07 ± 0.10	125	5.77 ± 0.20	22	0.14
IgG, mg/mL	18.71 ± 2.15	153	19.00 ± 2.21	125	15.28 ± 3.92	22	0.60
Cortisol, ng/mL	3.56 ± 0.34	153	3.46 ± 0.35	125	3.50 ± 0.61	22	0.93

[a]Data are pooled for 2006, 2007, 2008 calf crops.
[b]Young = 2 to 4 years; Mature = 5 to 9 years; Aged = ≥ 10 years.
[c,d]Means within a row without a common superscript differ ($P < 0.05$).

Table 2.12. The effect of calf sex on serum blood parameters in Brahman calves[a]

	Calf Sex				
	Heifer	n	Bull	n	*P* =
24 h					
Total protein, g/dL	6.97 ± 0.20	143	7.07 ± 0.16	157	0.71
IgG, mg/mL	25.34 ± 3.11	143	25.03 ± 2.58	156	0.93
Cortisol, ng/mL	10.85 ± 2.42	143	10.22 ± 2.38	157	0.47
d 21 to 24					
Total protein, g/dL	5.82 ± 0.13	143	6.03 ± 0.11	157	0.12
IgG, mg/mL	16.37 ± 2.72	143	18.96 ± 2.43	157	0.31
Cortisol, ng/mL	3.89 ± 0.42	143	3.11 ± 0.38	157	0.04

[a]Data are pooled for 2006, 2007, 2008 calf crops.

Calf Temperament and Blood Parameters. Calves classified as calm, intermediate, or temperamental based on EV measurements at d 21 to 24. Serum TP concentrations at 24 h were affected ($P = 0.02$; Figure 2.4) by the interaction of calf temperament classification at d 21 to 24 of age and cow temperament. Intermediate calves born to temperamental cows had lower concentrations of TP than temperamental calves which were similar to calm calves born to temperamental cows. No other parameter evaluated at 24 h or d 21 to 24 of age was affected by the interaction of calf temperament at d 21 to 24 and cow temperament ($P > 0.05$).

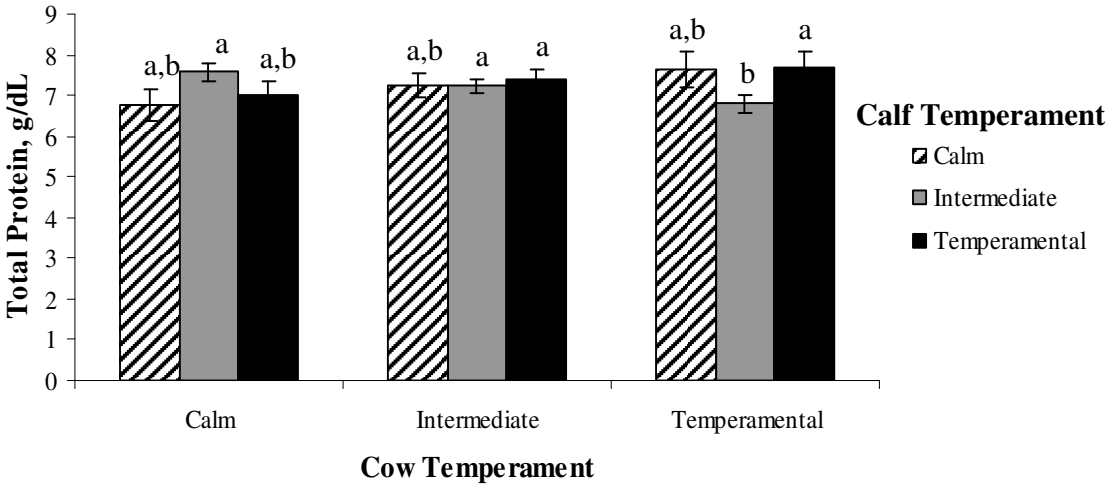

Figure 2.4. The effect of cow temperament by calf temperament as assessed by EV at d 21 to 24 on serum TP concentrations 24 h after birth in Brahman calves. Concentrations of serum TP were not affected by cow temperament or calf temperament. However, TP concentrations were affected ($P = 0.02$) by the interaction, with intermediate calves born to temperamental cows having lower concentrations of TP than temperamental but similar concentrations to calm calves.

Calf temperament assessed at d 21-24 of age using EV had no effect on serum TP ($P = 0.70$) or IgG ($P = 0.77$) at 24 h (Table 2.13). There was a trend ($P = 0.08$) for calf temperament to have an affect on CS concentrations at 24 h. Calm calves had numerically greater concentrations of CS than did intermediate or temperamental calves. Furthermore, at d 21 to 24 of age serum concentrations of TP ($P = 0.31$) nor IgG ($P = 0.25$) were affected by temperament. Cortisol concentrations were affected ($P = 0.01$) by calf temperament at d 21 to 24. Calm and intermediate calves had lower concentrations of CS than did the temperamental calves. There was no difference between calm and intermediate calves for concentrations of CS at d 21 to 24 of age (Table 2.13).

Calves were reevaluated for temperament and assigned a temperament score (TS) which was calculated using the average of PS and EV (TS = ((EV+PS/2)); King et al., 2006).

Table 2.13. The effect of calf temperament classification (EV) at 21 to 24 d of age on blood parameters in Brahman calves[a]

	Calf Temperament Classification[b]						
	Calm	n	Intermediate	n	Temperamental	n	*P* =
24 h							
Total protein, g/dL	7.21 ± 0.23	43	7.20 ± 0.14	203	7.37 ± 0.20	54	0.70
IgG, mg/mL	25.95 ± 3.27	43	28.34 ± 1.75	202	27.37 ± 2.89	54	0.77
Cortisol, ng/mL	12.13 ± 2.34	43	10.40 ± 2.25	203	10.35 ± 2.31	54	0.08
d 21 to 24							
Total protein, g/dL	5.86 ± 0.14	43	5.98 ± 0.10	203	6.10 ± 0.13	54	0.31
IgG, mg/mL	18.63 ± 2.74	43	17.96 ± 1.95	203	21.46 ± 2.52	54	0.25
Cortisol, ng/mL	3.41 ± 0.43[c]	43	3.34 ± 0.32[c]	203	4.27 ± 0.40[d]	54	0.01

[a]Data are pooled for 2006, 2007, 2008 calf crops

[b] Calm = TS was < 1 SD below the mean; Intermediate = TS was ± 1 SD above or below the mean; Temperamental = TS was > 1 SD above the mean.

[c,d]Means within a row without a common superscript differ (P < 0.05).

Average TS from 28 d prior to weaning and at weaning was then assigned to each calf. Calf TS had no affect on serum TP ($P = 0.69$) at 24 h. There was a trend for calf TS to have an affect on serum IgG concentration at 24 h. Temperamental calves (32.51 ± 2.73 mg/mL) had greater concentrations of IgG than did intermediates (26.93 ± 1.71 mg/mL) which had greater concentrations than calm calves (23.63 ± 4.06 mg/mL). Additionally, calf TS tended to have an affect on serum CS concentrations at 24 h. Temperamental calves had greater concentrations of CS than did calm calves and were slightly greater than intermediates. Serum TP ($P = 0.87$) and IgG ($P = 0.80$) at d 21 to 24 of age were not affected by calf TS. There was a significant affect ($P = 0.001$) of calf TS on serum CS concentrations at d 21 to 24 of age. While calm calves (2.71 ± 0.52 ng/mL) had numerically lower CS concentrations than intermediates (3.37 ± 0.36 ng/mL) both classes were lower than temperamental calves (4.31 ± 0.42 ng/ml; Table 2.14).

Table 2.14. The effect of calf temperament score (TS) classification measured at 28 d prior to and at weaning on blood parameters in Brahman calves[a]

	Calf Temperament Classification[b]						
	Calm	n	Intermediate	n	Temperamental	n	*P* =
24 h							
Total protein, g/dL	7.03 ± 0.27	45	7.18 ± 0.11	196	7.30 ± 0.18	59	0.69
IgG, mg/mL	23.63 ± 4.06	45	26.93 ± 1.71	195	32.51 ± 2.73	59	0.09
Cortisol, ng/mL	8.57 ± 2.53	45	10.84 ± 2.37	196	10.71 ± 2.42	59	0.08
d 21 to 24							
Total protein, g/dL	5.92 ± 0.16	45	5.96 ± 0.08	196	6.01 ± 0.11	59	0.87
IgG, mg/mL	18.54 ± 3.30	45	18.34 ± 2.01	196	19.73 ± 2.51	59	0.80
Cortisol, ng/mL	2.71 ± 0.52[c]	45	3.37 ± 0.36[c]	196	4.31 ± 0.42[d]	59	0.001

[a]Data are pooled for 2006, 2007, 2008 calf crops

[b] Calm = TS was < 1 SD below the mean; Intermediate = TS was ± 1 SD above or below the mean; Temperamental = TS was > 1 SD above the mean.

[c,d]Means within a row without a common superscript differ (P < 0.05).

Calf Temperament and Ultrasound Carcass Traits. Ultrasound measures were collected at weaning and 56 d post-weaning to evaluate the effects of temperament on ultrasound measurements. Calf TS had no effect on measures of REA ($P = 0.67$), REA:CWT ($P = 0.13$), IMF ($P = 0.75$), or back fat ($P = 0.49$) at weaning. Interestingly, calf TS tended ($P = 0.08$) to have an effect on rump fat at weaning. Temperamental calves (0.52 ± 0.16 cm) tended to have less rump fat than intermediate (0.60 ± 0.16 cm) or calm calves (0.59 ± 0.16 cm; Table 2.15).

Table 2.15. The effect of calf temperament score (TS) classification measured at 28 d prior to and at weaning on ultrasound carcass traits at weaning in Brahman calves[a]

	Calf Temperament Classification[b]						
Trait[c]	Calm	n	Intermediate	n	Temperamental	n	*P* =
REA, cm^2	35.92 ± 2.60	45	36.97 ± 2.36	195	36.67 ± 2.44	59	0.67
REA:CWT	1.32 ± 0.09	45	1.36 ± 0.08	195	1.40 ± 0.09	59	0.13
IMF, %	2.10 ± 0.21	45	2.02 ± 0.17	195	2.07 ± 0.19	59	0.75
Back fat, cm	0.23 ± 0.01	45	0.22 ± 0.01	195	0.21 ± 0.01	59	0.49
Rump fat, cm	0.59 ± 0.16	45	0.60 ± 0.16	195	0.52 ± 0.16	59	0.08

[a]Data are pooled for 2006, 2007, 2008 calf crops
[b]Calm = TS was < 1 SD below the mean; Intermediate = TS was ± 1 SD above or below the mean; Temperamental = TS was > 1 SD above the mean.
[c]REA = ribeye area, REA:CWT = ribeye area per 45.36 kg, IMF = intramuscular fat .

When evaluating the effect of TS on ultrasound carcass traits at 56 d post-weaning there was no effect of TS on REA ($P = 0.62$), REA:CWT ($P = 0.57$), IMF ($P = 0.77$), or back fat ($P = 0.11$). There was a significant effect ($P = 0.01$) of calf TS on rump fat. Calves classified as temperamental (0.38 ± 0.03 cm) had significantly less rump fat than intermediate (0.43 ± 0.03 cm), but were not different from calm calves (0.42 ± 0.04 cm). There was no significant difference in rump fat between calm and intermediate calves at 56 d post-weaning (Table 2.16).

Table 2.16. The effect of calf temperament score (TS) classification measured at 28 d prior to weaning and at weaning on ultrasound carcass traits at 56 d post-weaning in Brahman calves[a]

	Calf Temperament Classification[b]						
Trait[c]	Calm	n	Intermediate	n	Temperamental	n	*P* =
REA, cm^2	36.71 ± 1.71	45	36.86 ± 1.27	195	35.99 ± 1.42	59	0.62
REA:CWT	1.18 ± 0.04	45	1.19 ± 0.03	195	1.21 ± 0.03	59	0.57
IMF, %	2.77 ± 0.20	45	2.80 ± 0.16	195	2.86 ± 0.18	59	0.77
Back fat, cm	0.25 ± 0.02	45	0.23 ± 0.02	195	0.22 ± 0.02	59	0.11
Rump fat, cm	0.42 ± 0.04[d,e]	45	0.43 ± 0.03[d]	195	0.38 ± 0.03[e]	59	0.01

[a]Data are pooled for 2006, 2007, 2008 calf crops
[b] Calm = TS was < 1 SD below the mean; Intermediate = TS was ± 1 SD above or below the mean; Temperamental = TS was > 1 SD above the mean.
[c]REA = ribeye area, REA:CWT = ribeye area per 45.36 kg, IMF = intramuscular fat .
[d,e]Means within a row without a common superscript differ (P < 0.05).

Discussion

Results from a study conducted by Burdick (2007) using the 2006 calf crop promoted further investigation into the interrelationships among blood parameters, performance, and calf temperament. The results from that study found no affect of calf temperament on ADG during the pre-weaning phase of production. With regard to blood parameters and calf temperament, results from the initial study found no effect of calf temperament on serum Ig concentrations. Additionally, results related to TP and calf performance showed promise as an indicator of future performance in Brahman calves. Given these results further studies were conducted, and all calves evaluated over the three year period were included in the final analysis presented in this thesis.

Calf Performance and Temperament. Calf temperament as assessed at d 21 to 24 of age with EV had no affect on calf growth and performance, pre- or post-weaning. However, calf temperament as assessed with TS prior to weaning and at weaning had a significant affect on calf growth and performance during the pre-weaning phase. Temperamental calves were lighter at weaning and gained less per d from birth to weaning than did calm and intermediate calves. In a feedlot setting temperamental calves gained less weight per d than did calm calves (Burrow, 1997; Fell et al., 1999; Fordyce et al., 1988). Several additional studies by Müller and von Keyserlingk (2006) and Voisinet et al. (1997b) also found that calmer cattle had higher ADG than did more temperamental cattle.

Calf temperament as assessed at d 21 to 24 of age with EV was moderately correlated with calf TS at weaning. These results are in agreement with those reported

by Curley et. al. (2006) in which correlations between temperament assessments over time on Brahman bulls were positively correlated; EV ($r = 0.47$; $P < 0.01$) and PS ($r = 0.52$; $P < 0.0001$). One speculation regarding measures of temperament is that as animals become accustomed to human interaction temperament score would decrease. However, Grandin (1993) reported that behavioral agitation of animals was persistent over time. The results from these studies suggest that EV can be used early in life as an assessment of temperament with moderate consistency through weaning.

Calf Performance and Blood Parameters. Calf weaning weight and ADG from birth to weaning was affected by TP concentration 24 h after birth. Calves classified as high were significantly heavier at weaning and gained more weight per d from birth to weaning than calves classified as low. Reports relating to extensively managed beef calves with regard to passive transfer of immunity are less common than those for intensively managed dairy calves. However, Perino et al., (1993) reported that calves classified as having failure of passive transfer were at greater risk of being ill prior to weaning compared to calves classified as having adequate passive transfer. Wittum and Perino (1995) reported that passive immune status at 24 h did not directly affect calf weaning weight, but the association was indirect through the effect of neonatal morbidity on weaning weight. In addition to weaning weight performance, Wittum and Perino (1995) also reported that plasma protein concentrations at 24 h had an affect on feedlot performance. However this was an indirect affect as a result of decreased incidences of feedlot respiratory morbidity. It is important to note that the herd used in that study had a high rate of twinning and dairy maternal influence. These results and those of the

current study are in agreement with additional literature emphasizing the importance of passive immunity on calf performance.

Serum IgG concentrations at 24 h had a significant effect on calf weaning weight. Calves classified as high were significantly heavier than calves classified as low. These results are in agreement with literature published by Odde (1988) which reported that the correlation observed between IgG concentrations at 24 h and weaning weight reflected the increased resistance to disease and thus improved growth and performance. Similarly, Vann and Baker (2001) evaluated beef calves for the effects of serum Ig concentrations 24 h after birth on growth and performance through weaning. Calves classified as having adequate Ig concentrations were heavier at all weigh d and at weaning than the remaining calves classified as average or inadequate. In the present study serum IgG concentrations 24 h after birth showed a trend for calves classified as high to have greater ADG from birth to weaning than intermediate calves, which gained more weight per d than calves classified as low. These results are similar to those reported by Dewell et al. (2006), during the preweaning period calves with low IgG_1 had higher morbidity rates, higher mortality rates, and lower ADG. Additionally, calves with $\geq$ 2700 mg/dL IgG_1 concentrations weighed an estimated 3.35 kg more at 205 d of age than did calves with lower concentrations. Results from theses studies and the current study emphasize the importance of passive immunity in the neonate and its affects on future performance.

One significant difference between the current study and several previous studies related to passive immunity is the unknown colostral concentration of Ig. In order to

fully characterize the effects of passive transfer of immunity via Ig or TP, colostrum concentrations as well as calf intake are needed. Further studies evaluating the effect of passive immunity on calf performance should incorporate this measure to fully ascertain the link between colostrum consumption, passive immunity, and future performance in the calf.

Serum CS concentrations 24 h after birth had no affect on calf growth and performance through weaning or during the post-weaning period. Given the high concentrations of CS released by the fetus to induce parturition the response of the neonate to stressors may be limited in the early d of life. Serum CS concentrations in the calf decreased from 24 h after birth to d 21 to 24 of age. This decrease in CS is similar to other literature in cattle and pigs in which CS concentrations decreased with the initial 7 d after birth (Brown-Borg et al., 1993; Blum and Hammon, 2000).

Neither CS nor TP at d 21 to 24 of age had an affect on calf weaning weight, ADG from birth to weaning, or ADG from weaning to 56 d post-weaning. However, IgG at d 21 to 24 of age showed a tendency to have an affect on ADG from weaning to 56 d post weaning. This tendency may have been related to a decrease in incidences of illness in the calves.

Several factors including dystocia, twin birth, and age of the dam have been previously reported to be associated with the risk of preweaning morbidity (Wittum et al., 1994). While none of the calves born in this study were twins the herd was not without incidences of dystocia and had a small aged cow population. These factors

could have had an affect on calf performance with regard to passive transfer of immunity early in life.

Blood Parameters. The statistical model used for analysis included the interactions of cow temperament and calf sex for the blood parameters evaluated. There was a significant affect of this interaction on serum TP concentrations 24 h after birth and at d 21 to 24 of age. At both time points, bull calves born to calm cows had significantly higher concentrations of TP than did heifer calves born to calm cows. While the relationship between cow temperament and calf sex as related to serum TP concentrations is not well understood, it is interesting to observe the interaction and speculate about the mechanisms involved. Interestingly, bull calves had numerically higher concentrations of TP 24 h after birth than did heifers. Add this to the numerically higher concentrations of TP in calves born to calm cows when compared to calves born to temperamental cows and while not understood there is an interaction which exists between the cow temperament and calf sex.

Serum IgG concentrations 24 h after birth were also affected by the interaction of cow temperament and calf sex. The current study as well as Vann et al. (1995) found no effect of calf sex on serum Ig concentrations 24 h after birth, leaving the interaction elusive.

Analysis of serum TP, IgG, and CS included cow temperament, cow age classification, calf sex, and their interactions as fixed effects. Cow temperament had no affect on serum TP, IgG, or CS at 24 h or d 21 to 24 of age in Brahman calves. These results are contradictory to those published reports in which cows with poor

temperament produce less milk (Müller and von Keyserlingk, 2006; Drugociu et al., 1977; Breuer et al., 2000). This reduction in milk production would theoretically decrease TP and Ig available for absorption by the neonate.

Cows were classified based on age, those 2 to 4 yr of age classified as young, 5 to 9 classified as mature, and cows 10 yr and older classified as aged. Cow age classification had a significant affect on serum IgG concentrations 24 h after birth. Calves born to young cows had greater IgG concentrations than calves born to mature or aged cows. While limited research has been conducted on the relationship between cow age and Ig concentrations the results from our study are opposing to the results published in dairy cattle. Muller and Ellinger (1981) demonstrated the lack of statistical difference in colostral Ig concentrations based on parity in dairy cows, suggesting that age has no significant effect on colostral Ig concentrations in dairy cows. In that study, no difference was found in their 1st, 2nd, and 4th lactations or greater (59.1 g/L, 62.6 g/L, and 74.9 g/L, respectively); however, cows in their 3rd lactation did have significantly higher Ig concentration (81.5 g/L). While these studies were conducted in dairy cattle it is still interesting to note the lack of statistical difference in colostral Ig concentration, with the numerically highest concentration being found in the 3rd lactation. There was no effect of cow age on TP or CS 24 h after birth or TP, IgG, or CS at d 21 to 24 of age. Other than the work conducted by our group, to our knowledge no other studies have evaluated blood parameters at d 21 to 24 of age and their effect on calf performance. By 21 to 24 d of age the calf's ability to absorb large macro molecules including TP and IgG had disappeared. With gut closure estimates from 12 to 48 hours (Bush et al., 1971; Matte et

al., 1982; Stott et al., 1979b) and the most opportune time for absorption being within the initial 4 hours of life the calf is reliant on its own immune system for protection by 21 to 24 d of age.

Calf sex had no effect on TP, IgG, or CS 24 h after birth in Brahman calves. These results are similar to those reported by Vann et al. (1995) in which calf sex had no effect on serum concentrations of total Ig, IgG, IgG_1, IgG_2, IgM, or IgA.

Interestingly, serum CS concentrations at d 21 to 24 of age were affected by calf sex. Heifer calves had significantly greater concentrations of CS than did bull calves. Previous research conducted by our group has reported higher basal concentrations of CS in heifers than in bulls, suggesting sexual dimorphism between the two genders (Agado et al., 2009; Welsh et al., 2009).

Calf Temperament and Blood Parameters. Calves were evaluated for temperament at d 21 to 24 of age using EV, and then classified as calm, intermediate, or temperamental. While not affected by calf temperament alone, serum TP concentrations 24 h after birth were affected by the interaction of calf temperament at d 21 to 24 of age and cow temperament. Intermediate calves born to temperamental cows had lower concentrations of TP than temperamental calves born to temperamental cows. Calm calves born to temperamental cows had similar concentration of TP 24 h after birth when compared to temperamental calves born to temperamental cows. This interaction while not well understood suggests that the temperament of the cow may play a role in predisposing her offspring to a given temperament which when combined with calf sex have an affect on TP in the calf. An evaluation of temperament or behavioral activity at

24 h for the cow and the calf may assist in understanding this interaction and its future ramification.

Calf temperament at d 21 to 24 had no affect on serum TP or IgG concentrations 24 h after birth or d 21 to 24 of age in Brahman calves. As expected calf serum CS concentrations were elevated 24 h after birth and decreased by 21 to 24 d of age. High serum CS concentrations in the calf at birth are a result of the high concentrations secreted by the fetus to induce parturition (Mastorakos and Ilias, 2003). There was a trend for calf temperament to have an affect on CS concentrations at 24 h. Calm calves had numerically higher concentrations of CS than did intermediate or temperamental calves. While this is contradictory to traditional reports with CS concentrations being associated with temperamental calves, the increased concentrations at 24 h after birth could be partially explained by parturition. By 21 to 24 d of age temperamental calves had significantly higher concentrations of CS than did intermediate or calm calves. These findings at d 21 to 24 are in agreement with the literature; Curley et al. (2006) reported a positive relationship between temperament and serum CS concentrations in Brahman bulls. Additionally, Stahringer et al. (1990) observed a similar relationship in which excitable Brahman heifers had significantly greater concentration of CS than did calmer heifers.

Calf temperament was reevaluated 28 d prior to weaning and at weaning; assessed as the average of PS and EV, and termed TS. Calf TS had no effect on serum TP concentrations 24 h after birth. However, there was a tendency for calf temperament to have an affect on serum IgG concentrations at 24 h, with temperamental calves having

higher concentrations of IgG than did intermediates which had greater concentrations than calm calves. Blood parameters TP and IgG at 21 to 24 d of age were not affected by calf TS. Calf TS tended to have an association with CS concentration 24 h after birth and had a significant association with CS concentrations at d 21 to 24 of age. Calm and intermediate calves had significantly lower concentrations of CS than temperamental calves. Again this is in agreement with the literature reporting a positive relationship between temperament and CS concentrations (Curley et al., 2006; King et al., 2006; Stahringer et al., 1990).

Calf Temperament and Ultrasound Carcass Traits. Ultrasound measures were collected at weaning and 56 d post-weaning to evaluate the effects of calf TS on ultrasound carcass traits. No significant differences were observed among temperament classifications for REA, REA:CWT, IMF or back fat at weaning. There was a tendency for temperament to have an effect on rump fat at weaning, with calm and intermediate calves having increased rump fat when compared to temperamental calves.

Similar to results presented from weaning, ultrasound traits at 56 d post-weaning were largely unaffected by calf temperament. There was no difference in REA, REA:CWT, IMF, or back fat among the temperament classifications. Rump fat was significantly affected by calf temperament; temperamental calves had significantly less rump fat when compared to intermediate calves at 56 d post-weaning. There was no difference between calm and intermediate or intermediate and temperamental calves for rump fat at 56 d post-weaning. Several studies have reported the negative impacts of temperament on economically important traits in beef cattle, including lower weight gain (Burrow and Dillon, 1997; Voisinet et al., 1997b) and tougher meat (King et al., 2006). In those studies temperamental cattle had decreased weight gains and tougher meat when compared to calmer cattle. The result from this study are also contradictory to a recent study which found that as weaning EV increased (cattle became more temperamental) off pasture back fat increased (Behrends et al., 2009).

CHAPTER III

EVALUATION OF RESIDUAL FEED INTAKE AND REPRODUCTIVE DEVELOPMENT IN BRAHMAN BULLS

Introduction

Feed is the largest expenditure related to livestock production. Profitability for the beef cattle producer relies on the concept of decreasing inputs and maximizing outputs. Providing livestock with grain based or forage based feedstuffs is an inevitable part of production, adding to the cost and variability in profit for producers, which encourages the identification of efficient animals without sacrificing performance. Selection methods to identify animals which efficiently utilize the nutrients provided must be implemented in an effort to maximize profitability.

A good measure of feed efficiency accounts for variations in energy required for maintenance and weight gain among individual animals (Arthur et al., 1996). Historically, feed conversion ratio (FCR) has been the most common method of evaluating feed efficiency (Archer et al., 1999; Nkrumah et al., 2004). Feed conversion ratio can be described as the ratio of ADG to DMI (Arthur et al., 2001a; Arthur et al., 1996). Selection for feed efficiency based on FCR has led to an increase in mature cow size, resulting in an increase in maintenance requirement for the herd and thus increasing feed costs per animal to the producer. Additional measures of feed efficiency include partial efficiency of growth, calculated as the ratio of ADG to DMI available for growth, after accounting for maintenance requirements (Hennessy and Arthur, 2004). Partial

efficiency of growth is similar to FCR; however, it accounts for the differences in energy required for both maintenance and growth between animals (Hennessy and Arthur, 2004). Residual feed intake (RFI) is a measure of feed efficiency, introduced by Koch et al. (1963), which identifies those animals that consume less feed than predicted, at a given level of production. Residual feed intake is calculated as the difference between the actual feed consumed and the predicted feed intake. Based on this definition, an animal that consumes less feed than expected has a negative RFI value, while an animal that consumes more feed than expected has a positive RFI value. Selection for feed efficiency based on RFI targets animals with a negative RFI value and in turn an animal with decreased adipose tissue (Herd and Arthur, 2008).

Body composition, specifically the degree of fatness, is directly related to reproductive development. Selection based on RFI, as mentioned earlier, targets those animals with a negative residual, which translated into a decrease in adipose tissue stores. The degree of fatness needed to achieve the onset of puberty has been largely overlooked in males but has been reported in the literature in the female (Short and Bellows, 1971). Increased nutrient intake in prepubertal females initiated the onset of puberty at a younger age with heavier BW than nutritionally restricted females (Short and Bellows, 1971). The interdependent relationship between nutrition and reproduction encourages further research into the potential impacts which selection based on RFI may have on reproductive development and performance. Specifically research related to RFI selection and the potential impacts on reproduction in *Bos indicus* cattle is of

interest. The objective of this study was to evaluate the relationship between RFI classification, temperament, and reproductive development in Brahman bulls.

Materials and Methods

Brahman bulls were evaluated for residual feed intake and reproductive development. Temperament evaluation was also conducted on the bulls prior to weaning and at weaning. Temperament scores were assigned based on the average of exit velocity and pen score ((TS = ((EV+PS)/2)); King et al., 2006). Described below is the design for the 2 experiments, including animal handling, laboratory procedures, and statistical analysis.

Animals & Experimental Design – Residual Feed Intake. Brahman bulls (n = 41) born in the spring of 2007 were used in a 70-d feeding trial in the spring of 2008, using the Calan gate system (American Calan, Northwood, NH) at the Texas AgriLife Research Center in Overton. Bulls were weighed and initially penned as a group, and were fed the test ration at approximately 1.5% of BW (as-fed basis; gross BW) for acclimation to the diet (TDN = 61.7%; Table 3.1). Bulls were fed a 12% CP textured ration. Over a 4 d period the feeding rate was incrementally adjusted until the bulls were consuming 2.65% of BW.

Upon acclimation to the diet, bulls were weighed and assigned to pens based on initial BW and fitted with electronic keys. The keys were worn around their neck and allowed access to their respective feed bunks in the Calan gate system. Bulls were trained over a 7 d period to eat out of the gates, and those that did not train to the gates

were individually penned and fed accordingly. Initially the gates were fixed open, allowing bulls the opportunity to locate feed in the stanchions. The gates were then closed, latches being secured with tape allowing bulls to learn that pressure opened the gate. At the end of the 7 d training period the tape was removed and gates closed, allowing the bulls to open only their individual gate. Bulls were weighed weekly and feed allocations adjusted accordingly, to 2.65% of BW (as-fed basis; gross BW). Body weights were taken on the first d of each new week in the morning prior to feeding. Daily feedings for each bull were weighed out into individual sacks (7 per animal) at the beginning of the week, and stored in containers corresponding to the animal and their respective feed bunk. Bulls were fed one-half of the feed allocation twice daily, in the morning at 0800 h and again in the afternoon at 1600 h. Weekly orts, if any, were collected and weighed at the end of each week.

Following the conclusion of the feeding trial, bulls were pastured at the Texas AgriLife Research Center in Overton and maintained on coastal bermudagrass pasture with corn gluten supplementation at a rate of 0.91 kg per head per d. Bimonthly ejaculations were conducted before, during, and after the feeding trial to evaluate bulls for first sperm, puberty, and subsequent sexual maturity.

Table 3.1. Ingredients and nutrient content of the ration

Ingredient	As fed
Cottonseed hulls, pellets	25.00
Alfalfa, dehydrated	12.50
Soybean meal, 48%	10.47
Rice bran	10.00
Soybean hulls	7.45
Corn, cracked	2.00
Salt	0.85
Calcium carbonate	0.70
Magnesium, 56%	0.46
Potassium/magnesium/sulfate	0.32
855 Calf 2x	0.12
Dairy ADE vit premix	0.04
Vitamin A 30M UG	0.04
Trace mineral premix	0.03
Zinpro 100	0.01
Selenium, 0.06%	0.01
Nutrients (Dry-matter basis):	
CP, %	12.00
TDN, %	61.70
NE_m Mcal/kg	1.36
NE_g Mcal/kg	0.78

Residual Feed Intake Determination. Following the completion of the 70 d feeding trial, 41 bulls were ranked based on RFI. Residual feed intake was determined using the residual between actual and expected feed intake for each bull. Initial BW and ADG were computed using linear regression of BW on d of test using GLM procedures of SAS 9.2 (Statview, SAS Inst., Inc., Cary, NC). Using the initial BW and ADG with an adjustment of 3% for shrink, mid-test body weight was estimated for each bull. All bulls in the test were considered cohorts, RFI was determined as the residual from the

liner regression of feed intake, as mentioned above, on mid-test body weight $^{0.75}$ and ADG using GLM procedures of SAS. Bulls that were efficient had a negative RFI value; those considered inefficient had a positive RFI value.

Ultrasound Carcass Traits. Ultrasound measures were collected at weaning and at the conclusion of the feeding trial on all bulls evaluated for RFI. Measures of REA, REA:CWT (REA:45.36kg), IMF, fat over the 12th rib (Back fat), and rump fat were all collected by the same Ultrasound Guidelines Council certified technician. The Aloka 500V real-time ultrasound machine (Corometrics Medical Systems, Wallingford, CT) was equipped with a 17.2 cm, 3.5 MHz linear transducer which was fitted with a Superflab (Designer Genes Technologies, Inc., Harrison, AR) guide for image capture. Animals were brushed with a curry comb prior to the application of 100% vegetable oil to obtain proper acoustic contact. Images were interpreted using Beef Image Analysis Pro Plus Software 2.0.3 (Designer Genes Technologies, Inc., Harrison, AR).

Bull Classification. Bulls were classified based on RFI using two methods: in method I bulls 0.5 a SD above the mean were classified as inefficient, those 0.5 a SD below the mean were classified as efficient and all remaining were classified as intermediate. For RFI classification method II bulls with a positive RFI value were classified as inefficient and those with a negative RFI value were classified as efficient. Bulls (n = 41) were also classified based on temperament using the average TS collected 28 d prior weaning and at weaning. Bulls 1 SD above the mean were classified as temperamental, bulls 1 SD below the mean were classified as calm, and all remaining were classified as intermediate.

Insulin-like Growth Factor-I (IGF-I). Weekly blood samples were collected from each bull via coccygeal vessel puncture. Approximately 15 mL of whole blood was held at 4°C until centrifuged at 1700rcf X 4C x 30min (3200 rpm) 24 h after collection; serum was harvested and stored at -20°C until analysis. Samples collected at d 0 and d 70 were analyzed for IGF-I concentration, to determine the utility of IGF-I as an indicator of RFI. Serum IGF-I concentrations were determined using radioimmunoassay as described by (Bilby et al., 1999). The protocol included two modifications. The final primary antibody dilution was 1:120,000 and the goat-anti-rabbit secondary antibody dilution 1:60. The IGF-I antibody used was anti-hIGF-I (AFP4892898, A.F. Parlow, National Hormone and Peptide Program, Torrance, CA;(Caldwell, 2009). Unknown concentrations of IGF-I were calculated using Assay Zap software (Biosoft, Cambridge, UK) using counts per minute (cpm) obtained from a Cobra II auto-gamma-counter (Perkin Elmer, Waltham, MA). Final concentrations were calculated and are presented as ng/mL. Intraassay coefficient of variation was 9.55%.

Animals & Experimental Design – Reproductive Development. Brahman bulls (n = 41) born in the spring of 2007 were evaluated for reproductive development and testicular growth beginning in December of 2007. Body weight and scrotal circumference (SC) were collected at 14 d intervals following the initiation of the trial. Scrotal circumference was measured by palpating the testicles down into the scrotum with placement of the fingers and thumb at the neck of the scrotum. The scrotal tape was placed around the largest portion of the testicles and pulled snugly for a measurement reading. Testicular measurements of length, width, and depth were also

taken at the point of maximum dimension for each, using calipers. The epididymis was not included in the measurements. Once bulls reach a SC ≥ 25 cm a semen sample was collected via electroejaculation (Pulsator IV Auto Adjust, Lane Manufacturing, Denver, CO). Stimulus was administered in a rhythmic pattern of increasing intensity from initiation to erection and eventual ejaculation. After clearing pre-ejaculatory fluids, sample collection commenced, ending when fluid became clear or no further semen could be collected. Graduated collection vials were housed in an insulated water jacket to maintain temperature regulation during the collection process. Following collection, samples were immediately evaluated for motility and morphology. Semen collection occurred concurrently with body and testicular measurements on the bimonthly schedule. Electroejaculations continued through puberty; defined as an ejaculate containing 50 x 10^6 spermatozoa with at least 10 % motility (Barber and Almquist, 1975; Wolf et al., 1965). Ejaculations ceased once bulls achieved sexual maturity; defined as an ejaculate containing 50 x 10^7 spermatozoa (Lunstra and Echternkamp, 1982).

Semen Evaluation. Upon detection of spermatozoa in the ejaculate, samples were evaluated for motility, morphology, and total concentration. All equipment used to handle semen samples was maintained at 35 to 37°C. Slides and coverslips were kept on a slide warmer maintained at 35 to 37°C. Samples were kept in a water bath during evaluation to maintain temperature. Approximately 100 µl of neat semen was placed on a slide and covered with a coverslip (22 X 30 mm, No. 1½). Each sample was evaluated for overall motility (0-100%) and progressive motility (1-4; Sorensen, 1976).

Progressive motility as an estimate of spermatozoa progressing forward is expressed as follows:

1 – No forward movement, all dead

2 – Slow forward movement

3 – Moderate forward movement

4 – Fast forward movement

Morphology was evaluated along with motility on the slide. Defects including abnormal heads, abnormal tails, and cytoplasmic droplets were evaluated. Ten sperm cells were counted to obtain a percent abnormal/normal present in each ejaculate.

Total concentration of each ejaculate was determined using a hemacytometer (Improved Neubauer Phase Hemocytometer; 0.10 mm deep, St. Louis, MO). Semen was diluted (1:100) using a unopette with 19.8 ml physiological saline and a 20 µl pipette. The diluted sample was placed in both chambers of a hemacytometer and then counted, yielding a mean count which was used in the final determination of total sperm concentration per ejaculate.

$$\text{Total Concentration} = (\text{Average \# of particles counted on 10 squares} / 0.02\ \text{mm}^3) \times 100$$

$$(\text{Dilution Rate}) \times 1000\ (\text{Conversion to ml}) \times \text{Ejaculate Volume}$$

Statistical Analysis. All data were analyzed using MIXED MODEL procedures of SAS 9.2 (SAS Inst. Inc., Cary, NC) and the Satterthwaite approximation for df. To evaluate the effect RFI and temperament on reproductive development measures, IGF-I, and ultrasound carcass characteristics models which included RFI classification, temperament score classification, and their interactions were used.

Least square means were separated using the LSD procedure of SAS ($\alpha = 0.05$). Additionally, Pearson's correlation coefficients were calculated to evaluate the relationship among residual feed intake, reproductive development, ultrasound carcass traits, and temperament.

Results

Insulin-like Growth Factor-I. There were no significant correlations ($P > 0.05$) between concentrations of IGF-I at d 0 (r = 0.18; $P = 0.27$) or d 70 (r = -0.06; $P = 0.70$) and RFI. There was no effect of RFI classification method I on concentrations of IGF-I at d 0 ($P = 0.47$) or d 70 ($P = 0.54$; Table 3.2) of the feeding trial. Similarly, there was no effect of RFI classification method II on concentrations of IGF-I at d 0 ($P = 0.76$) or d 70 ($P = 0.76$; Table 3.3). These results suggest that concentrations of IGF-I are neither predictive nor reflective of RFI in Brahman bulls.

Table 3.2. Differences in concentrations of IGF-I at d 0 and d 70 of the feeding trail in Brahman bulls separated by RFI classification method I

	RFI Classification Method I[a]			
Trait[c]	Efficient	Intermediate	Inefficient	*P* =
Number of bulls	16	12	13	
RFI	-0.02 ± 0.00[c]	0.00 ± 0.00[d]	0.03 ± 0.00[e]	<0.01
IGF-I d 0, ng/mL	91.69 ± 15.39	113.94 ± 15.36	115.76 ± 14.86	0.47
IGF-I d 70, ng/mL	109.41 ± 8.96	110.23 ± 8.95	121.86 ± 8.66	0.54

[a]Efficient = RFI was > 0.5 SD above the mean; Intermediate = RFI was ± 0.5 SD above or below the mean; Inefficient = RFI was < 0.5 SD below the mean.
[c]RFI = residual feed intake; IGF-I d 0 = insulin-like growth factor-I at d 0; IGF-I d 70 = insulin-like growth factor-I at d 70.
[c,d,e] Means within a row without a common superscript differ (P < 0.05).

Table 3.3. Differences in concentrations of IGF-I at d 0 and d 70 of the feeding trail in Brahman bulls separated by RFI classification method II

	RFI Classification Method II[a]		
Trait[b]	Efficient	Inefficient	*P* =
Number of bulls	16	25	
RFI	-0.02 ± 0.01[c]	0.02 ± 0.02[d]	< 0.01
IGF-I d 0, ng/mL	112.48 ± 13.33	107.29 ± 10.73	0.76
IGF-I d 70, ng/mL	113.63 ± 7.78	118.20 ± 6.27	0.65

[a]Efficient = RFI was > 0.5 SD above the mean; Intermediate = RFI was ± 0.5 SD above or below the mean; Inefficient = RFI was < 0.5 SD below the mean.
[c]RFI = residual feed intake; IGF-I d 0 = insulin-like growth factor-I at d 0; IGF-I d 70 = insulin-like growth factor-I at d 70.
[c,d,] Means within a row without a common superscript differ (P < 0.05).

Temperament had no effect ($P = 0.99$) on RFI. Bulls were classified based on temperament scores as calm, intermediate, and temperamental and had mean RFI of -0.001 ± 0.03 kg/d, 0.001 ± 0.03 kg/d, and 0.000 ± 0.02 kg/d, respectively. Temperament had a significant effect ($P = 0.04$) on concentrations of IGF-I at d 0 of the feeding trial (Figure 3.1). Calm bulls had higher ($P = 0.04$) concentrations (135.03 ± 13.36 ng/mL) of IGF-I than temperamental (75.37 ± 17.64 ng/mL) bulls. At d 70 of the feeding trial temperament classification had no effect ($P = 0.24$) on concentrations of IGF-I (Figure 3.2).

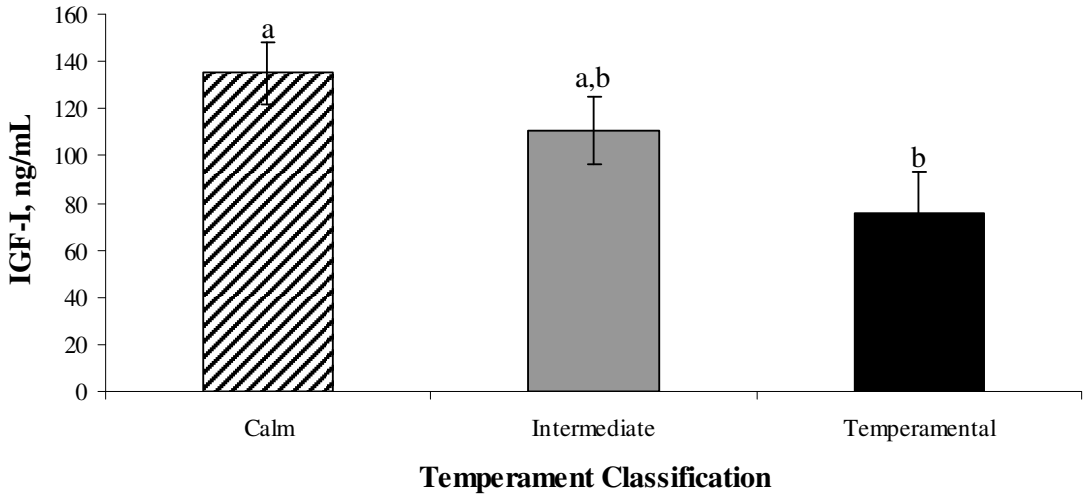

Figure 3.1. Mean concentrations of IGF-I at d 0 of the feeding trial in calm, intermediate, and temperamental Brahman bulls.
[a,b]Means without a common superscript differ ($P < 0.05$).

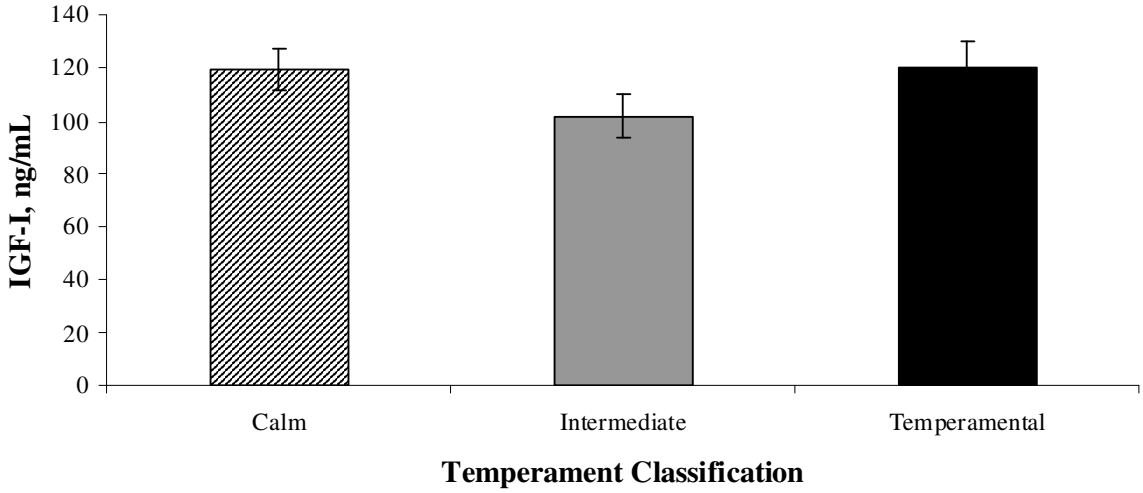

Figure 3.2. Mean concentrations of IGF-I at d 70 of the feeding trial in calm, intermediate, and temperamental Brahman bulls.

There was no RFI classification by temperament classification interaction affecting concentrations of IGF-I at d 0 of the feeding trial, for RFI classification method I ($P = 0.69$) or II ($P = 0.89$). However, there was an affect of the interaction of RFI classification by temperament classification, at d 70 of the feeding trial on concentrations of IGF-I for RFI classification method I ($P = 0.03$; Figure 3.3) and II ($P = 0.02$; Figure 3.4).

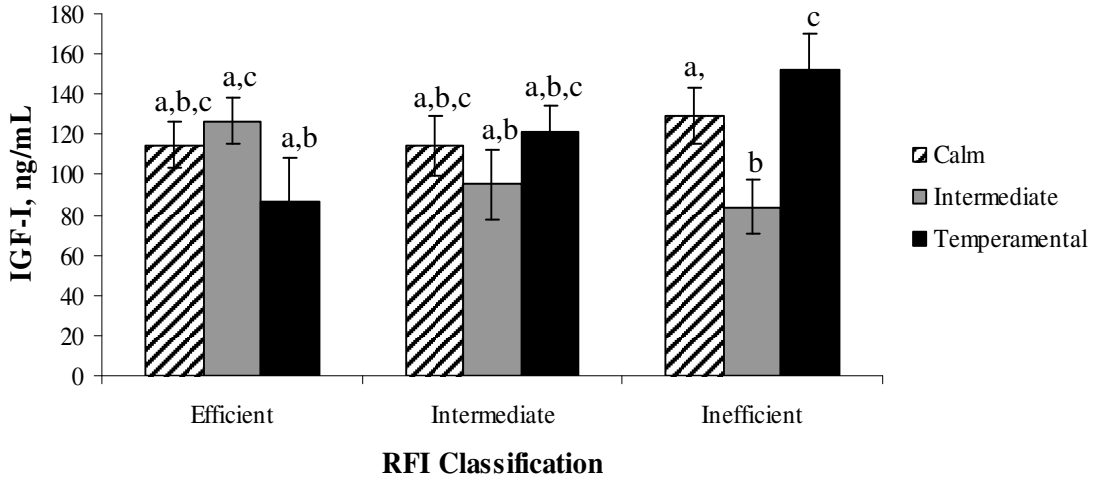

Figure 3.3. Mean concentrations of IGF-I at d 70 of the feeding trial separated by RFI classification method I and temperament classification in Brahman bulls. [a,b]Means without a common superscript differ ($P < 0.05$).

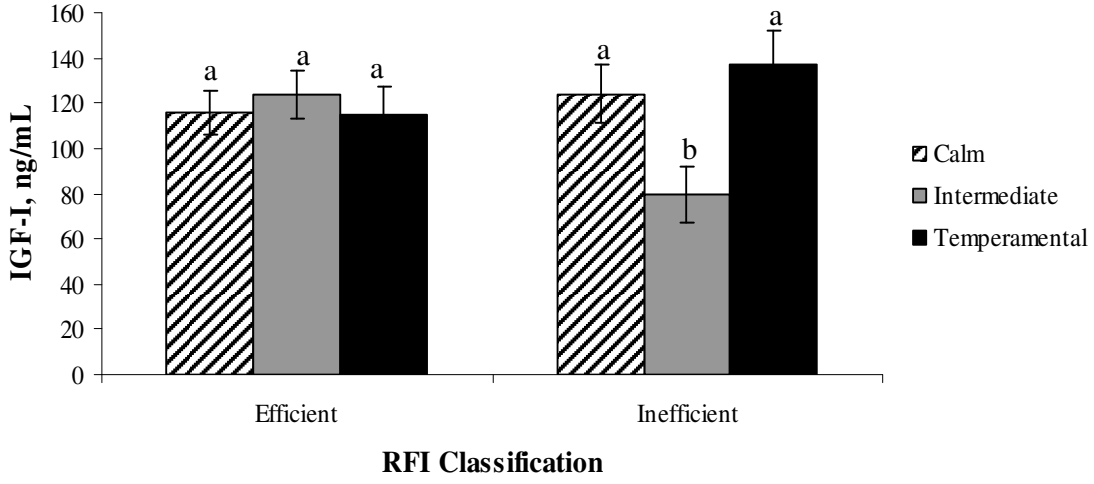

Figure 3.4. Mean concentrations of IGF-I at d 70 of the feeding trial separated by RFI classification method II and temperament classification in Brahman bulls. [a,b]Means without a common superscript differ ($P < 0.06$).

The results from this portion of the study suggest that concentrations of IGF-I are unrelated to RFI in Brahman bulls. Additionally, while RFI classification had no effect on concentration of IGF-I it is apparent that temperament classification may influence concentrations of IGF-I. The effect of temperament was most obvious at d 0 of the feeding trial for both RFI classification methods. At d 70 of the feeding trial the effect of temperament classification appeared as an interaction with RFI classification for both classification methods.

Reproductive Development. Brahman bulls averaged 290 (SD = 18.02) d of age, and had an average initial BW of 217 kg (SD = 20.30) at the start of the reproductive development trial. At the initiation of the feeding trial bulls averaged 376 (SD = 18.02) d of age, and had an initial BW of 311 kg (SD = 36.7). There were no significant differences between efficiency groups for RFI classification method I for any of the reproductive parameters measured (Table 3.4). Residual feed intake for each efficiency group differed significantly ($P < 0.01$). Efficient bulls had an average RFI of -0.02 ± 0.00 kg/d, intermediate bulls had a RFI of -0.00 ± 0.00 kg/d, and inefficient bulls has a RFI of 0.03 ± 0.00 kg/d. There were no differences in age at FS ($P = 0.99$), age at PUB ($P = 0.72$), or age at SM ($P = 0.79$) among the efficiency groups. There also were no differences in BW at FS ($P = 0.88$), BW at PUB ($P = 0.95$), or BW at SM ($P = 0.44$) among the efficiency groups. Additionally, there was no difference in SC at FS ($P = 0.44$), SC at PUB ($P = 0.54$), or SC at SM ($P = 0.17$) among the efficiency groups. Although it was not statically significant there was a numeric trend for efficient bulls to have smaller testis at SM than intermediate or inefficient bulls.

Table 3.4. Differences in reproductive development among efficient, intermediate, and inefficient Brahman bulls

Trait[b]	RFI Classification Method I[a]			*P*-Value
	Efficient	Intermediate	Inefficient	
Number of bulls	16	12	13	
RFI	-0.02 ± 0.00[c]	-0.00 ± 0.00[d]	0.03 ± 0.00[e]	<0.01
Age at FS, d	430 ± 11.3	430 ± 11.3	428 ± 10.9	0.99
Age at PUB, d	453 ± 13.7	462 ± 13.6	446 ± 13.2	0.72
Age at SM, d	480 ± 12.6	492 ± 13.0	490 ± 12.0	0.79
BW at FS, kg	354 ± 11.0	357 ± 11.0	361 ± 10.6	0.88
BW at PUB, kg	374 ± 13.6	380 ± 13.6	379 ± 13.1	0.95
BW at SM, kg	390 ± 13.1	403 ± 13.6	414 ± 12.5	0.44
SC at FS, cm	26.6 ± 0.34	26.0 ± 0.34	26.4 ± 0.33	0.44
SC at PUB, cm	27.2 ± 0.44	26.8 ± 0.44	27.4 ± 0.43	0.54
SC at SM, cm	27.6 ± 0.55	28.4 ± 0.57	28.9 ± 0.54	0.17

[a]Efficient = RFI was > 0.5 SD above the mean; Intermediate = RFI was ± 0.5 SD above or below the mean; Inefficient = RFI was < 0.5 SD below the mean.

[b]Age at FS = age at first sperm; Age at PUB = age at puberty; Age at SM = age at sexual maturity; BW at FW = body weight at first sperm; BW at PUB = body weight at puberty; BW at SM = body weight at sexual maturity; SC at FS = scrotal circumference at first sperm; SC at PUB = scrotal circumference at puberty; SC at SM = scrotal circumference at sexual maturity.

[c,d,e] Means within rows with different superscripts differ ($P \leq 0.01$).

There were no significant differences between efficiency groups for RFI classification method II for any of the reproductive parameters measured (Table 3.5). Residual feed intake for each classification differed significantly ($P < 0.01$). Efficient bulls had an average RFI of -0.02 ± 0.00 kg/d and inefficient bulls had an average RFI of 0.02 ± 0.00 kg/d. There was a trend ($P = 0.07$) for efficient bulls to be younger at FS (419 ± 7.68) than inefficient bulls (441 ± 9.38). There were no differences in age at PUB ($P = 0.31$) or age at SM ($P = 0.31$) between the efficiency groups. There also were no differences in BW at FS ($P = 0.31$), BW at PUB ($P = 0.40$), or BW at SM ($P = 0.25$) between the efficiency groups. While not statically significant there was a numeric trend for efficient bulls to be younger (d) and lighter (kg) at all three milestones of reproductive development. No differences were observed in SC at FS ($P = 0.13$), SC at PUB ($P = 0.23$), or SC at SM ($P = 0.98$) between the efficiency groups.

Residual feed intake or temperament score were not correlated ($P > 0.05$) with any of the reproductive development traits measured in this study. Age at FS was strongly correlated with age at PUB (r = 0.80; $P < 0.0001$), moderately correlated with age at SM (r = 0.62; $P < 0.0001$), BW at FS (r = 0.37; $P = 0.009$), SC at FS (r = -0.48; $P = 0.0006$), SC at PUB (r = -0.32; $P = 0.03$), and SC at SM (r = -0.46; $P = 0.0014$). Age at PUB was strongly correlated with age at SM (r = 0.83; $P < 0.0001$), moderately correlated with BW at FS (r = 0.32; $P = 0.009$), BW at PUB (r = 0.46; $P = 0.001$), and SC at FS (r = -0.48; $P = 0.0006$). Age at SM was moderately correlated with BW at PUB (r = 0.31; $P = 0.04$) and BW at SM (r = 0.39; $P = 0.009$). Body weight at FS was strongly correlated with BW at PUB (r = 0.89; $P < 0.0001$) and BW at SM (r = 0.80; $P <$

0.0001). Similarly, BW at PUB was strongly correlated with BW at SM (r = 0.85; $P <$ 0.0001). Body weight at SM was moderately correlated with SC at FS (r = 0.30; $P =$ 0.05) and SC at SM (r = 0.34; $P = 0.02$). Scrotal circumference at FS was moderately correlated with SC at PUB (r = 0.38; $P = 0.0087$) and SC at SM (r = 0.53; $P = 0.0002$). Scrotal circumference at PUB was moderately correlated with SC at SM (r = 0.50; $P =$ 0.0005; Table 3.6).

Table 3.5. Differences in reproductive development between efficient and inefficient Brahman bulls

	RFI Classification Method II[a]		
Trait[b]	Efficient	Inefficient	*P*-Value
Number of bulls	25	16	
RFI	-0.02 ± 0.00	0.02 ± 0.00	<0.01
Age at FS, d	419 ± 7.5	441 ± 9.3	0.07
Age at PUB, d	447 ± 9.3	462 ± 11.6	0.31
Age at SM, d	482 ± 8.3	496 ± 10.4	0.31
BW at FS, kg	352 ± 7.3	364 ± 9.1	0.31
BW at PUB, kg	373 ± 8.9	385 ± 11.1	0.40
BW at SM, kg	399 ± 9.1	417 ± 11.4	0.25
SC at FS, cm	26.7 ± 0.26	26.0 ± 0.32	0.13
SC at PUB, cm	27.4 ± 0.84	25.8 ± 1.04	0.23
SC at SM, cm	28.5 ± 0.44	28.5 ± 0.53	0.98

[a]Efficient = RFI < 0.00; Inefficient = RFI ≥ 0.00.
[b]Age at FS = age at first sperm; Age at PUB = age at puberty; Age at SM = age at sexual maturity; BW at FW = body weight at first sperm; BW at PUB = body weight at puberty; BW at SM = body weight at sexual maturity; SC at FS = scrotal circumference at first sperm; SC at PUB = scrotal circumference at puberty; SC at SM = scrotal circumference at sexual maturity.

Table 3.6. Partial correlations between residual feed intake, temperament score, and reproductive development in Brahman bulls

Trait[a]	RFI	TS	Age at FS	Age at PUB	Age at SM	BW at FS	BW at PUB	BW at SM	SC at FS	SC at PUB	SC at SM
RFI	1.00	0.08	0.06	-0.01	-0.01	0.02	0.01	0.07	-0.03	-0.03	0.04
TS		1.00	0.05	0.03	0.06	-0.22	-0.23	-0.24	-0.03	0.00	-0.14
Age at FS			1.00	0.80[b]	0.62[b]	0.37[b]	0.28	0.07	-0.48[b]	-0.32[c]	-0.46[b]
Age at PUB				1.00	0.83[b]	0.32[c]	0.46[b]	0.24	-0.48[c]	-0.17	-0.24
Age at SM					1.00	0.28	0.31[c]	0.39[c]	-0.24	-0.13	-0.03
BW at FS						1.00	0.89[b]	0.80[b]	0.19	0.03	0.01
BW at PUB							1.00	0.85[b]	0.11	0.12	0.14
BW at SM								1.00	0.23[c]	0.23	0.34[c]
SC at FS									1.00	0.37[b]	0.53[b]
SC at PUB										1.00	0.50[b]
SC at SM											1.00

[a]RFI = residual feed intake, TS = temperament score, Age at FS = age at first sperm, Age at PUB = age at puberty, Age at SM = age at sexual maturity, BW at FW = body weight at first sperm, BW at PUB = body weight at puberty, BW at SM = body weight at sexual maturity, SC at FS = scrotal circumference at first sperm, SC at PUB = scrotal circumference at puberty, SC at SM = scrotal circumference at sexual maturity.
[b]Correlations are different than zero ($P < 0.01$).
[c]Correlations are different than zero ($P < 0.05$).

Temperament, as assessed by the average of temperament scores from 28 d prior to weaning and at weaning, had no affect ($P > 0.05$) on reproductive development in Brahman bulls (Table 3.7). Calm bulls had an average TS of 1.29 ± 0.08, intermediate bulls had TS of 2.07 ± 0.08, and temperamental bulls had TS of 3.50 ± 0.09. Average daily gain, calculated for the course of the 70-d feeding trial, was not statistically different among the temperament classifications, but did show a trend ($P = 0.06$) for calm bulls to have higher ADG (1.15 ± 0.04 kg) than the intermediate (1.00 ± 0.04 kg) or temperamental (1.04 ± 0.05 kg) bulls. There were no differences in age at FS ($P = 0.91$), age at PUB ($P = 0.97$), or age at SM ($P = 0.70$) among calm, intermediate, and temperamental bulls. There also were no differences in BW at FS ($P = 0.26$) or BW at PUB ($P = 0.20$). Although, there was no statistical difference in BW at the two initial milestones it is interesting to note the numerical trend for calm bulls to be heavier than intermediates which were heavier than temperamental bulls at both FS and PUB. There was a trend ($P = 0.10$) for calm bulls to be heavier (432 ± 12.04 kg) than intermediate bulls (403 ± 12.25 kg) which were heavier than temperamental bulls (381 ± 14.89 kg) at SM. There was no difference in SC at FS ($P = 0.65$), SC at PUB ($P = 0.93$), or SC at SM ($P = 0.17$) among the calm, intermediate, or temperamental classifications. These data suggest that temperament had no effect on reproductive development measures of age and SC but may influence BW at SM.

Table 3.7. Differences in reproductive development and ADG among calm, intermediate, and temperamental Brahman bulls

	Temperament Classification[a]			
Trait[b]	Calm	Intermediate	Temperamental	*P*-Value
Number Bulls	16	15	10	
TS	1.29 ± 0.08[c]	2.07 ± 0.08[d]	3.50 ± 0.09[e]	<0.01
ADG, kg/d	1.15 ± 0.04	1.00 ± 0.04	1.04 ± 0.05	0.06
Age at FS, d	430 ± 9.86	426 ± 10.53	433 ± 13.00	0.91
Age at PUB, d	454 ± 11.91	456 ± 12.72	451 ± 15.72	0.97
Age at SM, d	483 ± 11.53	496 ± 11.73	484 ± 14.25	0.70
BW at FS, kg	371 ± 9.59	356 ± 10.24	345 ± 12.66	0.26
BW at PUB, kg	395 ± 11.86	378 ± 12.67	360 ± 15.66	0.20
BW at SM, kg	432 ± 12.04	403 ± 12.25	381 ± 14.89	0.10
SC at FS, cm	26.4 ± 0.30	26.1 ± 0.32	26.5 ± 0.40	0.65
SC at PUB, cm	24.1 ± 0.39	27.2 ± 0.41	27.0 ± 0.51	0.93
SC at SM, cm	29.0 ± 0.53	28.0 ± 0.53	27.9 ± 0.61	0.17

[a]Calm = TS was < 1 SD below the mean; Intermediate = TS was ± 1 SD above or below the mean; Temperamental = TS was > 1 SD above the mean.
[b]TS = temperament score; ADG = average daily gain during 70 d RFI feeding trial; Age at FS = age at first sperm; Age at PUB = age at puberty; Age at SM = age at sexual maturity; BW at FW = body weight at first sperm; BW at PUB = body weight at puberty; BW at SM = body weight at sexual maturity; SC at FS = scrotal circumference at first sperm; SC at PUB = scrotal circumference at puberty; SC at SM = scrotal circumference at sexual maturity.
[c,d,e]Means within rows with different superscripts differ ($P \leq 0.01$).

Ultrasound Carcass Traits. Ultrasound and temperament measures were collected at weaning for the analysis of the affect of temperament classification on ultrasound carcass traits. Bulls were classified based on temperament as, calm, intermediate, or temperamental. Partial correlations between temperament score and ultrasound measurements at weaning are presented in Table 3.8. Temperament score was moderately correlated with REA:CWT (r = 0.29; $P = 0.04$) at weaning. Temperament score was not correlated ($P > 0.05$) with any other ultrasound carcass traits measured in this study. Ribeye area was moderately correlated with REA:CWT (r = 0.54; $P < 0.0001$), back fat (r = 0.31; $P = 0.03$), and rump fat (r = 0.49; $P = 0.0004$).

Intramuscular fat was moderately correlated with back fat (r = 0.33; *P* = 0.02). Back fat was moderately correlated with rump fat (r = 0.41; *P* = 0.004).

Differences in ultrasound carcass traits at weaning among calm, intermediate, and temperamental Brahman bulls are presented in Table 3.9. Carcass composition traits at weaning were not different (*P* > 0.05) among the calm, intermediate, and temperamental bulls. There were no differences in REA (*P* = 0.55), REA:CWT (*P* = 0.29), IMF (*P* = 0.85), back fat (*P* = 0.83), or rump fat (*P* = 0.48) among the temperament classifications. These data suggest that temperament at weaning had no effect on ultrasound carcass traits in Brahman bulls.

Table 3.8. Partial correlations between temperament score and ultrasound carcass traits at weaning in Brahman bulls

Trait[a]	TS	REA	REA:CWT	IMF	Back fat	Rump fat
TS	1.00	0.10	0.29[c]	0.04	0.03	0.07
REA		1.00	0.54[b]	-0.07	0.31[c]	0.49[b]
REA:CWT			1.00	-0.17	0.16	0.18
IMF				1.00	0.33[c]	0.04
Back fat					1.00	0.41[b]
Rump fat						1.00

[a]TS = temperament score; REA:CWT = ribeye area per 45.36 kg; IMF = intramuscular fat.
[b]Correlations are different than zero (*P* < 0.01).
[c]Correlations are different than zero (*P* < 0.05).

Table 3.9. Differences in ultrasound carcass traits at weaning among calm, intermediate, and temperamental Brahman bulls

	Temperament Classification[a]			
Trait[b]	Calm	Intermediate	Temperamental	*P*-Value
Number of Bulls	16	15	10	
TS	1.29 ± 0.08[c]	2.07 ± 0.08[d]	3.50 ± 0.09[e]	<0.01
REA, cm^2	38.86 ± 2.01	36.92 ± 1.92	38.59 ± 2.13	0.55
REA:CWT	1.33 ± 0.07	1.34 ± 0.07	1.40 ± 0.08	0.29
IMF, %	1.88 ± 0.13	1.80 ± 0.13	1.88 ± 0.15	0.85
Back fat, cm	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.83
Rump fat, cm	0.39 ± 0.03	0.36 ± 0.02	0.40 ± 0.03	0.48

[a]Calm = TS was < 1 SD below the mean; Intermediate = TS was ± 1 SD above or below the mean; Temperamental = TS was > 1 SD above the mean.
[b]TS = temperament score; REA = ribeye area; REA:CWT = ribeye area per 45.36 kg; IMF = intramuscular fat.
[c,d,e]Means within rows with different superscripts differ ($P \leq 0.01$).

At the conclusion of the feeding trial, d 70, ultrasound measures were again collected to evaluate the effect of RFI classification on ultrasound carcass traits. At d 70 of the feeding trial ultrasound carcass traits were not different ($P > 0.05$) among the efficiency groups for RFI classification method I. There were no differences in REA ($P = 0.96$), REA:CWT ($P = 0.54$), IMF ($P = 0.29$), back fat ($P = 0.98$), or rump fat ($P = 0.50$) among the efficiency groups (Table 3.10). These results suggest that RFI classification is independent of ultrasound carcass traits in Brahman bulls. Moreover, selection based on RFI would not negatively affect economically important traits related to meat quality and yield.

Table 3.10. Differences in ultrasound carcass traits at d 70 among efficient, intermediate and inefficient Brahman bulls

	RFI Classification Method I[a]			
Trait[b]	Efficient	Intermediate	Inefficient	*P*-Value
Number Bulls	16	12	13	
RFI	-0.02 ± 0.00[c]	-0.00 ± 0.00[d]	0.03 ± 0.00[e]	<0.01
REA, cm^2	61.49 ± 3.32	60.96 ± 3.33	60.48 ± 3.29	0.96
REA:CWT	1.15 ± 0.04	1.15 ± 0.04	1.11 ± 0.04	0.54
IMF, %	1.97 ± 0.22	1.88 ± 0.22	2.20 ± 0.22	0.29
Back fat, cm	0.27 ± 0.02	0.26 ± 0.02	0.27 ± 0.02	0.98
Rump fat, cm	0.53 ± 0.04	0.51 ± 0.05	0.57 ± 0.04	0.50

[a]Efficient = RFI was > 0.5 SD above the mean; Intermediate = RFI was ± 0.5 SD above or below the mean; Inefficient = RFI was < 0.5 SD below the mean.
[b]REA = ribeye area; REA:CWT = ribeye area per 45.36 kg; IMF = intramuscular fat.
[c,d,e]Means within rows with different superscripts differ ($P \leq 0.01$).

For RFI classification method II, ultrasound carcass traits at d 70 of the feeding trial were not different ($P > 0.05$) between the efficiency groups. There were no differences in REA ($P = 0.49$), REA:CWT ($P = 0.45$), IMF ($P = 0.34$), back fat ($P = 0.48$), or rump fat ($P = 0.83$) between efficiency groups (Figure 3.11).

Table 3.11. Differences in ultrasound carcass traits at d 70 between inefficient and efficient Brahman bulls

	RFI Classification Method II[a]		
Trait[b]	Efficient	Inefficient	*P*-Value
Number Bulls	25	16	
RFI	-0.02 ± 0.00	0.02 ± 0.00	<0.01
REA, cm^2	62.13 ± 2.60	60.22 ± 2.90	0.49
REA:CWT	1.15 ± 0.03	1.12 ± 0.04	0.45
IMF, %	1.98 ± 0.20	2.13 ± 0.21	0.34
Back fat, cm	0.28 ± 0.02	0.26 ± 0.02	0.48
Rump fat, cm	0.54 ± 0.04	0.55 ± 0.05	0.83

[a] Efficient = RFI < 0.00;Inefficient = RFI ≥ 0.00.
[b]REA = ribeye area; REA:CWT = ribeye area per 45.36 kg; IMF = intramuscular fat.

Residual feed intake was not correlated ($P > 0.05$) with any of the ultrasound carcass traits measured at d 70 of the feeding trial. Average daily gain was moderately correlated with REA (r = 0.63; $P < 0.001$), back fat (r = 0.42; $P = 0.003$), and rump fat (r = 0.50; $P < 0.001$). Ribeye area was moderately correlated with REA:CWT (r = 0.53; $P < 0.001$), back fat (r = 0.32; $P = 0.02$), and rump fat (r = 0.60; $P < 0.001$). Back fat was moderately correlated with rump fat (r = 0.53; $P < 0.001$; Table 3.12).

Table 3.12. Partial correlations between residual feed intake and ultrasound carcass traits at d 70 in Brahman bulls

Trait[a]	RFI	ADG	REA	REA:CWT	IMF	Back fat	Rump fat
RFI	1.00	-0.3	-0.08	-0.09	0.18	-0.05	0.03
ADG		1.00	0.63[b]	-0.09	0.14	0.42[b]	0.50[b]
REA			1.00	0.53[b]	-0.15	0.32[c]	0.60[b]
REA:CWT				1.00	-0.27	-0.14	0.12
IMF					1.00	0.26	0.13
Back fat						1.00	0.53[b]
Rump fat							1.00

[a]RFI = residual feed intake; ADG = average daily gain during 70 d RFI feeding trial; REA = ribeye area; REA:CWT = ribeye area per 45.36 kg; IMF = intramuscular fat.
[b]Correlations are different than zero ($P < 0.01$).
[c]Correlations are different than zero ($P < 0.05$).

Discussion

Insulin-like Growth Factor-I (IGF-I). Residual feed intake is a measure of feed efficiency that is heritable in beef cattle (Arthur et al., 2001b), but is expensive to measure. Targeting a non-invasive and economically feasible method to identify efficient animals is essential to the implementation of efficiency based selection. Circulating concentrations of IGF-I have been linked to several economically important traits in beef cattle, leading to the investigation of the use of IGF-I as a possible selection tool for feed efficiency in beef cattle (Wood ., 2004).

Concentrations of IGF-I at the initiation of the feeding trial were not predictive of RFI. Additionally, concentrations of IGF-I at the conclusion of the trial were not reflective of RFI. These results are similar to recent reports in the literature; Lancaster et al. (2007) found no significant correlation between RFI and concentrations of IGF-I when evaluating Brangus heifers. However, previous studies in *Bos taurus* cattle have proposed a link between IGF-I and RFI (Moore et al., 2008; Wood et al., 2004). Additionally, Johnston et al. (2002) suggested that IGF-I may be a predictor of RFI in *Bos taurus* cattle. Caldwell (2009) found differences in concentrations of IGF-I among various breeds, and suggests that these differences are likely due to breed composition. Simpson et al. (1997) found similar results that revealed that Brahman cows have greater concentrations of IGF-I than Angus cows. This difference in concentration of IGF-I could possibly be due to a greater quantity of IGFBP3. Simpson et al. (1997) also demonstrated that Brahman cows had greater IGFBP3 binding activity compared to Angus. Since IGF-I has not been demonstrated to be stored in tissue, the pool being

circulated by IGFBPs is the only form of storage for the growth promoting peptide. The IGFBP3 has a very high binding affinity to IGF-I, higher even than that of the type I IGFR (Baxter, 1986). This high binding affinity has been known to act in an inhibitory manner on the actions of IGF-I (Baxter, 1988). Presumably, the high concentrations of IGF-I seen among the tropically adapted breeds are resultant from greater concentrations of IGFBPs.

Selection based on temperament was initially used to identify animals which could potentially cause injury to handlers, and often times varied among production operations. With improvement in efficiency and performance in the cattle industry being a primary concern, the impact of temperament on a variety of economically important traits has been further assessed. Concentrations of IGF-I are not removed from the effects of temperament as evident from the results of this study. Temperament had a significant affect on concentrations of IGF-I at the initiation of the feeding trial among all efficiency groups within each RFI classification method. Additionally, the interaction of RFI by temperament classification had a significant affect at d 70 of the feeding trial on concentrations of IGF-I among all efficiency groups. These results are surprising but explainable. The role of cortisol in stress response and temperament has an affect in modulating the release and activity of growth hormone (GH). There is a direct relationship between GH and IGF-I; hypersecretion of GH is accompanied with increased concentrations of IGF-I (Baxter, 1986). A decrease in GH related to increased cortisol would explain the lower concentrations of IGF-I in temperamental animals.

While the interactions are not completely clear, the results of this study suggest that temperament does have an affect on concentrations of IGF-I in Brahman bulls.

Reproductive Development. Evaluation related to residual feed intake has been limited mostly to research settings due to the expensive and laborious nature of evaluating animals. Additionally, most reports related to RFI focus on traits other than reproduction, but it is important to understand the potential impact of RFI based selection on reproductive efficiency and performance of beef cattle. No differences in age at FS, age at PUB, or age at SM were observed among the efficiency groups for RFI classification method I. However, there was a trend ($P = 0.07$) for efficient bulls to be younger at FS than inefficient bulls for RFI classification method I. Age at FS and PUB was greater in this study than has been previously reported in the literature; however, the majority of these reports have been for temperate *Bos taurus* cattle in northern climates (Almquist and Barber, 1974; Wolf et al., 1965). Bulls in this study also were older at FS and PUB, lighter at PUB and SM, and had smaller scrotal circumferences at PUB and SM than Brahman bulls from a study conducted by Tatman (2004). These differences can possibly be attributed to differences in sampling schedules. Additionally, no differences in BW or SC at any of the milestones of reproductive development were observed among the different efficiency groups for either classification method.

Previous research conducted by our group reported a tendency for RFI to be negatively correlated ($r = -0.44$; $P < 0.08$) with age at puberty, where inefficient bulls reached puberty at a younger age than did efficient bulls (Ramirez et al., 2008). These differences may be due to the differences in diet and testing duration. Bulls evaluated in

the Ramirez et al. (2008) study were fed a pelleted ration; split into limit and ad libitum fed, and then switched back for a second iteration of the trial. Bulls in the current study were fed a textured ration for only one 70-d testing period.

As expected, strong correlations existed between ages at the three milestones of reproductive development. Historically, age at FS has been reported to be indicative of age at PUB and SM. While the SC for each milestone was not significantly different, the results of this study are in agreement with the literature that scrotal circumference is an indicator of puberty, but as suggested by Fields et al. (1982) may vary within populations of Brahman bulls.

Ultrasound Carcass Traits. Variations in ultrasound carcass traits have been reported between RFI classifications. Carstens et al. (2002) reported a negative correlation between RFI and ultrasound measurements of 12^{th} rib fat thickness ($r = 0.22$; $P = .004$) and rump fat thickness ($r = 0.18$; $P = 0.02$) in *Bos taurus* crossbred cattle. In these studies there were no correlations observed between RFI and ribeye area or intramuscular fat in the crossbred steers. Herd et al. (2003) reported that high RFI *Bos taurus* cattle had greater fat depth over the ribs (11.6 ± 0.3 and 10.2 ± 0.3; $P < 0.05$) and rump (14.8 ± 0.4 and 13.1 ± 0.4; $P < 0.05$), as determined by ultrasound measurement, than low RFI cattle prior to slaughter. Hot carcass rump fat thickness was also different between high and low RFI cattle (16.5 ± 0.5 and 14.9 ± 0.5; $P < 0.05$) following slaughter. These studies indicate that low RFI cattle may produce a leaner carcass than high RFI cattle.

In the current study, RFI was not correlated with any of the ultrasound carcass traits. Additionally, no differences were observed among efficiency groups for any of the ultrasound carcass traits measured. Lancaster et al. (2009) observed no significant difference in IMF among RFI classifications when evaluating a set of Angus bulls. Moreover, he suggests that adjusting RFI for carcass composition will facilitate selection to reduce feed intake in cattle without affecting rate or composition of gain. Baker et al. (2006) reported results similar to those of this study in which there were no differences among RFI classifications for hot carcass weight, ribeye area, fat thickness, kidney pelvis and heart fat, yield grade, marbling score, or quality grade when evaluating Angus steers.

Numerous publications in the literature report the negative impacts of temperament on economically important traits in beef cattle, including lower weight gain (Burrow and Dillon, 1997; Voisinet et al., 1997b), tougher meat (King et al., 2006; Voisinet et al., 1997a), and increased amounts of bruise trim (Fordyce et al., 1988). Studies also indicate that calmer cattle had a higher average daily gain (ADG) than did more temperamental cattle (Müller and von Keyserlingk, 2006; Voisinet et al., 1997b). No significant difference was observed among temperament classifications for any of the ultrasound carcass traits evaluated in this experiment. This contradiction to the literature may be due to differences in temperament assessment and the fact that the animals in this study were intact males. Temperament for bulls in this study was assessed 28-d prior to weaning and at weaning by averaging the exit velocity and pen score at both time points. In conjunction with the ultrasound carcass traits, the effect of temperament

classification on ADG over the course of the 70-d feeding trial also was evaluated. The trend observed for calm bulls to be heavier than intermediate and temperamental bulls is in agreement with several reports; Fell et al. (1999) and Voisinet et al. (1997) found that temperamental cattle had lower ADG when compared to calmer cattle.

CHAPTER IV

GENERAL CONCLUSIONS

Chapter II: Evaluation of Growth, Performance, and Temperament in Brahman Calves

The results from this study have a variety of implications, and emphasize the need for further investigation into several factors affecting calf growth and performance throughout the multiple phases of production. The findings from this experiment are similar to previous reports in the literature in regard to the effect of temperament on performance in cattle. There was no effect of temperament evaluated at d 21 to 24 of age on the measures of performance for the pre-weaning and post-weaning stages of production. Temperament evaluated at weaning had a significant affect on weaning weight and ADG from birth to weaning. It can be concluded that, based on these data and other reports, temperament has a negative impact on growth and performance in Brahman calves. Additionally, while not having an effect on calf performance temperament as assessed at d 21 to 24 using EV was moderately correlated with temperament as assessed at weaning using TS. This suggests that EV can be used earlier in life than at weaning to assess temperament with relative consistency through weaning.

Secondly, results from this study suggest that assessment of performance using blood parameters including TP and IgG are more accurate and indicative at 24 h after birth than at d 21 to 24 of age. Serum TP at 24 h may be a more appropriate indicator of future performance in the suckling Brahman calf than IgG measured at 24 h.

Additionally, serum IgG concentrations at 24 h had a significant affect on calf weaning weight. Cumulatively, the results for TP and IgG further emphasize the importance of passive immunity on future calf performance.

In regard to blood parameters including TP, IgG, and CS, cow temperament is not related to calf serum concentrations of these parameters at either 24 h after birth or d 21 to 24 of age in Brahman calves. Additionally, the results from this study suggest that cow age may have an effect on colostral IgG concentration or absorption, as reflected by decreased IgG concentration in calf serum 24 h after birth as dam age increases. Culling of aged cows should reduce the adverse effects of decreased TP concentrations observed in calves born to aged cows, this resulting in potential increases in calf performance. Calf sex has a significant affect on serum CS concentrations at d 21 to 24 d of age, with heifer calves having higher concentrations of CS than bull calves. This difference observed between genders reinforces previous reports from our group suggesting a sexually dimorphic nature of CS in Brahman cattle. This relationship between calf sex and CS concentrations is not fully understood and warrants further investigation into the mechanisms involved in these differences. At the current time an unexplainable interaction between cow temperament and calf sex exists, influencing TP concentrations at 24 h and d 21 to 24 of age as well as IgG concentrations at 24 h.

Concurrently, the lack of differences among calf temperament groups at d 21 to 24 of age and weaning suggest that serum TP concentrations at 24 h and d 21 to 24 may be independent of calf temperament.

Lastly, this study found no significant differences in REA, REA:CWT, IMF, or back fat at weaning or 56 d post-weaning among temperament classifications. Rump fat at weaning tended to be influenced by calf temperament. Additionally, there was a significant affect of calf temperament on rump fat 56 d post-weaning. These results are inconclusive, warranting further investigation into relationships between early life blood parameters and calf ultrasound carcass traits at and around weaning.

Cumulatively, the results from this study offer interesting insight into the roles of calf temperament, early life blood parameters, cow temperament, cow age, and calf sex on growth and performance in Brahman calves. While not entirely conclusive these data do offer valuable information related to potential selection tools, measures of performance, and other resources for increasing calf survival and performance.

Chapter III: Evaluation of Residual Feed Intake and Reproductive Development in Brahman Bulls

The results from this study are multifaceted and have a variety of implications. The findings of this experiment suggest that concentrations of IGF-I are unrelated to RFI in Brahman bulls. While concentrations of IGF-I are unrelated to RFI, temperament had a significant affect on IGF-I in Brahman bulls. Moreover, it can be concluded that based on this research, and similar reports, concentrations of IGF-I are not a reliable indicator of residual feed intake in Brahman bulls. Variations in concentrations of IGF-I may be due to breed composition as reported by Caldwell (2009) as well as temperament as evident from the results of this study.

Secondly, results from this study suggest that RFI can be used as a selection criterion without negatively affecting reproductive development in Brahman bulls. With no difference in age, body weight, or scrotal circumference among the efficiency groups it is evident that RFI had little influence on reproductive development.

Concurrently, the lack of differences between efficiency groups with regard to ultrasound carcass traits suggests that RFI did not influence any of these traits in Brahman bulls. Selection for feed efficiency based on RFI should not negatively affect those economically important traits related to carcass quality or yield. Additionally, this study found no significant difference in ultrasound carcass traits among temperament classifications, but caution should be used when interpreting these limited results as numerous reports in the literature have found a link between temperament and undesirable carcass traits. Lastly there was agreement with the literature relating to the effects of temperament on ADG. With these results in mind, selection against those cattle with a more excitable temperament would yield an increase in ADG and lessen negative effects on carcass traits of economic importance.

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APPENDIX A

INSULIN-LIKE GROWTH FACTOR-I (IGF-I) RADIOIMMUNOASSAY

PROTOCOL FOR BOVINE SERUM

Reagent Preparation

1. IGF-I Assay Buffer

   0.40 g Protamine (grade II) SO_4 (Sigma S-4380)
   8.28 g Sodium phosphate (monobasic) (Sigma S-0751)
   1.0 ml Tween 20 (Sigma P-1379)
   0.40 g Sodium azide (Sigma S-2002)
   7.44 g EDTA (Sigma E-5134)

   Mix above reagents in double-distilled water (ddH_20). pH solution to 7.5 with NaOH and bring volume to 2.0 liters. Store solution at 4ºC for one month. **(Caution: Sodium azide is highly toxic.)**

2. 1M Glycine

   75.07 g Glycine (Sigma G-8898)

   Mix glycine in approximately 850 ml ddH_20. Using 38% HCl, adjust pH of solution to 3.2 and bring volume to 1.0 liter. Make fresh 1M glycine for each extraction and store at 4ºC.

3. 0.5N NaOH

   Add 50 ml of 2.5N NaOH to 200 ml ddH_20 (1:5 dilution) or dissolve 5.0 g NaOH pellets into 250 ml ddH_20. Store at 4ºC.

4. 12.5% Polyethylene Glycol (PEG)

   250 g Carbowax PEG 8000 (Sigma P-2139)

   Mix PEG in approximately 1800 ml ddH_20, cover and mix at room temperature until solution is clear (~6 h). Adjust pH of solution to ~8.6 and store at 4ºC overnight. Allow solution to come to room temperature while spinning. Adjust pH of solution to 8.6 and bring volume to 2.0 liters. Store at 4ºC for up to 3 months.

5. Primary Antibody (Anti-Human IGF-I)

   (Source: A. F. Parlow, National Hormone and Peptide Program, Harbor-UCLA Medical Center, 1000 West Carson St., Torrance, CA 90509)

Antibody comes lyophilized at a 1:10 dilution in PBS. Use antibody at a final dilution of 1:120,000 in IGF-I Assay Buffer (7.5 µl stock into 90 ml IGF-I Assay Buffer). Prepare fresh daily at least one hour before use and store at 4ºC.

6. Secondary Antibody (GARGG)

(Source: Calbiochem, San Diego, CA; Goat Anti-Rabbit y-Globulin cat#. 539845)
Add 667 µl of stock GARGG to 39.33 ml IGF-I Assay Buffer (1:60 dilution). Prepare fresh daily and store at 4ºC.

7. Normal Rabbit Serum (NRS) (IgG Corporation, Cat# IgG-NRS)

Prepare at 1:100 dilution in IGF-I Assay Buffer (500 µl stock into 49.5 ml Buffer).

8. [^{125}I] Tracer

(Source: MP Biomedicals Inc., Cat# 68128)
Calculation of required activity:
1µCi isotope = 22.20 x 10^6dpm.
2.22 x 10^6 dpm = 1.665 x 10^6 cpm (At 75% counting efficiency estimate [^{125}I]).

(n) RIA tubes x 21,000 cpm x µCi = Required Activity (µCi)
1 tube 1,665,000 cpm
= approx. 15 µCi/1000 RIA tubes (~800 RIA tubes per 10 µCi batch)

Prepare trace at RIA working dilution of 21,000 cpm/100 µl. Prepare and store in an appropriately labeled HD polypropylene bottle set behind lead-block shielding in 4ºC walk-in. Survey and thaw raw trace shipment under hood. Calculate the final working dilution as above. Make final dilution and store, preferably overnight, before use.

9. IGF-I Standards

Absolute range of IGF-1 standards @1:200 sample dilution is 19.54 through 5000 ng/ml serum. (Dilutions are 1:100 at sera extraction, and 1:2 in RIA; final = 1:200). Expected biological range should be approximately 40 to 250 ng/ml sera, therefore most samples should be represented by the range between the 0.98 and 1.56 ng/ml standards.

hIGF-1, BIO: Lot #01, sample #1168, 134 µg/vial, lyophilized.
Source: A.F. Parlow, National Hormone and Peptide Program,
Harbor-UCLA Medical Center, 1000 West Carson St, Torrance CA

Reconstitute lyophilized standard stock with 1.00 ml ddH_2O (IGF-1 STD Stock I). Note: this resulted in a previous shipment of this specific standard (sample #1168) having a concentration of 134 µg/ml (vial's specific mass listed on same

by FJP). Construct 1 µg/ml IGF-1 STD Stock II. Transfer 74.63 µl (i.e. 10 µg) to a 10 ml volumetric containing approximately 8.0 ml IGF-1 RIA buffer. Bring to volume and allow for equilibration. Aliquot and freeze if not used immediately.

Prepare serial dilutions of IGF-1 standards fresh for each RIA series. Use liquid-to-liquid transfer, and allow for equilibration. The resulting STD A = 25 ng/ml. Continue preparation of serial dilutions by volume. Mix by gentle vortexing then allow to equilibrate for a minute or two before continuing with the next 1:1 by volume dilution (STD B = 12.5 ng/ml). Continue serial dilutions through STD I (0.098 ng/ml).

IGF-I Standards chart:

ng/ml	ng/tube	equivalence
IGF-1 STD	ng/tube	ng/ml sera
STD A	25.000	5000.00
STD B	12.500	2500.00
STD C	6.250	1250.00
STD D	3.125	625.00
STD E	1.563	312.50
STD F	0.781	156.25
STD G	0.391	78.13
STD H	0.195	39.06
STD I	0.098	19.53
STD J	0.000	0.00

10. IGF-1 Composite Pools for RIA:

For verification of inter-and intra-RIA performance over the expected biological IGF-1 concentration range, construct a “normal” pool and a “high” pool from a composite sub-set of acidified serum samples.

Pool (n = 30) 200 µl aliquots from a random set of acidified serum samples. Pipette 3.00 ml of this to a 13 x 100 mm PP culture tube (to be used for the “high” pool preparation). Aliquot remainder of “normal” pool at 500 µl, freeze and store until use.

For the “high” pool, prepare a 100 ng/ml IGF-1 stock through a 1:10 dilution of 1 µg /ml IGF-1 Standard Stock II. Pipette 20 µl of the 100 ng/ml stock into 2980 µl of the “normal” pool aliquot to produce the “high” pool stock (e.g. back-pipette 20 µl from the 3.0 ml and replace). This results in the addition of 0.667 ng/ml at RIA or, after accounting for the 1:200 final sample dilution following RIA, a delta at RIA of 133.3 ng/ml. (e.g. “normal” IGF-I concentration plus 133 ng/ml at the serum level). Vortex and aliquot “high” pool at 500 µl, freeze and store until use. Laboratory wide “Welsh” pool should be acid extracted along with unknown samples.

IGF-I Assay Protocol:

A. Acidification of Samples

1. Pipette 10 µl of each bovine serum sample into polypropylene eppendorf tubes. (of 1M glycine to each sample.
2. Add 500 µl Polypropylene tubes must be used due to low pH. Number tubes in even numbers so that samples can be assayed in duplicate.)
3. Add 400 µl of IGF-I Assay Buffer to each sample.
4. Cap tubes and incubate in 37ºC water bath for 48 h.
5. Add 90 µl of 0.5N NaOH to all samples and vortex to mix. (Continue assay immediately.) (Sample dilution is now 1:100)

B. Assay Procedure

1. Each assay should include at least triplicate tubes of total (T), non-specific binding (NSB), zero tubes (B_0), standards, and pools. Single acidified unknown samples should be in duplicate for the RIA.
2. Pipette 400 µl of IGF-I Assay Buffer into NSB tubes.
3. Pipette 300 µl of IGF-I Assay Buffer into B_0 tubes.
4. Pipette 200 µl of IGF-I Assay Buffer into standard tubes.
5. Pipette 200 µl of IGF-I Assay Buffer into all sample tubes and pools.
6. Add 100 µl of each standard to each designated standard tube.
7. Add 100 µl of each acidified serum sample into each designated tube pair.
8. Add 100 µl of acidified pools into control pool tubes.
9. Pipette 100 µl of primary antibody to all tubes except NSB and T.
10. Carefully shake tubes to mix and cover with foil.
11. Incubate for 24 h at 4ºC.
12. Pipette 100 µl of [^{125}I]-IGF-I Tracer to all tubes.
13. Cover tubes with foil and shake the tubes carefully to mix.
14. Incubate for 16 h at 4ºC.
15. Pipette 50 µl of NRS to all tubes except totals.
16. Pipette 50 µl of GARGG to all tubes except totals.
17. Pipette 300 µl of PEG to all tubes except totals.
18. Carefully shake tubes to mix and cover with foil.
19. Incubate tubes at room temperature for 30 minutes. (NO LONGER)
20. Centrifuge tubes at 3000 rcf for 25 minutes at 4ºC. (3220 rpm on Sorvall RC3C)
21. Decant tubes (except totals) immediately into radioactive waste container.
22. Allow tubes to remain upside down on absorbent towels for 5 minutes.
23. Remove all visible droplets by tapping tube bottoms.
24. Count tubes on Beckman gamma counter for 1 minute per sample.
25. Use AssayZap to calculate concentrations of unknowns in comparison to a known standard curve. (Final ng/mL concentrations are determined by multiplying mean unknown by 1000)

APPENDIX B

CORTISOL RADIOIMMUNOASSAY FOR BOVINE SERUM

Reagent Preparation

1. Charcoal-Dextran
   3.75 g Charcoal Norit SPXX
   0.375 g Dextran Pharmacia T-70
   600 mL PBSG

   Mix charcoal and dextran into 600mL PBSG. Store at 4ºC for up to 1 month. Mix well before each use.

2. Standard Chart

pg/ml Cortisol STD	
STD 1	7.80
STD 2	15.60
STD 3	31.25
STD 4	62.50
STD 5	125.00
STD 6	250.00
STD 7	500.00
STD 8	1000.00
STD 9	2000.00
STD 10	4000.00
STD 11	8000.00

3. Tracer Preparation
   To make working trace, dilute stock solution with PBSG.
   For these assays 50 mL PBSG: 500 µl stock tracer.
   Counts for the working trace solution should be approximately 12,000 CPM.

Cortisol Assay Procedure

1. Each assay should include at least triplicate tubes of total (T), non-specific binding (NSB), zero tubes (B_0), standards, and pools. Single unknown samples should be in duplicate for the RIA.
2. Pipette 50 µl of sample into 12x75 mm tubes.
3. Add 50 µl of PBSG to each tube.
4. Cover tubes with foil, shake well, and place in 70°C water bath for 1 hour.
5. Allow samples to cool for 30 minutes, and then proceed.
6. Pipette 100 µl of each standard to each designated standard tube.
7. Pipette 100 µl of each pool to each designated pool tube.
8. Add 400 µl of PBSG to each TC tube.
9. Add 300 µl PBSG to each NSB tube.
10. Add 200 µl PBSG to each B_o tube.
11. Add 100 µl of antibody to each tube except TC and NSB.
12. Add 100 µl of [^{3}H]-Cortisol Tracer to all tubes.
13. Cover tubes with foil and shake carefully.
14. Incubate at 4°C for 18 h.
15. Prepare scintillation vials – 5 mL of scintillation cocktail into each tube.
16. Prepare Charcoal-Dextran as described.
17. Add 200 µl of Charcoal-Dextran to all tubes except TC at 4°C.
18. Shake racks and allow tubes to sit for 15 minutes at 4°C.
19. Centrifuge tubes at 3000 rcf for 25 minutes at 4ºC. (3220 rpm on Sorvall RC3C)
20. Keep tubes at 4°C, and begin pour-off.
21. Decant supernatant into scintillation vials.
22. Cap scintillation vials, shake each tube individually, and shake the rack,
23. Load vials in βeta counter, count for 1 minute/tube.
24. Use AssayZap to calculate concentrations of unknowns in comparison to a known standard curve. (Final ng/mL concentrations are determined by dividing mean unknown by 50).

APPENDIX C

DOUBLE ANTIBODY SANDWICH ELISA PROTOCOL

Reagent Preparation

1. Coating Buffer
    a. Mix a 1:100 dilution of your primary antibody in coating buffer (ex: for 20 mL mix 19.8 mL coating buffer and add 200 µL primary antibody).
    b. Add 100 µL to each well of a 96-well plate
    c. Incubate on shaker for 1 hour at room temperature and then place in the refrigerator over night
    d. Wash plate 3 times with wash buffer.

2. Blocking Buffer (Wash Solution)
    a. Add 200 µL blocking buffer to each well
    b. Incubate on shaker for 1 hour at room temperature
    c. Wash plate 3 times with wash buffer

3. Sample
    a. Add 100 µL standards to appropriate wells
    b. Add 100 µL of diluted sample (1:250,000) to appropriate wells
    c. Incubate on the shaker for 2 h at room temperature.
    d. Wash plate 4 times with wash buffer.

4. Secondary Antibody
    a. Mix a 1:1600 dilution of the secondary antibody using sample diluent.
    b. Add 100 µL secondary to each well.
    c. Incubate on the shaker for 1 hour at room temperature.
    d. Wash plate 4 times with wash buffer.

5. Addition of ABTS
    a. Add 5 µL of 30% H_2O_2 to 1 tube of ABTS.
    b. Add 100 µL of ABTS to each well of plate and place in the dark.
    c. Read plate at 405 nm at 10 minutes after ABTS addition.

Assay Procedure

1. Addition of coating antibody
    a. Dilute coating antibody (sheep anti-bovine Ig) 1:100 with coating buffer.
    b. Add 100 µL to each well on a 96-well plate.
    c. Cover plate with parafilm and incubate at room temperature on platform shaker for 30 minutes.
    d. Incubate plate at 4 C overnight.
    e. Wash plate 4 times with wash buffer.

2. Addition of Blocking Buffer
    a. Add 200 µL blocking buffer to each well.
    b. Cover plate and incubate at room temperature on platform shaker for 1 hour.
    c. Wash plate 4 times with wash buffer.

3. Addition of Samples and Standards
    a. Add 100 µL standards and diluted samples to appropriate wells.
    b. Cover plate and incubate at room temperature on platform shaker for 2 h.
    c. Wash plate 5 times with wash buffer.

4. Addition of Secondary Antibody
    a. Add 100 µL of appropriately dilute secondary antibody (Sheep anti-bovine Ig, HRP-conjugated) to each well.
    b. Cover plate and incubate at room temperature on platform shaker for 1 hour.
    c. Wash plate 5 times with wash buffer.

5. Addition of enzyme substrate
    a. Add 5 µL of 30% H_2O_2 to each tube of ABTS and mix well.
    b. Add 100 µL ABTS to each well.
    c. Incubate in dark for 10 - 60 minutes
    d. Read absorbance at 405 nm using spectrophotometer.

APPENDIX D

TOTAL PROTEIN COLORMETRIC ASSAY

Reagent:

Total Protein Reagent - Product Number T 1949

Assay Procedures:

The Total Protein Reagent is linear to 160 mg/ml. If the total protein level is greater than 160 mg/ml, dilute the sample with an equal volume of buffer and reassay. Multiply the result by 2 to compensate for the dilution.

1. Set up a series of test tubes labeled Reagent Blank, Standard, and Test Samples
2. Pipette 20 µl of deionized water, protein standard, and test samples into the appropriately labeled test tubes.
3. Pipette 1.0 ml of the Total Protein Reagent into each tube
4. Mix by gentle inversion.
5. Pour off each tube into cuvettes
6. Incubate each tube for 10 minutes at ambient temperament.
7. Read and record the absorbance at 540 nm (A_{540}) of all tubes verse the Reagent Blank as the reference. The biuret color is stable for at least 1 hour.
8. To determine total protein concentration (mg/ml) in the samples, refer to the calculation:

    - Total Protein (mg/ml) = $\dfrac{A_{540}\text{ (sample) X Concentration of Standard (mg/ml)}}{A_{540}\text{ (standard)}}$

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