POSTNATAL GROWTH, FEEDING BEHAVIOR AND SEXUAL DEVELOPMENT OF PRENATALLY STRESSED BRAHMAN BULLS

A Thesis

by

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ABSTRACT

We attempted to manipulate fetal development and performance of Brahman calves by subjecting gestating mothers to prenatal stress or late gestation and early lactation yeast cell wall supplementation. The following objectives were pursued the effect of yeast cell wall supplementation during late gestation and early lactation on cow performance and calf growth and white blood cells. Additionally, the effect of prenatal stress and postnatal temperament on feeding behavior and sexual development at first sperm, puberty and sexual maturity in in post-weaning Brahman bulls.

Pregnant Brahman cows were assigned to a control (n=42; C) or transport group (n=43; PNS, to receive transportation stress during gestation). Bulls were selected at weaning for these studies and temperament was measured. PNS bulls were heavier at first sperm (P=0.04). Control bulls had a greater scrotal circumference per 100 kg of body weight (P=0.05), indicating the PNS bulls had slower development based on body weight at first sperm. Temperamental bulls had a greater (P>0.01) time interval (69.25 ± 10.73 d) from puberty to sexual maturity than calm (27.21 ± 6.05 d) or intermediate bulls (38.60 ± 9.05 d). Additionally, a GrowSafe system was used to record feeding behavior. PNS bulls had a great head-down time per meal and average meal size. Temperamental bulls had a greater number of visits, meal events, head-down time and head-down time per meal than calm or intermediate.

Yeast supplementation did not affect cow prepartum or postpartum performance or the postpartum interval. Calf birth weight was not affected; however, control males on

d 14 and weaning tended to be heavier (P=0.08, 0.07, respectively). Treatment did not affect the white blood cell profile of calves on d 0 or 28 (P>0.2).

From the experiments we concluded that yeast cell wall supplementation of late gestating and early lactating cows did not affect cow or calf performance, temperament affected feeding behavior in a bunk feed system; therefore, temperament should be considered in the design of future feeding studies and prenatal stress and postnatal temperament cause delays in sexual development in bulls; therefore, prenatal conditions need to be evaluated and considered when determining potential future reproductive performance.

DEDICATION

To my Mom, Dad and Sister who have always been there for me and encouraged me to pursue further education. Also to my husband, David, who has helped and supported me throughout my journey.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Introduction

Fetal programming is defined as the "alteration in fetal nutrition or endocrine status that results in developmental adaptations that permanently change structure, physiology and metabolism" (Godfrey and Barker, 2001). It can be accomplished by manipulation such as over nutrition (Gunn et al., 1995), under nutrition (Borwick et al., 1997), or physical and chemical, such as rough handling and exogenous ACTH (Lay et al., 2011).

We attempted to manipulate fetal development and performance of Brahman calves by subjecting gestating mothers to prenatal stress or late gestation yeast cell wall supplementation by investigating the following:

1) the effect of prenatal stress on the onset of puberty and sexual maturity in bull calves; 2) the effect of prenatal stress on feeding behavior of bull calves; and, 3) the effect of yeast cell wall supplementation during gestation on cow performance and calf performance and immunity.

Hypothalamic-Pituitary-Adrenal Axis

The main function of the hypothalamic-pituitary-adrenal (HPA) axis is to maintain basal and stress-related homeostasis. The system can respond to circadian, neurosensory, blood-borne and limbic signals. This can also include cytokines released by the immune system such as tumor necrosis factor alpha, interleukin-1 and interleukin-

6 (Chrousos, 1995). When a perceived threat or stress is observed, neurons activate the release of corticotropin-releasing hormone (CRH) into the hypophyseal portal blood to the anterior pituitary. In the anterior pituitary, adrenocorticotropin-releasing hormone (ACTH), β-endorphin, β-lipotropin, and α-melanotropin are released (von Borell, 2001). The neurohormone CRH not only activates the anterior pituitary but it also acts as a neurotransmitter in the brain and lead to increased plasma catecholamines, arterial blood pressure and heart rate (von Borell, 2001). Circulating ACTH is the main regulator of cortisol secretion in the adrenal cortex, which play a role in homeostasis of the animal and a regulator in basal activity of the HPA axis (Tsigos and Chrousos, 2002). Cortisol acts as an inhibitory feedback loop on the hypothalamus and anterior pituitary to limit the exposure time of the body to glucocorticoids.

Glucocorticoids can have an impact on reproductive function and growth. CRH subdues gonadotropin releasing hormone (GnRH) neurons in the arcuate nucleus of the hypothalamus. Cortisol can cause interference in the anterior pituitary leading to disruption in gonadal utility (Tsigos and Chrousos, 2002). The mechanism of disruption in the testes was determined *in vitro* using dexamethasone, which is a synthetic glucocorticoid. The conclusion was that there was decreased progesterone to testosterone production within the Leydig cell when treated with dexamethasone and this resulted from decreased cAMP production and 17-alpha-hydroxylase (Welsh et al., 1982).

Development of the HPA axis is highly species specific, for example a majority of brain development and neuroendocrine development take place *in utero* in animals that give birth to mature young, such as primates, cattle and sheep. However, animals

that give birth to immature young, such as rats and mice, will have significant postnatal development of the HPA axis. Due to these differences, manipulation of the HPA axis at various stages of development will impact species differently (Kapoor et al., 2006). Synthetic glucocorticoids, prenatal stress, and nutrient restriction are all examples of prenatal HPA axis function alterations that can have a negative impact on the postnatal function of the HPA axis (Kapoor et al., 2006).

Hypothalamic-Pituitary-Testicular Axis

Expression of the male genetic sex is important for testicular formation and inhibition of the development of the female reproductive organs (Amann and Schanbacher, 1983). The testes have two main functions: 1) endocrine production (i.e. testosterone production) and 2) spermatogenesis. The hypothalamic-pituitary-testicular axis is a self-regulating system with a negative feedback loop that secretes luteinizintg hormone and testosterone in a pulsatile manner. Gonadotropin releasing hormone (GnRH) is a decapeptide released from the hypothalamic-hypophyseal portal system and triggers the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the gonadotrophs of the anterior pituitary (Amann and Schanbacher, 1983). Scientists in the 1920-1930s provided evidence of testicular regression following hypophysectomy (Smith, 1927). Within the testes are the Sertoli cells and Leydig cells both of which are highly dependent on FSH and LH secretions, respectively. Sertoli cells are the only somatic cell within seminiferous tubule and provide communication across blood-testes barrier (Amann and Schanbacher, 1983). FSH acts on Sertoli cells, which in turn secretes androgen binding protein (ABP) and inhibin which help regulate

spermatogenesis within the seminiferous tubules. Leydig cells are located in the interstitial space of the testes. They are the primary source of testicular hormones, such as testosterone, and are active in the early embryonic stage, regress during later development but then restart during the onset of puberty (Hooker, 1970). LH stimulates steroid hormone production within the Leydig cells and the amount of smooth endoplasmic reticulum within the Leydig is correlated with steroid production (Amann and Schanbacher, 1983). Testosterone then acts as the negative feedback to the hypothalamus and anterior pituitary along with estrogen and inhibin.

Puberty and Sexual Maturity in Bulls

Sexual development of cattle has been extensively studied including parameters and requirements for puberty and sexual maturity and also factors that could affect those parameters. Puberty and sexual maturity are not synonymous. Puberty is defined as an ejaculate that contains 50 X 10^6 sperm with at least 10% progressive motility (Lunstra and Echternkamp, 1982). Sexual maturity is defined as an ejaculate that contains 500 X 10^6 with at least 50% motility (Wolf et al., 1965; Killian and Amann, 1972; Barber and Almquist, 1975). Puberty is associated with rapid testicular growth, increased circulating concentrations of LH and testosterone and the initiation of spermatogenesis (Amann and Schanbacher, 1983). The hypothalamus plays a key role in puberty due to the interactions with the pituitary gland and gonads. It is suggested that puberty is made up of multiple actions beginning at birth with the feedback inhibition by gonadal steriods, which leads to an increased frequency of LH pulses and increased circulating testosterone, followed by the differentiation of Sertoli cells and then initiation of

spermatogenesis (D'occhio et al., 1982). Bulls are often characterized as sexually mature by a breeding soundness exam (BSE) consisting of an external and internal exam, which includes scrotal circumference, sperm morphology and motility, and a physical exam (Tatman et al., 2004). Many factors have been shown to influence sexual development including inheritance, prenatal environment, postnatal environment and nutrition. These factors can affect the central nervous system, which, coordinates with the endocrine system causing alterations in development.

Fetal Programing

The hypothalamic-pituitary-adrenal (HPA) axis is subject to programming during fetal and neonatal life (Lay et al., 1997). A hostile or high predation environment can cause alterations with the biology of the offspring to help them better adapt to their surroundings (Matthews, 2002). However, when nutrient restriction, placental insufficiency or chronic stress are involved it could have detrimental affects on the offspring such as low birth weight, increased blood pressure, cardiovascular disease and insulin resistance (Matthews, 2002). Phillips (2007) demonstrated that an adverse early environment could increase HPA activity, which, in various studies, affects growth, increased HPA activity, which reduced maintenance, and inhibition of reproductive function. All of these factors led to the potential for decreased skeletal and organ growth, suppressed immune function, lower birth weight and altered behavior of the offspring.

Due to environmental and nutritional factors, reproductive performance could be altered such as development of the fetal testes and ovaries (Rhind et al., 2001). When ewes were given a supplementary feed during late gestation and early lactation the

progeny had a higher incidence of multiple births when they became sexually mature (Gunn et al., 1995). This demonstrates the impact of fetal programming even much further in life than the birth weight and overall health of the offspring. Under nutrition in early gestation led to reduced fetal growth, delayed fetal ovarian structural development and altered testicular steriodogenesis when comparing high (150 or 100%) and low (50%) percentages of energy requirements for maintenance during gestation (Rhind et al., 1989; Borwick et al., 1997; Rae et al., 2001).

Prenatal Stress

Livestock experience or encounter unavoidable stressors due to exposure to various required managerial processes such as transportation, restraint in a squeeze chute, and social regrouping following weaning or livestock auctions etc. Prenatal stress has been reported to alter behavior, HPA axis function, and sexual development in many species (Lay et al., 2011; Diz-Chaves et al., 2013; Gutierrez-Rojas et al., 2013). Specifically, in a study using mice, bright light was administered to the mothers for 45 minutes, 3 times a day, starting at 12 days of gestation and continuing through parturition. At four months of age, prenatally stressed and control male progeny were exposed to a lipopolysaccharide (LPS) challenge. Prenatally stressed male mice had increased interleukin one beta and tumor necrosis factor alpha responses to the LPS challenge (Diz-Chaves et al., 2013). Depending on management and available resources, different stressors and species have been used to study the effects of prenatal stress. To mimic stress or induce a stressful circumstance, a bright light was used as a stressor of mice, whereas exogenous ACTH was used to pharmacologically mimic

stress-induced activation of the pig's adrenal cortex. In addition, rough handling has been used to activate a stress response in laboratory animals and livestock (Diz-Chaves et al., 2013; Lay et al., 2008). It is difficult to compare studies that used various types of stressors such as rough handling and ACTH (Lay et al. 2011). Lay et al. (2008) demonstrated a reduction in anogenital distance in the progeny of sows that experienced either rough handling or exogenous ACTH (1IU/kg of BW) once a week between d 42 and 77 (of the typical 112-d gestation length for pigs) relative to the non-challenged control group. Specifically, the anogenital distance was the least in pigs from the ACTH group, intermediate in pigs from the roughly handled group and greatest in pigs from the non-challenged control group. The crown-rump length: anogenital distance (CRL:AGD) for the male progeny was determined to be 2.01 ± 0.03, 1.91 ± 0.02, 1.87 ± 0.03 for ACTH, rough handling and control group respectively with $P = 0.03$. The CRL:AGD can be used to determine masculinization and the reduction of the anogenital distance and increased ratio suggested that the prenatally stressed male pigs were demasculinized. The CRL:AGD was not measured in the females due to the reduced distance for the anogenital and not enough variation occurred.

Not only does prenatal stress affect the behavior of the offspring it can also alter HPA axis function. Due to significant differences in ACTH and cortisol concentrations in offspring that were prenatally stressed, it was proposed that there are regulatory differences between prenatally stressed and control rats or pigs (Henry et al., 1994; Batuev et al., 1996; Haussmann et al., 2000). It has been suggested that the phenotype of the HPA axis depends on the timing and duration of the prenatal stimulation

(Matthews, 2002). Development of the gonads occurs early in gestation with the testes secreting markedly greater concentrations of testosterone by 45 d of gestation in the bovine (Dominguez et al., 1988). Development of the gonads continues between 3 and 4 months of gestation. Stress such as transportation for cattle during this time could have an effect on gonadal development. The bovine adrenal gland also has significant development during this time as well (Wrobel and Suss, 1999). On d 35 of gestation the first precursor cells of the adrenal gland become visible. From d 50 to 60 the medullary and cortical precursors are present and there is a high proliferation rate (Wrobel and Suss, 1999). The adrenal medulla has more concentrated nerves than the cortex when separation begins. Knowledge of the timeline of adrenal gland development can assist in better understanding how the timing of the stressor will affect the fetus in postnatal life.

Prenatal stress causes changes in adrenal and brain morphology that lead to changes in function of those structures (Kapoor et al., 2006). Also prenatal stress affects neuroendocrine functions, neurotransmission systems and transcription factors (Kapoor et al., 2006). Timing of the exposure is important to consider, there are species such as cattle that give birth to relatively mature young so that much of the neuroendocrine maturation of the HPA axis occurs in utero whereas species such as mice and rats give birth to immature young and much of the HPA development occurs following birth (Kapoor et al., 2006). It could be suggested from previous rodent studies that an increase in neonatal handling and stress increases thyroid activity, which then affects the hippocampus and could influence HPA activity (Kapoor et al., 2006).

When pregnant sows were physically stressed their neonatal progeny exhibited a compromised immune system along with altered adrenal function (Tuchscherer et al., 2002). In the agricultural industry more emphasis is put on post-natal development and less on prenatal development due to what is economically recognizable such as meat and milk that are physical products of the industry. However, if a breeding animal was exposed to prenatal stress this could subsequently result in offspring with an altered brain, HPA and hormonal function that could lead to delayed puberty and sexual maturity. When a gestating animal is stressed due to transportation habitation can occur however cortisol concentrations have been shown to increase and range between 25-35 mg/ml (Lay et al., 1996). 11b-hydroxysteroid dehydrogenase type 2 (11b-HSD2) is expressed by the placenta, which can decrease the exposure of the fetus to maternal glucocorticoids by inactivating it into11-dehydrocorticosterone (Brunton, 2013). Additionally, exposure to repeated stress during pregnancy could reduce the capacity to regulate the activity of placental 11b-HSD2, which could cause the increase fetal exposure to maternal glucocorticoids (Brunton, 2013). Understanding the consequences of transportation stress on the offspring can allow the industry to modify and accommodate for these affects. Lambs born to ewes that were prenatally stressed explored their novel environment more and jumped less when stimulated than control lambs (Roussel-Huchette et al., 2008). It can be speculated from the research that prenatal stress could cause more excitable animals leading to a more dangerous environment for both the animal and handler as well as more time on feed to gain the desired result.

Brahman cattle have been known to mature later than *Bos taurus* breeds such as Angus or Hereford (Fields et al., 1979). Once Brahman bulls reach sexual maturity, it has been shown that they exhibit a larger scrotal circumference when compared with Angus bulls. However, Brahman bulls were found to have lower testosterone concentrations than Angus bulls although testosterone increases linearly until puberty (Fields et al., 1982). Testicular weight has been closely correlated with semen production in breeding bulls (Coulter and Foote, 1979). However, testicular weight is not a measurement easily obtained from live bulls so in order to accommodate for that, scrotal circumference and testicular length, width and depth are measured to determine testis volume (Coulter and Foote, 1979). Scrotal circumference has also been reported to be a better indicator of puberty than body weight or age regardless of breed (Lunstra et al., 1978). *Bos taurus* breeds have mean scrotal circumferences at puberty similar to Brahman bulls, which were 27.9 cm for both, although Brahman bulls reached puberty at a later age (Neuendorff et al., 1985).

In a study by Gipson et al., (1985), polled Hereford and Simmental bulls were divided by breed and scrotal circumference into three groups. The first group was comprised of bulls with SC < 32 cm, the second group was > 32 cm and the breed average of SC, while the third group was made up of any bull above the breed average for SC. Within breed, the average body weight was statistically significant between all the groups. There were also differences in live sperm and number of sperm in the ejaculate with the greatest difference being between the first group with a SC < 32 cm and the groups with a SC > 32 cm. For other sperm ejaculate measurements such as

motility and sperm concentration there was a significant difference among the groups with the first group with a SC < 32 cm having the lowest average scores. This study along with many others indicates that there is a strong correlation between scrotal circumference and overall reproductive performance for bulls (Coulter and Foote, 1976; Coulter and Keller, 1982; Gipson et al., 1985; Godfrey et al., 1990).

Early maturing bulls are heavier at puberty with greater scrotal circumferences and as previously stated scrotal circumference can be the best predictor of sexual maturity. However, age, weight, and scrotal circumference combined have been shown to be key indicators of sexual maturity (Brito et al., 2004). In a study by Brito et al. (2012), the goal was to determine if the rate of growth and gain in bulls from 6 to 16 months had an effect on their reproductive development. Scrotal circumference, age at puberty and maturity, paired-testes volume and weight, semen production and morphology and testicular histology were parameters of the study. It was found that while body weight was negatively correlated with age at puberty and maturity and body weight was positively correlated with paired-testes weight and seminifereous tubule volume, there was no significant correlation between average daily gain and any reproductive markers investigated. Their study demonstrated that sexual development is perhaps more associated with pre-weaning development where there is an initial rise in gonadotropin secretion. Calves that are born to first-time dams were demonstrated to have smaller scrotal circumferences indicative that nutrition in the early part of life is essential for later sexual development (Barth et al., 2008). Additionally, Holstein bulls were fed three different diets during the pre-weaning period, 60-75, 100 and 140-160%

of NRC requirements for 80 wk. For the high intake diet, bulls were heavier and reached puberty sooner than the medium and low intake diets (Bratton et al., 1956). It can be concluded that nutrition during the pre-weaning period is perhaps more important in the sexual development of bull calves than during the post-weaning period in the sexual development of bull calves (Barth et al., 2008).

Lay et al. (1997) stated that prenatal stress has a lasting effect on the post-natal calf with increased ACTH secretion, which would lead to higher cortisol concentrations in those calves that were exposed to prenatal stress. Also increased concentrations of CRH have been shown to suppress GnRH through cortisol and beta-endorphins (Klimek et al., 2005). When GnRH is suppressed FSH concentrations are also decreased which causes lower levels of sperm production (Klimek et al., 2005). Men being treated for infertility were used in a study to determine if there was a correlation between infertility and ACTH. The men were divided into three groups according to their concentration of ACTH with the first group ranging from 5-10 pg/ml of ACTH, the second group was 11-30 pg/ml and the third group was classified as having concentrations greater than 31 pg/ml. Based on the analysis of Klimek's study, increased secretion of ACTH was negatively correlated with semen volume. There were decreased numbers of motile sperm cells and increased numbers of immobile sperm cells with the increased ACTH concentrations. Testosterone, FSH, LH and cortisol were determined and there were no statistically significant differences in hormone concentrations except for a positive correlation between cortisol and ACTH. A rise in ACTH and cortisol concentrations

could hinder the Leydig cells by inhibiting testosterone synthesis causing lower sperm concentrations leading to infertility or subfertility (Klimek et al., 2005).

Feeding Behavior

Prenatal stress has been demonstrated to affect temperament (Littlejohn et al., 2012) and temperamental cattle have decreased body weight at weaning and reduced average daily gain in a feedlot system (Francisco et al., 2012; Voisenet et al., 1997). When determining the influence of temperament on the performance of feedlot cattle, flight speed had a negative phenotypic correlation ($r = -0.34$) with dry matter intake (DMI) and a negative genetic correlation ($r = -0.56$) with head down duration (Nkrumah et al., 2007). These finding demonstrate that temperamental cattle spent less time feeding than calmer cattle. Feeding behavior and temperament in a feedlot or feeding situation have recently been examined because they could play a major role in areas such as acidosis, feed intake, and overall performance. Variation in feed intake by individual animals within a pen needs more assessment to fully understand the impact of certain clinical disorders caused by feeding disturbances such as increased ruminal pH (Schwartzkopf-Genswein et al., 2003). Feed intake is very important because if fluctuation is great enough it can cause decreased daily weight gain and increased feed to gain ratio (Galyean et al., 1992). However, when fluctuation of feed intake is extended to a weekly variation there was no significant difference in gain or the feed to gain ratio when compared to constant intake.

During the last week of pregnancy, rats were administered bright light for three times a day as a stressor. The male progeny were aged until 23 mo then after a 24-hr

fasting period, prenatally stressed male rats exhibited an increase in feed intake and increased glucose concentrations after a period of fasting (Lesage et al., 2004). This could demonstrate an alteration in feeding behavior under stressful conditions for prenatally stressed progeny. Increased concentrations of glucocorticoids and an increased exposure to maternal stressful situations have been thought to increase anxiety-like behavior and certain emotional disorders in the offspring of humans. When assessed in a questionnaire, mothers with increased prenatal anxiety and depression had an increased prevalence of emotional disorders in their children (Rice et al., 2007). For cattle, this behavior could be translated into decreased feed intake or a variation in feed intake that could result in reduced performance in the feedlot. There needs to be further examination of the effects of both prenatal stress and temperament on the feeding behavior of cattle when being transitioned from range type management to a feedlot type management system.

Role of Probiotics (Yeast Cell Wall)

The cell wall of yeast consists of alpha-D-mannan and beta-D-glucan which are two polysaccharides that have been shown to increase immune function and responsiveness to a microbial attack in pigs (Kogan and Kocher, 2007). It has been proposed that beta-D-glucan can bind to specific sites on monocytes, macrophages and granulocytes to create a response by the bone marrow colony to increase cytokine release, increase antibody production, and alter production of white blood cells. Alpha-D-mannose prevents bacterial pathogens from colonizing by adhering to the lectin type receptors of the bacteria (Kogan and Kocher, 2007). In a contrasting study, sows were

either fed beta-glucan or vaccinated for *Actinobacillus pleuropneumoniae*, which contributes to bacterial pneumonia in swine. Total milk IgG or pig serum IgG was not elevated for either treatment group; however, increased serotypes for *A. pleuropneumoniae* were observed for the vaccinated group (Chau et al., 2009). It was proposed from the study that beta-glucan can be beneficial as a direct fed product in addition to vaccination.

Through both human and animal studies, yeast cell wall and its component could have a positive effect on preparing and altering the immune system for pathogen attacks (Eicher et al., 2006; Volman et al., 2008). Neonatal calves were supplemented with various yeast cell wall extracts, either 2% or 70% beta-glucan and subjected to a transportation stress. This resulted in an increase in E. coli shedding and feed intake in the yeast cell wall experiment group (Eicher et al., 2010).

In nursing and weaned pigs, yeast cell wall along with other various modified yeast cultures have been used to determine if there is an increase in body growth and feed digestibility. Antibiotic growth promoters (AGP) have been used to improve the health of weaned pigs; however, supplementation with a yeast culture was reported to increase average daily gain, digestibility of dry matter, and have a positive effect on jejunal villus and villus height: crypt depth ratio (Shen et al., 2009). The increase in villi height and crypt depth increases surface area in the lumen can increase absorption of nutrients. The comparable effects of AGP and yeast culture suggest that yeast culture could be a good alternative to antibiotic use. However, in another study there was no effect of a yeast culture or yeast cell wall on gut integrity or blood cell composition (van

der Peet-Schwering et al., 2007). The amount of yeast culture varied between studies, which can make it difficult to determine the effectiveness of the yeast.

In dogs, yeast cell wall supplementation tended to increase total nutrient digestibility and immunoglobulin A, which plays a critical role in mucosal immunity. Increases in IgA concentrations suggest an increase in resistance to antigen invasion due to IgA binding to the antigen, which will not allow them to pass the mucosal membrane. The total white blood cell profile and eosinophil counts tended to decrease with supplementation. Additionally fecal *E. coli* concentrations decreased with supplementation (Middelbos et al., 2007). Yeast cell wall, along with other derivatives, were demonstrated to have varying degrees of effectiveness in species such as cattle, pigs and dogs. Increases in digestibility and alteration of immune function have been exhibited when directly fed to postnatal animals; however, maternal dietary supplementation during gestation and its consequent effect on progeny has been less investigated.

Prenatal supplementation of the dam with specific nutrients and nutraceuticals has long been looked at as a methodology to increase health and productivity in progeny. Passive immune transfer is the only way to provide neonatal ruminants the immunity they need. Prenatal supplementation could boost passive transfer of immunity and this includes yeast cell wall extracts. Colostrum is the primary source of immunity in the neonatal calf due to the lack of particular immunoglobulin transfer through the placenta. It contains immunoglobulins, nonprotein nitrogen, fat, ash, vitamins and minerals in greater quantities than milk (Quigley and Drewry, 1998). *Saccharomyces cereuisiae* has

been used as a microbial additive and could benefit ruminant nutrition by improving the viability of the ruminal microbes leading to increased milk production and live weight gain may function similarly to ionophores (Wallace and Newbold, 1994). Yeast cell wall has been shown to mediate toxic effects of consumption of tall fescue grass. Fescue toxicity can cause decreased peripheral blood circulation, decreased reproductive efficiency and elevated body temperature. Pregnant Angus X Hereford cows were either fed 0, 20, 40 or 60 g/d of yeast cell wall. No differences were observed in prepartum BW change but YCW supplemented cows gained more postpartum. Additionally, milk production was increased as increased YCW was fed as well as serum prolactin. This is beneficial because fescue toxicity can cause a decrease in prolactin (Merrill et al., 2007).

There have been multiple proposals for the mode of action for yeast and yeast cultures. Increased viable ruminal microbes can be achieved by decreased lactate production and increased pH following feeding. This would then lead to an increased rate of fiber digestion and flow of microbial protein, which would lead to increased feed intake followed by improved milk production and growth of the supplemented animal (Wallace and Newbold, 1994). Increased milk production has led to increased weaning weights (Clutter and Nielsen, 1987); however, studies have shown that increased colostrum production might be negatively correlated with immunoglobulin concentrations (Pritchett et al., 1991). Research has been conducted on yeast, and its derivatives, for postnatal supplementation; however, there is very little research on prenatal supplementation and the benefits to the progeny.

Based on the foregoing assessment of current literature the following objectives were pursued:

1. Assessment of yeast cell wall supplementation during late gestation and early lactation on cow performance and calf growth and white blood cells
2. The effect of prenatal stress and postnatal temperament on feeding behavior in post-weaning Brahman bulls
3. The effect of prenatal stress and postnatal temperament on age, body weight, scrotal circumference and paired-testes volume at first sperm, puberty and sexual maturity in Brahman bulls

CHAPTER II

THE EFFECT OF A YEAST CELL WALL SUPPLEMENT DURING LATE GESTATION ON COW PERFORMANCE AND CALF GROWTH AND WHITE BLOOD CELLS

Introduction

The cell wall of yeast consists of 90% alpha-D-mannon and beta-D-glucan of the dry cell wall weight. These are two polysaccharides that have been shown to increase immune function and responsiveness to a microbial attack in pigs (Kogan and Kocher, 2007).

Based upon data derived of yeast cell wall (YCW) components in both human and animals, it appears the YCW could have a positive effect on preparing and altering the immune system for pathogen attacks (Eicher et al., 2006; Volman et al., 2008). Neonatal calves that were supplemented with various yeast cell wall extracts were subjected to a transportation stress, this resulted in an increase in E. coli shedding and feed intake (Eicher et al., 2010). The increased *E. coli* shedding could indicate clearance from the intestines and a lack of colonization. Different yeast extracts can cause modifications in moderation of immune function in livestock that are faced with stressors, which could be contributing to the varying degrees of effectiveness or lack of effectiveness when comparing studies.

Antibiotic growth promoters (AGP) have been used to improve the health of weaned pigs. Recent studies have sought non-antibiotic replacements for the existing AGPs. Yeast cell wall, along with other various modified yeast cultures, have been used

as supplements to determine if digestibility of feedstuffs is increased and if growth of nursing and weaned pigs is improved. For example, a yeast culture increased 1) average daily gain, 2) digestibility of dry matter, and 3) jejunal villus and villus height:crypt depth ratio reducing the need for AGP (Shen et al., 2009). The comparable effects of AGP and yeast culture suggest that yeast culture could be a good alternative to antibiotic use. In dogs, yeast cell wall supplementation tended to increase total nutrient digestibility and immunoglobulin A, which plays a critical role in mucosal immunity. Increases in IgA concentrations suggest an increase in resistance to antigen invasion due to IgA binding to the antigen, which will not allow them to pass the mucosal membrane. The total white blood cell and eosinophil counts tended to decrease with supplementation (Middelbos et al., 2007). Yeast cell wall supplementation of gestating cows has not been investigated in great detail so the objective of this study was to determine a yeast cell wall supplement fed during late gestation influences cow performance and postnatal calf growth and immunity.

Materials and Methods

Forty-eight multiparous cows were grouped by calving date, and then divided into two treatment groups with 24 cows per group. They were fed 0.23 kg of yeast cell wall top dressed with 1.81 kg of a 4:1 corn gluten and soybean meal ration. These cows were fed in groups of twelve where an even distribution of feed was regulated during the last trimester of gestation through 28 d post-calving. Body weight and body condition scoring were collected monthly prepartum. Body condition score is characterized by a scale from 1-9; 1 = emaciated and 9 = obese (Richards et al., 1989). Cows were weighed

and received body condition scores within 24 hours of calving and on d 14 and 28 after calving. They also continued to receive either the cracked corn for the control or yeast cell wall supplement cracked corn based on treatment group pre-calving for 28 d post-calving. Postpartum interval was determined using the date of calving and the date of first detected visual heat.

Within 24-h of calving, the calves were weighed and blood was collected from the jugular vein into three tubes: a tube without any additive, a 15-ml tube coated with EDTA and a 5-ml tube coated with EDTA. These blood samples were placed on ice and processed within 30 min of collection. Serum and plasma was processed in a refrigerated centrifuge at 5^{o}C at 3,000 rpm for 30 min. Serum was stored at 20^{o}C and calf plasma was stored at -80^{o}C (Burdick et al., 2009). Serum concentrations of cortisol were determined by running duplicates using a single antibody radioimmunoassay (Coat-A-Count Cortisol Kit # TKC02, Siemens Medical Solutions Diagnostics, USA). Polypropylene tubes are coated with antibodies to cortisol. A ^{125}I –labeled cortisol competes for an antibody site with the patient sample. The minimum detectable cortisol concentrations for this assay were 3.5 ng/mL and the intra- and inter-assay coefficients of variation were 2.8% and 6.9%, respectively. Using a gamma counter and a standard curve, the cortisol concentrations of the unknown samples were determined. Blood collected in the 5-ml EDTA coated tube was used for blood smears to be analyzed at a later time for a white blood cell profile. These blood collections were repeated on 14 and 28-d after calving. Blood smears were done manually by placing a small drop of blood on the edge of the microscope slide. Using another slide as a spreader placed at a 45^{o}

angle, smoothly and quickly pull the blood drop to the opposite side of slide (Benattar and Flandrin, 1999).

To determine temperament of the calves, pen score and exit velocity were determined on d 14 and 28 after birth. Pen score was determined by placing 3 to 5 calves in a pen and rating their reactiveness to the observer, 1 = docile and 5 = very temperamental (Curley et al., 2008). The exit velocity was the rate in meters per second the calf exits the squeeze chute. This was determined by using a timer with a beam right in front of the squeeze chute and a beam 1.83 m from the squeeze chute. Once the head gate opens, the calf crossed the first beam starting the timer. After traveling 1.83 m the calf crossed the second beam, the timer stopped. The time recorded divided by 1.83 m resulted in the exit velocity (Curley et al., 2008).

Statistical Analysis

Data for the cow weight, BCS and postpartum interval additionally the calf growth and whiteblood cell profile were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) and the Satterthwaite approximation for degrees of freedom. The model for cow performance included treatment as a fixed effect. The models for calf growth and white blood cell profile included treatment, calf sex, temperament class, and day as fixed effects. Random effect included calf sire. Data for pregnancy rate were analyzed using the FREQ procedure of SAS. The model included treatment as the fixed effect.

Results

Cow Performance

Summarized in Table 1, yeast supplementation did not affect the change in cow prepartum BW or BCS or the change in cow postpartum BW or BCS. There was no affect on the postpartum interval or pregnancy rate of the cows.

Table 1. Change[1] in BW and BCS prepartum and postpartum, PPI and pregnancy rate in Brahman cows.

Variable	Control	YCW[2]	*P-Value*
n=	23	23	
Prepartum BW change[1] (kg)	31.05 ± 3.39	35.88 ± 3.39	0.27
Postpartum BW change[1] (kg)	16.39 ± 4.36	14.71 ± 4.36	0.79
Prepartum BCS change[1]	0.15 ± 0.22	0.32 ± 0.22	0.38
Postpartum BCS change[1]	0.26 ± 0.12	0.24 ± 0.12	0.87
PPl (d)	56.18 ± 3.26	56.26 ± 3.19	0.99
Pregnancy Rate (%)	82.61	82.61	

[1]Change in BW and BCS prepartum was for the last third of gestation during supplementation and change in BW and BCS postpartum was 28 d supplementation
[2] YCW: prenatal yeast cell wall supplementation.
BW = Body Weight
BCS= Body Condition Score
PPI= Postpartum Interval

Calf White Blood Cells

There was no interaction of treatment by day ($P > 0.1$) but data are summarized in Table 3. Treatment did not affect the white blood cell profile of calves at 24-h or 28-d as C and YCW calves had similar percentages ($P > 0.2$) of lymphocytes, monocytes, segmented neutrophils and banded neutrophils as summarized in Table 2. Lymphocytes and segmented neutrophils were significantly affected by day ($P < 0.0001$) with lymphocytes having a greater percentage on 28-d of age and segmented neutrophils having a greater percentage at 24-h of age. There was a tendency ($P = 0.076$) for a treatment by sex interaction with control females having a greater percentage of monocytes than the control males, yeast females or yeast males as summarized in Figure 1. Also there was a tendency ($P = 0.09$) for temperament to affect monocytes with temperamental calves having a greater percentage of monocytes (6.17 ± 0.86) than either calm (4.68 ± 0.80) or intermediate (4.06 ± 0.74) calves.

Table 2. The effect of prenatal yeast cell wall supplementation on total white blood cells (%) in Brahman calves between 24-h and 28-d of age.

WBC[1]	Control	YWC[2]	*P-Value*
n=	23	21	
Lymphocytes	44.16	42.87	0.59
Monocytes	4.92	5.01	0.91
Segmented Neutrophils	48.06	49.21	0.64
Banded Neutrophils	2.23	2.80	0.22

[1]WBC: White blood cells
[2]YCW: prenatal yeast cell wall supplementation, calves of cows that were supplemented the last third of gestation.

Table 3. The effect of day on prenatal yeast cell wall supplementation in Brahman calves on white blood cells (%) with an effect of day of age.

	24-h of Age		28-d of Age		
Treatment	Control	YCW[1]	Control	YCW[1]	*P-Value*
n=	23	21	23	21	
Lymphocytes	28.22	29.49	60.11	56.00	0.22
Monocytes	4.14	4.41	5.72	5.77	0.86
Segmented Neutrophils	64.77	63.51	31.36	34.94	0.30
Banded Neutrophils	2.38	2.76	2.09	2.85	0.68

[1]YCW: prenatal yeast cell wall supplementation, calves of cows that were supplemented the last third of gestation.

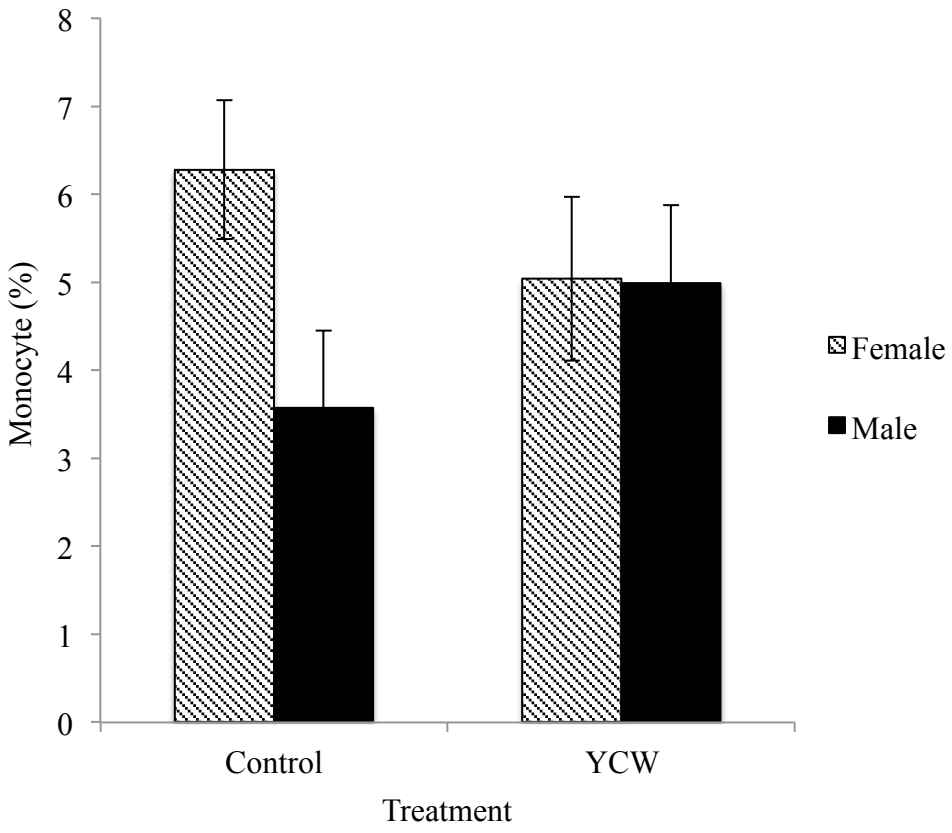

Figure 1. The effect of prenatal YCW[1] supplementation on the total monocytes of Brahman calves between 24-h and 28-d of age and sex, ($P = 0.08$). [1]YCW: prenatal yeast cell wall supplementation, calves of cows that were supplemented the last third of gestation.

Calf Growth and Performance

Calf weights did not differ between yeast or control treatment groups on 24-h or 14-d of age; however, there was a tendency ($P > 0.09$) for control calves to be heavier than yeast calves on 28-d as demonstrated in Table 4. As expected male calves were significantly heavier than female calves however control males tended ($P > 0.08$) to be heavier than either yeast males or control and yeast females on 14-d and was significant ($P > 0.02$) on 28-d as summarized in Table 5. Treatment by day was significant ($P = 0.0047$) with control calves being heavier than yeast calves summarized in Table 6.

Table 7 describes the correlation between lymphocytes at 28 d of age and cortisol on 14-d (Cort14), cortisol on 28-d (Cort28), average of 14-d and 28-d cortisol (AvCort) and body weight on 28-d (BW28). Correlations were calculated for overall, sex and temperament class. Cortisol at 28-d and lymphocytes at 28-d were ($r = -0.44$) negatively correlated ($P < 0.05$) for females and there was also a negative correlation for intermediate calves. Intermediate calves also had a tendency ($P = -0.55$) to be negatively correlated with cortisol at 28-d of age and body weight at 28-d of age. Overall calves had a positive correlation between lymphocytes and body weight at 28-d. Average daily gain (ADG) was also negatively correlated with cortisol at 28-d for all contemporary groups except for temperamental calves ($r= 0.13$).

Prenatal yeast cell wall supplementation did not have an affect on cow performance prepartum or postpartum. Pregnancy rates were not different between treatment groups and the postpartum interval was not improved with fewer days between calving and first recorded estrus. The white blood cell profile was not directly affected by prenatal yeast cell wall supplementation; however, there was a tendency for control females to have a greater percentage of monocytes overall than either females of the yeast group or males of either treatment group. Body weight was affected with control calves being significantly heavier than yeast calves and there was a treatment by sex interaction with control males being heavier than yeast males or yeast and control females. These data suggest that prenatal YCW supplementation to healthy mature cows in a low stress environment does not benefit cow or calf performance.

Table 4. The effect of prenatal yeast cell wall supplementation on BW (kg) in Brahman calves.

Age	Control	YCW[1]	*P-Value*
n=	23	21	
24-h	37.35 ± 1.33	35.79 ± 1.39	0.25
14-d	51.13 ± 1.37	48.74 ± 1.46	0.15
28-d	66.73 ± 1.33	63.34 ± 1.44	0.09

[1]YCW: prenatal yeast cell wall supplementation, calves of cows that were supplemented the last third of gestation.

Table 5. The effect of prenatal yeast cell wall supplementation and sex on BW (kg) in Brahman calves at 24-h, 14 and 28-d of age.

Sex	Female		Male		
Treatment	Control	YCW[1]	Control	YCW[1]	*P-Value*
n=	13	11	10	10	
24-h	34.60 ± 1.54	35.01 ± 1.72	40.11 ± 1.68	36.57 ± 1.69	0.14
14-d	47.15 ± 1.67	47.75 ± 1.91	55.13 ± 1.88	49.73 ± 1.89	0.08
28-d	61.10 ± 1.73[a]	62.57 ± 2.02 [a]	72.35 ± 2.02 [b]	64.10 ± 2.05 [a]	0.02

[1]YCW: prenatal yeast cell wall supplementation, calves of cows that were supplemented the last third of gestation.

Table 6. The effect of prenatal yeast cell wall supplementation on BW (kg) from d -140 preweaning to weaning in Brahman calves.

	Treatment	
Day[2]	Control	YCW[1]
n=	23	21
-140	73.67 ± 3.64	68.08 ± 4.07
-112	96.73 ± 3.50	89.66 ± 3.79
-84	126.52 ± 3.50	115.77 ± 3.79
-56	153.12 ± 3.50	137.49 ± 3.79
-28	179.64 ± 3.50	163.67 ± 3.79
Weaning	208.02 ± 3.50	193.11 ± 3.79

[1]YCW: prenatal yeast cell wall supplementation, calves of cows that were supplemented the last third of gestation, $P = 0.0047$.
[2]Day indicates the number of days preweaning with weaning being day 0.

Table 7. Pearson correlation coefficients between lymphocyte percent at 28-d of age and cortisol on 14-d, cortisol on 28-d, average of 14-d and 28-d cortisol and body weight on 28-d for overall, sex and temperament class[5].

Contemporary Group	Cort14[1]	Cort28[2]	AvCort[3]	BW28[4]
Overall	-0.13	-0.13	-0.19	0.28*
Female	-0.08	-0.44**	-0.21	0.13
Male	-0.13	-0.13	-0.16	0.22
Calm	0.21	-0.11	0.11	0.01
Intermediate	-0.35	-0.41*	-0.50**	0.50**
Temperamental	-0.19	0.01	-0.08	0.40

* $P < 0.1$, ** $P < 0.05$

[1]Cort14: serum cortisol concentration at 14-d of age.

[2]Cort28: serum cortisol concentration at 28-d of age.

[3]AvCort: average serum cortisol concentration between 14 and 28-d of age.

[4]BW28: body weight (kg) at 28-d of age.

[5] Temperament class was based on temperament scores, calm (< 1.78), intermediate (1.78-2.90) and temperamental (> 2.90).

Discussion

Cow Performance

Cow prepartum change in BW and BCS did not differ significantly between treatment groups, additionally postpartum change in BW and BCS did not significantly differ between yeast and control cows. Dann et al., (2000) demonstrated that BCS did not change prepartum and postpartum in Jersey cows fed yeast culture. In the previous study cows were only fed 21-d prepartum and continued through 140-d postpartum, additionally supplemented yeast culture cows had increased dry matter intake and lost less weight quickly during the postpartum period than control cows (Dann et al., 2000). During prepartum and postpartum portions of the current study, cows gained body weight however there was no significant difference between treatment groups. Previous studies had proposed that yeast cell wall and other yeast additives benefit the rumen by improving the viability of the rumen microbes which would lead to increased feed intake, fiber digestion and microbial protein which would all lead to improved weight gain and milk production (Wallace and Newbold, 1994). We did not find improved weight gain in the current study suggesting that microbial activity was not affected to a magnitude that was of benefit to the cow's digestive system.

Yeast supplementation continued through 28-d post-calving; however, it did not have a significant affect on the postpartum interval or pregnancy rate between treatment groups. Pregnancy rates for the yeast cows (86.21%) and the control cows (86.21%) were comparable to results found when fed adequate nutrient requirements in Brahman cows (Browning et al., 1994; Randel, 1990). The postpartum interval was shorter than

previous research when comparing Brahman control cows (Henao et al., 2000; Tolleson and Randel, 1998). All cows analyzed for the postpartum interval and pregnancy rates were suckling calves. The lack of affect on weight change or BCS change could result in the lack of a difference in the postpartum interval and pregnancy rate. In a review by Dunn and Kaltenbach (1980), the postpartum interval increases as the prepartum weight decreases; however, we did not see a difference in weight change prepartum between yeast and control cows so it could be expected that the postpartum interval for this study did not change and consequently the pregnancy rates were similar between yeast and control cows.

Calf White Blood Cells

Prenatal yeast supplementation did not affect the total lymphocyte, monocyte, segmented or banded neutrophil counts. There was also no affect between treatment groups at 24-h or 28-d; however, there was a day effect for lymphocytes and segmented neutrophils. Lymphocytes were at a greater percentage on 28-d than at birth and segmented neutrophils were at a greater percentage at birth and reduced on d 28. This follows a similar trend as seen by Brun-Hansen et al. (2006), in which the mean neutrophil count was higher in the first week than the lymphocyte count and this was reversed even after the first two weeks. However, in their study it was found that monocytes increased during the first four weeks whereas monocytes did not change by day in the current study.

Breed, calf age, gender and health of the animal can affect immune cells. There was a tendency for a treatment by sex interaction in which the control females had a

greater percentage of monocytes than the control males, yeast females and yeast males. In Mohri et al. (2007) Holstein dairy calves did not show any significant sex interaction, which differs from the current study. Mohri et al. (2007), demonstrated a consistent trend in the regression of segmented neutrophils and increase in lymphocytes from 24-h to 28-d of age. Mirzadeh et al. (2010) reported there was no significant difference in the total WBC between male and female calves.

In this study, temperament had a tendency to affect monocytes with temperamental calves having a greater percentage of monocytes than calm or intermediate calves. Temperament has been shown to decrease ADG, increase basal concentrations of cortisol and overall decrease performance in cattle. Impaired clearance of bacteria due to increased glucocorticoids has been demonstrated and cortisol suppresses the immune system and immune cells (Martin, 2009). There are also recent studies suggesting that increased glucocorticoids have a positive effect on immune function and can help redistribute cells to various organs (Dhabhar et al., 2010).

Calf Growth and Performance

Treatment did not affect calf weight at 24-h and 14-d of age however there was a tendency ($P = 0.09$) for control calves to be heavier than yeast calves on 28-d of age. Sexual dimorphism was also observed in which males were significantly heavier than females at 24-h, 14-d, 28-d of age and at 180-d adjusted weaning ($P < 0.02$). Control males were significantly ($P = 0.02$) heavier than yeast males. Weaning weights and birth weights were comparable to previous research (Browning et al., 1994; Thrift, 1997). Pre-weaning data demonstrates that while prenatal yeast supplementation did not improve

growth of the calves, it did not have a deleterious effect when compared to previously documented Brahman and *Bos indicus* research.

Cortisol on 28-d of age was negatively correlated with lymphocytes at 28-d and ADG for all contemporary groups except temperamental calves; the cause of this is unknown since temperament did not significantly affect cortisol in this study. Also in Burdick et al., (2009) cortisol did not show any correlation with sex and temperament class as well; however, body weight gain (ADG) was negatively correlated with cortisol, which is consistent with the findings of the current study. From previous research, lymphocytes and body weight have been negatively correlated to cortisol both during a specific stressor, such as shipping stress, and under non-stressful situations in the neonate (Mao et al., 1994; McGlone et al., 1993). Cortisol at 14-d did not show a strong correlation with lymphocyte percentages at 28-d, which means that 14-d cortisol, may not be a good indicator of future lymphocyte progression.

While prenatal yeast cell wall supplementation did not improve cow and calf performance it did not have a deleterious effect on the cow or calf. Postpartum interval and pregnancy rates were similar between treatment groups as well as prepartum and postpartum BCS and BW. Calf weights and leukocytes did not differ by treatment but there was some sexual dimorphism in which females had a greater percentage of monocytes than males. These results suggest that prenatal yeast cell wall supplementation does not benefit cow or calf performance when the cows are not challenged by a pathogen or nutrition.

CHAPTER III

THE EFFECTS OF PRENATAL STRESS AND TEMPERAMENT ON POST WEANING FEEDING BEHAVIOR IN BRAHMAN BULLS

Introduction

Transportation is a very common stressor in the cattle industry. Activation of the hypothalamic-pituitary-adrenal axis (HPA) lead to the potential for decreased skeletal and organ growth, suppressed immune function, lower birth weight and altered behavior of the offspring. Prenatal stress can have a lasting affect on an animal both physically and mentally such as increased temperamental behavior. Additionally, prenatal stress can alter the stress response such as increased cortisol, increased heart rate and decreased clearance rate of plasma cortisol (Lay et al., 1997; Littlejohn et al., 2012). Prenatal stress has been demonstrated to affect temperament and temperamental cattle have reduced average daily gain in a feedlot system and decreased body weight at weaning (Francisco et al., 2012; Voisinet et al., 1997). Flight speed, an indicator of temperament, in feedlot cattle was negatively correlated with dry matter intake ($r = -0.34$) and head down duration ($r = -0.56 \pm 0.38$). This demonstrates that temperamental cattle spend less time feeding than calmer cattle. Prenatally stressed rats exhibited an increase in feed intake and increased glucose concentrations after a period of fasting (Lesage et al., 2004). Exposure to maternal stressful situations and resulting increased concentrations of glucocorticoids have been thought to increase anxiety-like behavior and certain emotional disorders in offspring. Children from mothers with increased prenatal anxiety and depression have exhibited increased prevalence of emotional

disorders, such as anxiety and ADHD (Rice et al., 2007). The objective of this study was to determine whether prenatal stress and postnatal temperament influences the post-weaning feeding behavior of Brahman bulls.

Materials and Methods

Post-weaning bulls used for the adaptation period feeding behavior study were derived from a previous study (Price et al., 2012). Specifically, eighty-five pregnant Brahman cows were matched by age and parity then randomly assigned to 1 of 2 treatment groups. Forty-two were control cows left on the farm and forty-three cows were transported for 2 h on d 60, 80, 100, 120 and 140 ± 5 of gestation to create prenatal stress. These calves were born during March and April of 2012. From the calves born, there were forty bull calves with sixteen prenatally stressed and twenty-four control bull calves. At weaning, pen score and exit velocity was determined on these bull calves. Pen score was determined by placing 3 to 5 calves in a pen and rating their reactiveness to the observer, 1 = docile and 5 = very temperamental (Curley et al., 2008). The exit velocity was the rate in meters per second the calf exited the squeeze chute. This was determined by using a timer with a beam right in front of the squeeze chute and a beam 1.83 meters from the squeeze chute. Once the head gate opens, the calf crosses the first beam starting the timer. After traveling 1.83 m the calf crossed the second beam, the timer stopped. The 1.83 m is divided by the recorded time to calculate the exit velocity (m/s). A temperament score was calculated from the average of the pen score and exit velocity (Curley et al., 2008). The temperament scores were then grouped into temperament classes of calm (< 1.78), intermediate (1.78-2.90) and temperamental (>

2.90). Temperament classes were assigned based on 0.5 standard deviation from the mean.

The bulls were characterized for feeding behavior parameters using a GrowSafe (GrowSafe Systems Ltd., Airdrie, Alberta, Canada) system at the Brown Loam Experiment Station in Raymond, MS. Before being placed in pens each bull received a plastic electronic tag that included identification number as well as a passive radio frequency transponder positioned 5 to 6 cm from the base of the right ear (Wang et al., 2006). Measurements were taken immediately following the application of the electronic identification tag to determine the adaptation of the bulls to a novel feeding system and location. The bulls were divided into 2 pens with 4 bunks per pen. Bulls were randomly assigned to 1 of 2 pens based on bull identification number. Feeding behavior was measured for 14-d during January of 2013, which was the adaptation feeding period. The GrowSafe system collected data using analysis software that determined the amount of feed consumed per meal, duration of meals and also time between meals. This was determined when the transponder of the bull was detected and ended when the time between the last 2 transponder readings was greater than 300 s (Lancaster et al., 2009). Using these data, feeding measurements to describe the feeding behavior were:

1. Number of visits per day, number of total feeding events
2. Number of meal events per day, number of feeding events in which feed was consumed
3. Head down time, min/d, total feed time per day
4. Head down time per meal, min/meal, total feed time per meal

5. Average meal size, kg/meal,
6. Feeding rate, g/s
7. Feed intake per 24 h period, kg/d
8. Gain: feed ratio
9. Gain over 14-d period, kg
10. Feed intake, % of BW

The number of visits is defined as the total number of feeding events whether feed was consumed or not. The meal events are defined as the number of feeding events in which feed was consumed. The ration consisted of 41% cottonseed hull pellets, 41% soybean hull pellets, 15% premix pellet and 3% condensed distillers solubles (as-fed basis). Crude protein was 25.2% and TDN was 58.3% of the concentrate (as-fed basis).

Statistical Analysis

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) and the Satterthwaite approximation for degrees of freedom. The model included treatment, temperament class, day and their interactions were included as fixed effects for number of visits, meal events, head down time, head down time per meal, average meal size, feeding frequency and feed intake per day. The random effect included bull sire. For gain over the 14-d adaptation period, gain:feed ratio and intake as a percentage of BW, MIXED procedure was used. The model included treatment, temperament class and the interaction. The random effect included bull sire. All data are reported as the least squares means ± standard errors.

Results

Prenatal stress and temperament class interactions were observed for head down time and head down time per meal, all other feeding behavior and efficiency characteristics did not have prenatal stress by temperament class interactions. Head down time per day was significantly affected by a prenatal stress by temperament interaction (P = 0. 0.02) with the results summarized in Figure 2, additionally head down time per meal was significantly affected by a treatment by temperament interaction (P = 0.0010) with the results summarized in Figure 3. The number of visits also had a significant interaction of temperament class and day (P = 0.0532) as summarized in Figure 4. Additionally, feed intake per day with respect to prenatal treatment and temperament class are summarized in Figure 5 and 6.

The effect of prenatal stress treatment on feeding behavior is reported in Table 8. Prenatal stress did not affect the number of visits (P = 0.33), meal events (P = 0.29), head down time (P = 0.21), feeding rate (P = 0.22), feed intake per day (P = 0.15), gain (P = 0.87), feed intake as a percentage of BW (P = 0.88) or G:F ratio (P = 0.66). Average meal size was affected by prenatal treatment with PNS bulls (0.82 ± 0.04) having a greater average meal size (P = 0.02) than control bulls (0.72 ± 0.04). Head down time per meal was greater for prenatally stressed bulls compared with control bulls per meal (4.07 ± 0.32, 3.14 ± 0.31, respectively, P = 0.0003).

Temperament had a significant affect on the number of visits (P = 0.0061), meal events (P = 0.0035), head down time (P = 0.0016), and head down time per meal (P = 0.0026) and feeding rate (P = 0.0001) as summarized in Table 9.There was no effect of

temperament on average meal size (P = 0.34), total feed intake per day (P = 0.37), gain (P = 0.92), feed intake as a percentage of BW (P = 0.99) or G:F ratio (P = 0.54).

Table 8. The effect of prenatal stress on feeding behavior and feeding efficiency in yearling Brahman bulls.

Variable	Control	PNS[1]	*P-Value*
n=	25	18	
[4]BW (kg)	222.47 ± 6.70	231.11 ± 6.80	0.37
Gain (kg)[2]	17.49 ±1.92	17.03 ± 1.95	0.87
Number of visits (visits/d)	12.64 ± 0.71	12.13 ± 0.73	0.33
Meal events (meals/d)	11.45 ± 0.63	10.93 ± 0.64	0.29
Head down time (min/d)	33.13 ± 3.14	36.39 ± 3.20	0.21
Head down time per meal (min/meal)	3.14 ± 0.31	4.07 ± 0.32	< 0.01
Feed intake per day (kg/d)[3]	7.28 ± 0.23	7.64 ± 0.24	0.15
Average meal size (kg/meal)	0.72 ± 0.04	0.82 ± 0.04	0.02
Feeding rate (g/s)	4.61 ± 0.30	5.10 ± 0.31	0.22
Feed Intake, % of BW/d[3]	3.32 ± 0.17	3.28 ± 0.17	0.88
G:F Ratio (kg)[3]	0.27 ± 0.04	0.30 ± 0.04	0.66

[1]Prenatal stress (PNS): Cows were transported for 2 h on d 60, 80, 100, 120 and 140 ± 5 of gestation.
[2]Gain over 14-d adaptation period.
[3]As-fed basis.
[4]BW: body weight

Table 9. The effect of temperament class[1] on feeding behavior and feeding efficiency in yearling Brahman bulls.

Variable	Calm	Intermediate	Temperamental	*P-Value*
n=	26	9	8	
[4]BW (kg)	222.21 ± 5.38	228.69 ± 9.23	229.49 ± 9.53	0.72
Gain (kg)[2]	17.91 ± 1.55	16.93 ± 2.65	16.93 ± 2.74	0.92
Number of visits (visits/d)	11.61 ± 0.70[a]	12.16 ± 0.76[ab]	13.37 ± 0.79[b]	< 0.01
Meal events (meals/d)	10.43 ± 0.62[a]	11.00 ± 0.67[ab]	12.15 ± 0.70[b]	< 0.01
Head down time (min/d)	31.46 ± 3.05[a]	31.71 ± 3.39[a]	41.11 ± 3.56[b]	< 0.01
Head down time per meal (min/meal)	3.26 ± 0.30[a]	3.37 ± 0.33[a]	4.18 ± 0.35[b]	< 0.01
Feed intake per day (kg/d)[3]	7.26 ± 0.22	7.52 ± 0.27	7.59 ± 0.29	0.37
Average meal size (kg/meal)	0.80 ± 0.04	0.78 ± 0.05	0.73 ± 0.05	0.34
Feeding rate (g/s)	5.84 ± 0.27[a]	4.55 ± 0.39[b]	4.17 ± 0.41[b]	< 0.01
Feed Intake, % of BW/d[3]	3.3 ± 0.13	3.32 ± 0.23	3.28 ± 0.24	0.99
G:F Ratio (kg)[3]	0.32 ± 0.03	0.26 ± 0.05	0.28 ± 0.05	0.54

[1]Temperament class was based on temperament scores, calm (< 1.78), intermediate (1.78-2.90) and temperamental (> 2.90).
[2]Gain over 14-d adaptation period.
[3]As-fed basis.
[4]BW: body weight
[a,b]Within a row means without a common script differ by ($P \leq 0.05$).

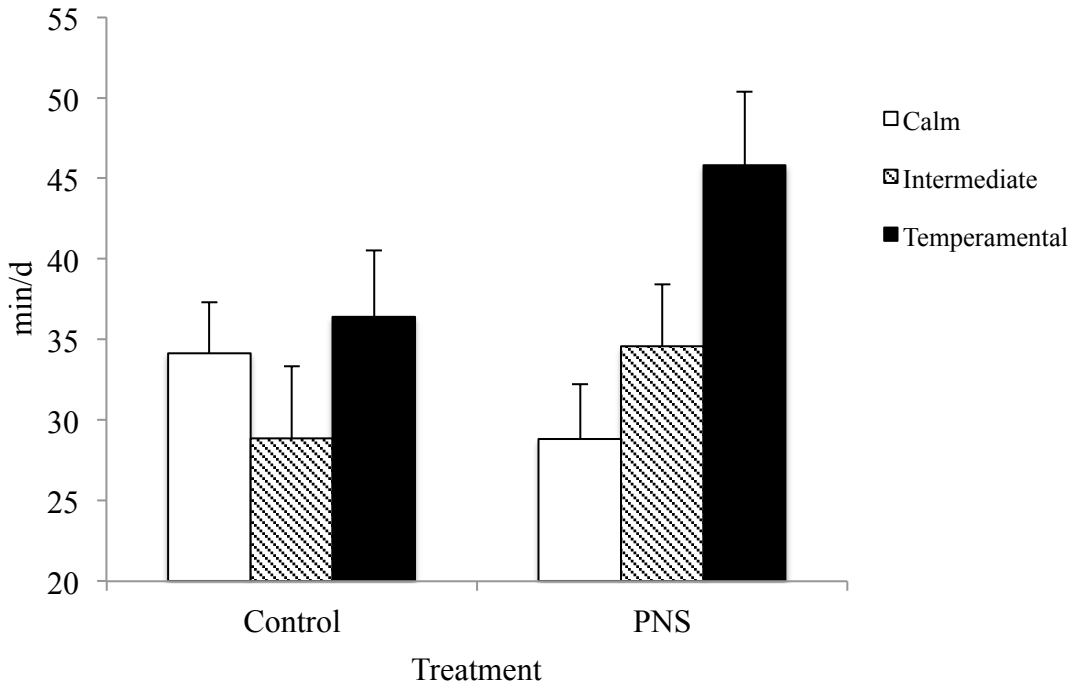

Figure 2. Feeding behavior for a 14-d adaptation period. Head down time per day (min/d) with a treatment[1] by temperament class[2] interaction in post-weaning Brahman bulls (P = 0. 0.02). [1]Prenatal stress (PNS): Cows were transported for 2 h on d 60, 80, 100, 120 and 140 ± 5 of gestation, PNS bulls were the progeny. [2]Temperament class was based on temperament scores, calm (< 1.78), intermediate (1.78-2.90) and temperamental (> 2.90).

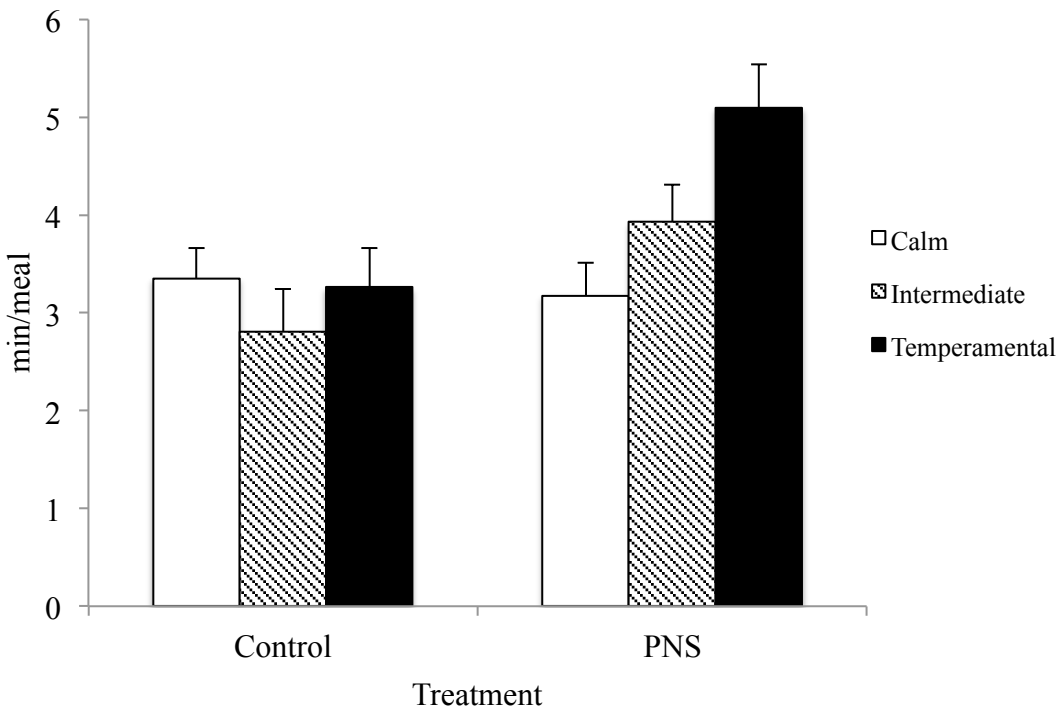

Figure 3. Feeding behavior for 14-d adaptation period. Head down time per meal (min/meal) with a treatment[1] by temperament class[2] interaction in post-weaning Brahman bulls ($P = 0.001$). [1]Prenatal stress (PNS): Cows were transported for 2 h on d 60, 80, 100, 120 and 140 ± 5 of gestation, PNS bulls were the progeny. [2]Temperament class was based on temperament scores, calm (< 1.78), intermediate (1.78-2.90) and temperamental (> 2.90).

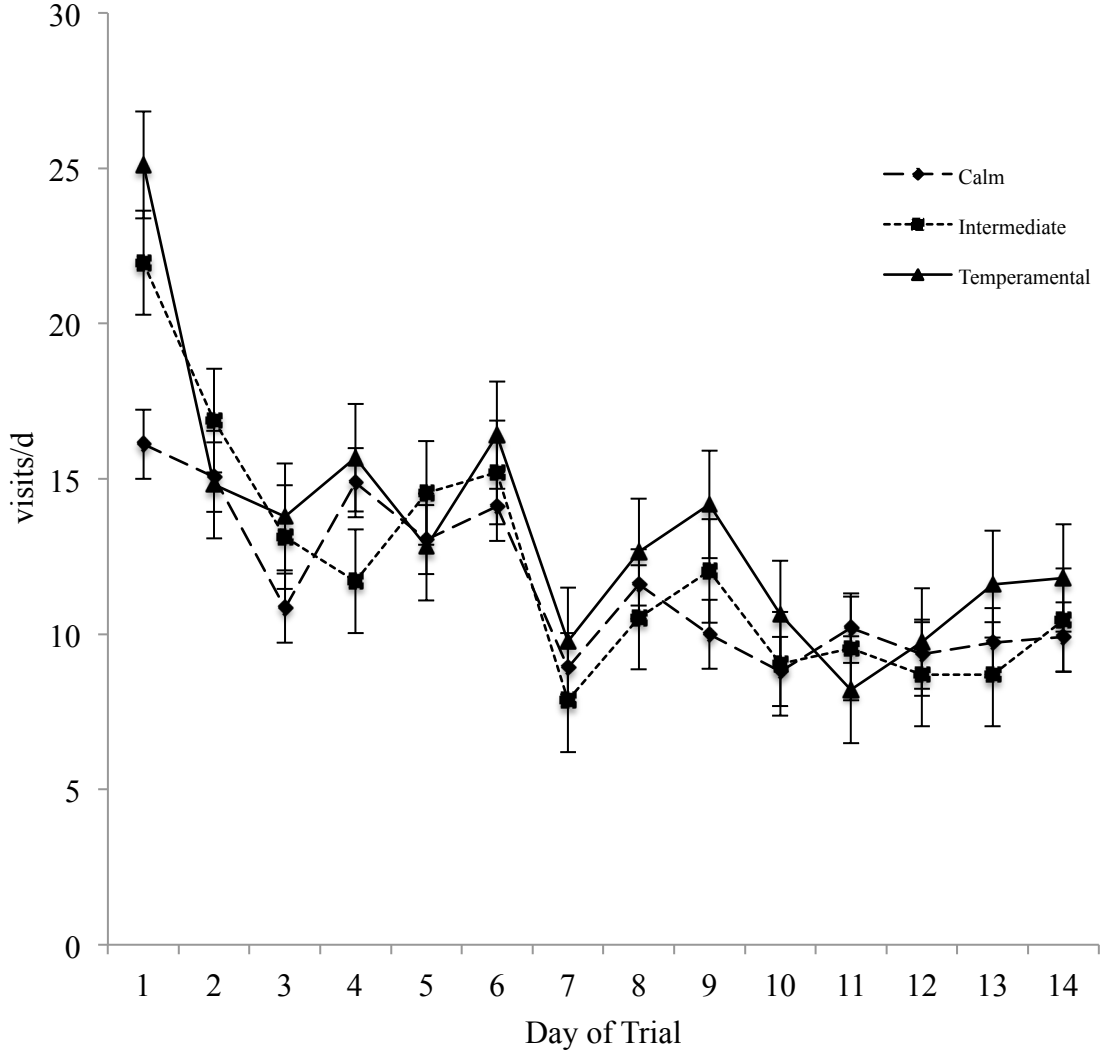

Figure 4. Number of visits[1] for a 14-d adaptation period. Temperament class[2] by day interaction ($P = 0.0532$) in post-weaning Brahman bulls. [1] Number of visits is defined as the number of visits whether feed was consumed or not. [2]Temperament class was based on temperament scores, calm (< 1.78), intermediate (1.78-2.90) and temperamental (> 2.90).

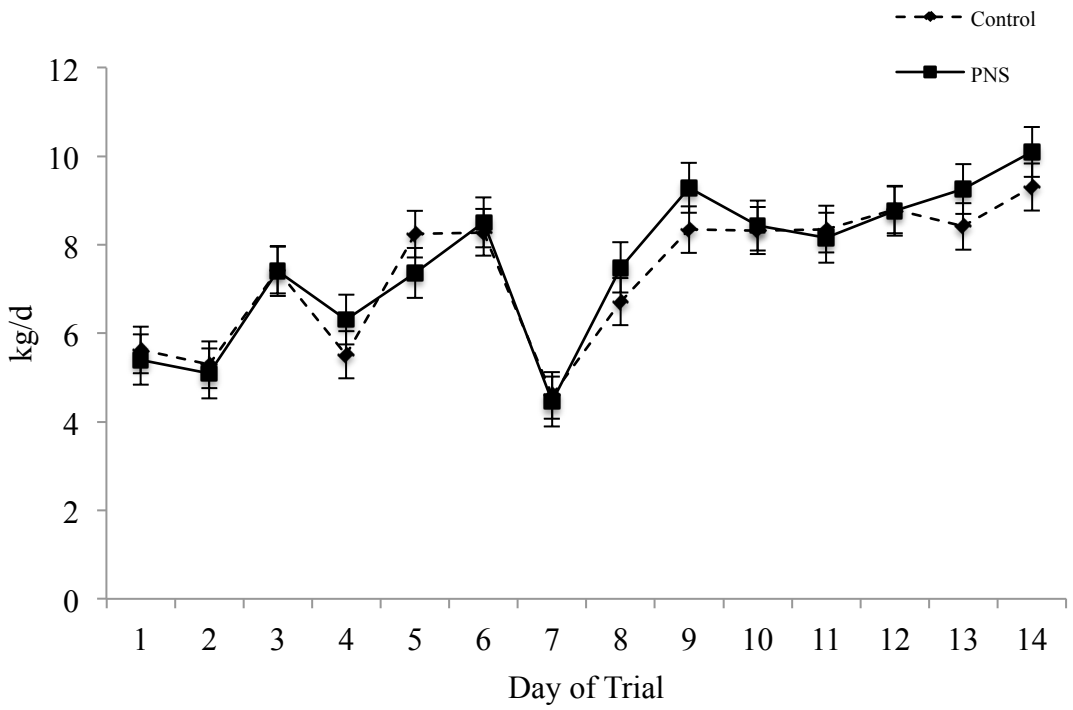

Figure 5. Feed intake per day by prenatal treatment[1] for a 14-d adaptation period in post-weaning Brahman bulls ($P = 0.72$). [1]Prenatal stress (PNS): Cows were transported for 2 h on d 60, 80, 100, 120 and 140 ± 5 of gestation, PNS bulls were the progeny.

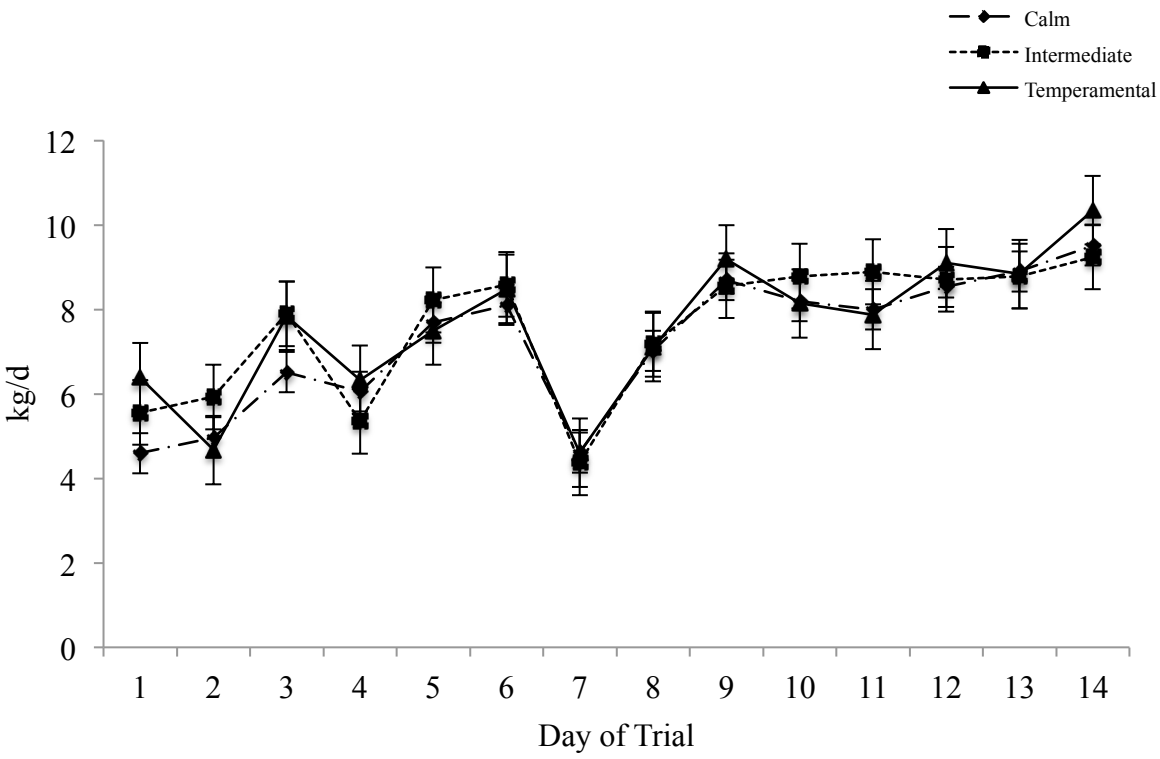

Figure 6. Feed intake per day by temperament class[1] for a 14-d adapation period in post-weaning Brahman bulls (P =0.99). [1]Temperament class was based on temperament scores, calm (< 1.78), intermediate (1.78-2.90) and temperamental (> 2.90).

Discussion

Feeding behavior was assessed in prenatally stressed yearling Brahman bulls during a typical receiving period of 14-d. Number of visits to the feed bunk and meal events were not significantly affected by prenatal treatment; however, both feeding behavior characteristics were significantly affected by temperament. Specifically, temperamental bulls had a greater number of visits and meal events than either calm or intermediate bulls. Temperament has also been found to affect growth rates, feed intake, time spent eating and meat quality (Café et al., 2011). Additionally, Brahman cattle had a stronger association with temperament, growth rates, feed intake, time spent eating and meat quality than Angus cattle (Café et al., 2011). Average daily gain was not calculated in this study for the two-week acclimation period. This was due to the potential lack of accuracy in the ADG since the recommended length to calculate ADG is 63-d when using the GrowSafe system (Wang et al., 2006).

Prenatal stress had a significant affect on head down time per meal and average meal size while it did not have an affect on any other feeding behavior characteristic. Prenatal stress has been demonstrated to affect temperament within these bulls (Littlejohn et al., 2013). Therefore, temperament of these bulls also affected head down time, head down time per meal and feeding rate. In previous studies cattle with more excitable temperaments had decreased ADG, BCS and DMI along with a negative genetic correlation with head down time (Nkrumah et al. 2007; Petherick et al. 2002). The differences between previous studies and the current study could be due to the calculation of head down time which was determined by the start of a feeding event in

which the bull consumed feed and ended when the time between the last two readings was greater than 300s (Chen et al., 2014). However, from the current study, the more excitable bulls could have come back to the feed bunk more frequently within one feeding event and all of that activity counts as the same head down time, while a calm bull might stay at the bunk for the entirety of the meal event. This could explain the significant difference between temperament classes in which the temperamental bulls had greater number of visits and meal events over the intermediate and calm bulls.

From these results, postnatal temperament of the bull had a greater effect than prenatal stress. The interactions of temperament and prenatal stress did reveal that prenatally stressed bulls, that were also classified as temperamental bulls had a greater amount of head down time and head down time per meal. Café et al. (2011) found that for every one meter per second increase in flight speed from the chute, used as a measure of temperament, there was a 17.6 min/d reduction in feeding time and a tendency for reduced feed conversion ratios. This previous research contradicts the findings from the current study. The Café et al. (2011) study used a flight speed instead of a combination of exit velocity (objective) and a pen score (subjective) to determine the temperament of the cattle.

In the current study average meal size was significantly affected by prenatal treatment and temperament class which affected feeding rate. These results differ from Golden et al. (2007), who reported that feeding rate did not differ between low or high RFI groups. This could indicate that feed efficiency of the bull is independent of the feeding rate. In rats, a stressor has been shown to cause reduced meal size and duration

of the meal; however, in the prenatally stressed bulls average meal size and head down time per meal were significantly increased. This could suggest that prenatally stressed bulls can cope more with a significant stressor such as a new environment by frequenting the bunk multiple times within one meal event, which would extend the total time at the bunk, while also increasing the average meal size.

Total feed intake per day was not affected by treatment or temperament. Feed The temperature dropped 7°C between d 6 to 7 of the trial which could explain the reduction in feed intake for day 7 of the trial. Francisco et al. (2012) found similar results studying the post-weaning acclimation period in which there were no significant differences in temperament and ADG. However, through the growing and finishing period there was a tendency for decreased HCW with the excitable calves. Additionally, steers were either acclimated to the facility or left untouched as a control and the results demonstrated that the acclimated steers had a decreased temperament score, but there was decreased ADG and gain to feed ratio and also a tendency for decreased DMI within the acclimated steers. It was suggested that there was a greater stress response due to a tendency for a higher cortisol concentration in the serum for the acclimated steers than the control steers. These findings are indicative that acclimating *Bos taurus* steers to extensive handling did not affect feedlot performance in a positive manner. In contrast, Nkrumah et al. (2007) found that DMI had negatively correlated with flight. Differences in feed intake among different studies could be due to sex, age and breed. Also this current study examined the differences in prenatal stress and temperament on the feeding

behavior in the first two weeks after transportation and most studies have an adjustment period before data is collected.

It can be concluded that postnatal temperament had a greater affect on feeding behavior in yearling Brahman bulls than prenatal stress. However it has been shown that prenatal stress can affect postnatal temperament (Littlejohn et al., 2012). Average daily gain was not calculated in this study however temperamental bulls had a greater head down time and head down time per meal than calm or intermediate bulls and prenatally stressed bulls had a greater head down time per meal than the control bulls. When examining a receiving period, DMI as a percentage of body increased between d-1 and d-28 after transportation. Additionally, the willingness of calves to eat after a transportation stress increases as the receiving period continues (Hutcheson and Cole, 1986). The feeding behaviors in this study differs with reports in the literature. This could be explained based on the examination of the adjustment period in this study and not on overall feeding behavior after an adjustment period, also the utilization of *Bos indicus* yearling bulls.

CHAPTER IV

THE EFFECTS OF PRENATAL STRESS AND POSTNATAL TEMPERAMENT ON AGE, BODY WEIGHT, SCROTAL CIRCUMFERENCE AND PAIRED TESTES VOLUME AT FIRST SPERM, PUBERTY AND SEXUAL MATURITY IN BRAHMAN BULLS

Introduction

Postnatal exposure to stressors is a well-recognized situation in livestock production. For example, livestock experience or encounter unavoidable stressors due to exposure to various required managerial processes such as transportation, restraint in a squeeze chute, and social regrouping following weaning or marketing through livestock auctions. In more recent years, questions have arisen as to whether prenatal exposure to stressors could affect the fetus in utero and/or the aspects of health, performance or behavior of progeny at some point in postnatal life.

Brahman cattle have been known to mature later than *Bos taurus* breeds such as Angus or Hereford (Fields et al., 1979). Once Brahman bulls reach sexual maturity, they exhibit a larger scrotal circumference when compared with Angus bulls. Brahman bulls exhibit some seasonality when compared to Hereford bulls suggesting there is sensitivity of Brahman testes when comparing sperm quality and testosterone (Godfrey et al., 1990). Testicular weight has been closely correlated with semen production in breeding bulls (Coulter and Foote, 1979). Brito et al. (2012) found that body weight was negatively correlated with age at puberty ($r = -0.48$) and sexual maturity ($r = -0.68$) and body weight was positively correlated with paired-testes weight ($r = 0.42$) and

seminiferous tubule volume (r = 0.57). This could indicate that pre-weaning development is more closely associated with sexual development than post-weaning and feedlot development. Many factors and events can influence sexual development of bulls. Bulls with higher serum testosterone concentrations reached puberty earlier than bulls with lower testosterone concentrations (Lunstra et al., 1978). Foote (1978) stated that environment is a major contributor to sexual development and semen quality because various environmental conditions can lead to either temporary or permanent changes that can hinder spermatogenesis. Environmentally induced permanent changes, such as season, mostly occur during the prenatal and prepubertal periods of development. Season of birth has also been shown to influence the age at sexual maturity and interval between puberty and sexual maturity with spring born bulls reaching sexual maturity sooner and heavier, indicating that photoperiod could also influence sexual development in *Bos indicus* bulls (Tatman et al., 2004). Prenatal stress has been reported to alter behavior, HPA axis function, and sexual development in many species (Lay et al., 2011; Diz-Chaves et al., 2013; Gutierrez-Rojas et al., 2013). As reported by Lay et al. (1997), prenatal stress has increased ACTH secretion continuously in the post-natal calf. Increased activation of the HPA axis can suppress GnRH through cortisol (Borg et al, 1991; Welsh et al., 1982).

The objective of this study was to determine whether prenatal stress and postnatal temperament influence 1) testicular dimensions and measurements, 2) the appearance of the first sperm in the ejaculate, 3) age and body weight at puberty and 4) age and body weight at sexual maturity of Brahman bulls.

Materials and Methods

The bull calves used in the current study were derived from a prior experiment (Price, 2013). Specifically, eighty-five pregnant Brahman cows were matched by age and parity then randomly assigned to one of two treatment groups. Forty-two were control cows left on the farm and forty-three were transported for 2 h on d 60, 80, 100, 120 and 140 ± 5 of gestation. These calves were born during March and April of 2012. From the calves born, sixteen were born to prenatally stressed dams and twenty-four were born to control dams.

Sexual maturation was assessed beginning January 15, 2013 from which time body weight of each bull was recorded at 2-wk intervals in order to determine the change in weight from weaning to sexual maturity and to calculate each bull's average daily gain (kg/d). A scrotal circumference measurement of the bull calves was taken by palpating the testes into the lower part of the scrotum. While holding the testes down within the scrotum, the scrotal tape was then looped around the largest circumference of the testes as stated by Tatman et al. (2004). Measurements of the right and left testes of each bull were taken at the point of maximum length using calipers. Paired testes volume (PTV) was calculated by the formula PTV= [0.0396125 X (average testes length) X (scrotal circumference)^2] (Lunstra and Schanbacher, 1988).

Once the scrotal circumference reached 24 cm or greater, semen was collected using electroejaculation (Standard Precision Electronics, Denver, CO). Electroejaculation was conducted in a squeeze chute with one person maintaining the probe in the rectum of the bull to prevent expulsion and the other person to clean and cut

the hair around the preputial orifice, if needed, and maintain the power of the electroejaculator unit. Starting on the first power level the intensity was increased in a pulsatile fashion 4-5 times on each level. Once the pre-ejaculatory fluid was cleared, the semen was collected (Furman et al., 1975). Semen collection was performed the same day that weight and scrotal measurements were taken. Semen samples were analyzed for sperm motility, which was visually assessed as a percentage of sperm with forward, fast motility. Also concentration of the ejaculate was determined using a hemocytometer. This was done by diluting the ejaculate (1:200) with saline then placing the mixture into both grids of the hemocytometer. After dilution of the semen, a coverslip was placed on top of the hemocytometer between the two rails, which held the cover slip in place. Using the micropipette for drawing semen into the dilution unipette, 10-15µl of solution containing sperm was placed under the coverslip across the grid of the hemocytometer. While viewing the sample with the 40X objective, average sperm count for five squares for both grids was calculated. The average sperm count from the hemocytometer and the volume of ejaculate were used to calculate the concentration of sperm per ejaculate (Anzar et al., 2009). The formula for sperm concentration using a hemocytmeter was [(average of the grids/0.02) X 100,000)] = concentration/ml.

The age at first sperm for each individual bull was defined as the age at which the first visible sperm was present in the bull's ejaculate whether or not the sample exhibited progressive motility. Puberty was classified as an ejaculate that contained 50 X 10^6 sperm with at least 10% motility. Collection of data was concluded once a bull reached sexual maturity, which was classified as an ejaculate that contained 500 X 10^6

sperm with at least 50% motility (Wolf et al., 1965; Killian and Amann, 1972; Barber and Almquist, 1975). Once each bull reached sexual maturity as defined, collection of data related to body and testicular growth ceased.

Statistical Analysis

Data were analyzed using the MIXED procedure (SAS Inst. Inc., Cary, NC) and the Satterthwaite approximation for degrees of freedom. The model included treatment, temperament class and the interaction was included as fixed effects for first sperm, puberty and sexual maturity. The random effect included bull sire. Data are reported as the least squares means ± standard errors.

Results

There were no interactions between prenatal stress treatment and temperament class for all sexual development characteristics. Age, scrotal circumference and paired testes volume (PTV) were similar between control and prenatally stressed (PNS) bulls at the appearance of first sperm. However, body weight was significantly greater (P = 0.04) in PNS bulls at first sperm as shown in Table 10. Table 11 and Table 12 show the averages between control and PNS for age, BW, SC, PTV, SC per 100 kg of BW and PTV per 100 kg of BW at puberty and sexual maturity. The time interval (d) between first sperm and puberty (P = 0.32) and puberty to sexual maturity (P = 0.96) were not affected by PNS. However, temperamental bulls had a greater (P > 0.01) time (69.25 ± 10.73 d) from puberty to sexual maturity than calm (27.21 ± 6.05 d) or intermediate bulls (38.60 ± 9.05 d). The effect of temperament class on the time interval between puberty and sexual maturity is summarized in Figure 7. There is about a 40 d average

difference in the time interval between calm and temperamental bulls showing a significant delay in sexual development after puberty in temperamental bulls.

Temperament of the bulls did not have a significant effect on age, BW, SC, PTV or SC per 100 kg of BW at first sperm, puberty or sexual maturity. Scrotal circumference at first sperm was greater ($P = 0.05$) for temperamental bulls than calm or intermediate bulls and PTV tended ($P = 0.06$) to be greater in temperamental bulls than calm or intermediate bulls. Tables 13, 14 and 15 summarized the effects of temperament class on age, BW, SC, PTV, SC per 100 kg of BW and PTV per 100 kg of BW at first sperm, puberty and sexual maturity.

Scrotal circumference and paired-testes volume per 100 kg of body weight was also calculated at first sperm, puberty and sexual maturity. At first sperm, the SC/100 kg BW was greater ($P = 0.05$) in the control bulls (7.37 ± 0.23 cm/kg) than in the PNS bulls (6.78 ± 0.24 cm/kg), as demonstrated in Table 10. There was also a tendency ($P = 0.07$) for PTV/100 kg BW (cm^2/kg) to be greater at first sperm in the control bulls (72.59 ± 3.59 cm^2/kg) than in the PNS bulls (62.52 ± 4.07 cm^2/kg). At sexual maturity, there was a tendency for both the calm (76.41 ± 5.61 cm^2/kg) and temperamental (75.03 ± 6.88cm^2/kg) bulls to have a greater PTV/100 kg BW than the intermediate bulls (63.62 ± 6.11 cm^2/kg, $P = 0.06$). This indicates that at first sperm and sexual maturity, PNS bulls needed to attain a greater body weight compared to the controls in order to reach first sperm and sexual maturity.

Prenatal stress did not affect age, SC or PTV at first sperm or age, body weight, SC or PTV at puberty and sexual maturity; however, body weight at first sperm was

affected by treatment with PNS bulls being heavier than control bulls. Additionally, SC/100 kg and BW were also significantly greater ($P = 0.05$) in control bulls at first sperm. These data suggest that temperamental bulls had delayed sexual development between puberty and sexual maturity because of the extended time between puberty and sexual maturity.

Table 10. The effect of prenatal stress on first sperm[2] characteristics in yearling Brahman bulls.

Variable	Control	PNS[1]	*P-Value*
n=	21	15	
Age (d)	412.25 ± 18.07	426.89 ± 18.97	0.47
BW (kg)	353.20 ± 10.55	382.16 ± 11.29	0.04
SC (cm)	26.00 ± 0.34	25.50 ± 0.38	0.34
PTV (cm^2)	252.19 ± 10.80	235.89 ± 12.26	0.33
SC/100 kg of BW (cm/kg)	7.37 ± 0.23	6.78 ± 0.24	0.05
PTV/100 kg of BW (cm^2/kg)	72.59 ± 3.59	62.53 ± 4.07	0.07

[1]Prenatal stress (PNS): Cows were transported for 2 h on d 60, 80, 100, 120 and 140 ± 5 of gestation.

[2]First sperm is defined as the as the age at which the first visible sperm was present in the bull's ejaculate whether or not the sample exhibited progressive motility.

BW: Body weight

SC: Scrotal circumference

PTV: Paired-testes volume

Table 11. The effect of prenatal stress on puberty[2] characteristics in yearling Brahman bulls.

Variable	Control	PNS[1]	*P-Value*
n=	21	15	
Age (d)	439.88 ± 16.08	447.15 ± 17.25	0.73
BW (kg)	371.66 ± 8.67	391.39 ± 9.64	0.13
SC (cm)	26.97 ± 0.48	26.54 ± 0.54	0.56
PTV (cm^2)	277.50 ± 12.17	260.19 ± 13.66	0.35
SC/100 kg of BW (cm/kg)	7.21 ± 0.19	6.88 ± 0.20	0.19
PTV/100 kg of BW (cm^2/kg)	73.15 ± 3.86	68.17 ± 4.17	0.34

[1]Prenatal stress (PNS): Cows were transported for 2 h on d 60, 80, 100, 120 and 140 ± 5 of gestation.

[2]Puberty is defined as an ejaculate that contained 50 X 10^6 sperm with at least 10% motility.

BW: Body weight

SC: Scrotal circumference

PTV: Paired-testes volume

Table 12. The effect of prenatal stress on sexual maturity[2] characteristics in yearling Brahman bulls.

Variable	Control	PNS[1]	*P-Value*
n=	21	15	
Age (d)	483.33 ± 15.15	484.60 ± 16.98	0.95
BW (kg)	398.73 ± 10.05	413.17 ± 11.56	0.35
SC (cm)	27.49 ± 0.69	27.62 ± 0.74	0.88
PTV (cm^2)	286.21 ± 20.14	297.04 ± 21.46	0.66
SC/100 kg of BW (cm/kg)	6.91 ± 0.21	6.73 ± 0.23	0.54
PTV/100 kg of BW (cm^2/kg)	69.80 ± 5.74	73.57 ± 5.96	0.51

[1]Prenatal stress (PNS): Cows were transported for 2 h on d 60, 80, 100, 120 and 140 ± 5 of gestation.

[2]Sexual maturity is defined as an ejaculate that contained 500 X 10^6 sperm.

BW: Body weight

SC: Scrotal circumference

PTV: Paired-testes volume

Table 13. The effect of temperament class[1] on first sperm[2] characteristics in yearling Brahman bulls.

Variable	Calm	Intermediate	Temperamental	*P-Value*
n=	22	8	6	
Age (d)	417.39 ± 17.26	428.02 ± 19.91	413.30 ± 22.93	0.80
BW (kg)	356.61 ± 9.83	364.52 ± 12.37	381.90 ± 14.53	0.28
SC (cm)	25.32 ± 0.28[a]	25.12 ± 0.46[a]	26.80 ± 0.54[b]	0.05
PTV (cm^2)	228.87 ± 9.27	227.71 ± 14.63	275.54 ± 17.35	0.06
SC/100 kg of BW (cm/kg)	7.19 ± 0.21	6.94 ± 0.26	7.10 ± 0.31	0.68
PTV/100 kg of BW (cm^2/kg)	64.84 ± 3.08	63.87 ± 4.85	73.98 ± 5.76	0.34

[1]Temperament class was based on temperament scores, calm (< 1.78), intermediate (1.78-2.90) and temperamental (> 2.90).

[2]First sperm is defined as the as the age at which the first visible sperm was present in the bull's ejaculate whether or not the sample exhibited progressive motility.

BW: Body weight

SC: Scrotal circumference

PTV: Paired-testes volume

Table 14. The effect of temperament class[1] on puberty[2] characteristics in yearling Brahman bulls.

Variable	Calm	Intermediate	Temperamental	*P-Value*
n=	22	8	6	
Age (d)	450.32 ± 14.96	447.41 ± 18.97	432.82 ± 22.30	0.76
BW (kg)	373.89 ± 7.40	378.35 ± 11.43	392.34 ± 13.55	0.49
SC (cm)	26.68 ± 0.40	25.84 ± 0.65	27.75 ± 0.77	0.19
PTV (cm^2)	263.71 ± 10.00	246.24 ± 16.47	296.59 ± 19.53	0.16
SC/100 kg of BW (cm/kg)	7.16 ± 0.17	6.85 ± 0.23	7.13 ± 0.27	0.46
PTV/100 kg of BW (cm^2/kg)	71.27 ± 3.54	64.96 ± 4.68	75.76 ± 5.52	0.27

[1]Temperament class was based on temperament scores, calm (< 1.78), intermediate (1.78-2.90) and temperamental (> 2.90).

[2]Puberty is defined as an ejaculate that contained 50 X 10^6 sperm.

BW: Body weight

SC: Scrotal circumference

PTV: Paired-testes volume

Table 15. The effect of temperament class[1] on sexual maturity[2] characteristics in yearling Brahman bulls.

Variable	Calm	Intermediate	Temperamental	*P-Value*
n=	22	8	6	
Age (d)	467.58 ± 14.22	482.32 ± 19.42	501.99 ± 22.99	0.42
BW (kg)	389.17 ± 9.09	408.08 ± 13.06	420.59 ± 16.13	0.20
SC (cm)	27.66 ± 0.67	26.56 ± 0.80	28.45 ± 0.94	0.22
PTV (cm^2)	292.80 ± 19.54	260.34 ± 22.83	321.74 ± 26.57	0.13
SC/100 kg of BW (cm/kg)	7.10 ± 0.20	6.54 ± 0.25	6.81 ± 0.30	0.15
PTV/100 kg of BW (cm^2/kg)	76.41 ± 5.61	63.62 ± 6.11	75.03 ± 6.88	0.06

[1]Temperament class was based on temperament scores, calm (< 1.78), intermediate (1.78-2.90) and temperamental (> 2.90).
[2]Sexual maturity is defined as an ejaculate that contained 500 X 10^6 sperm.
BW: Body weight
SC: Scrotal circumference
PTV: Paired-testes volume

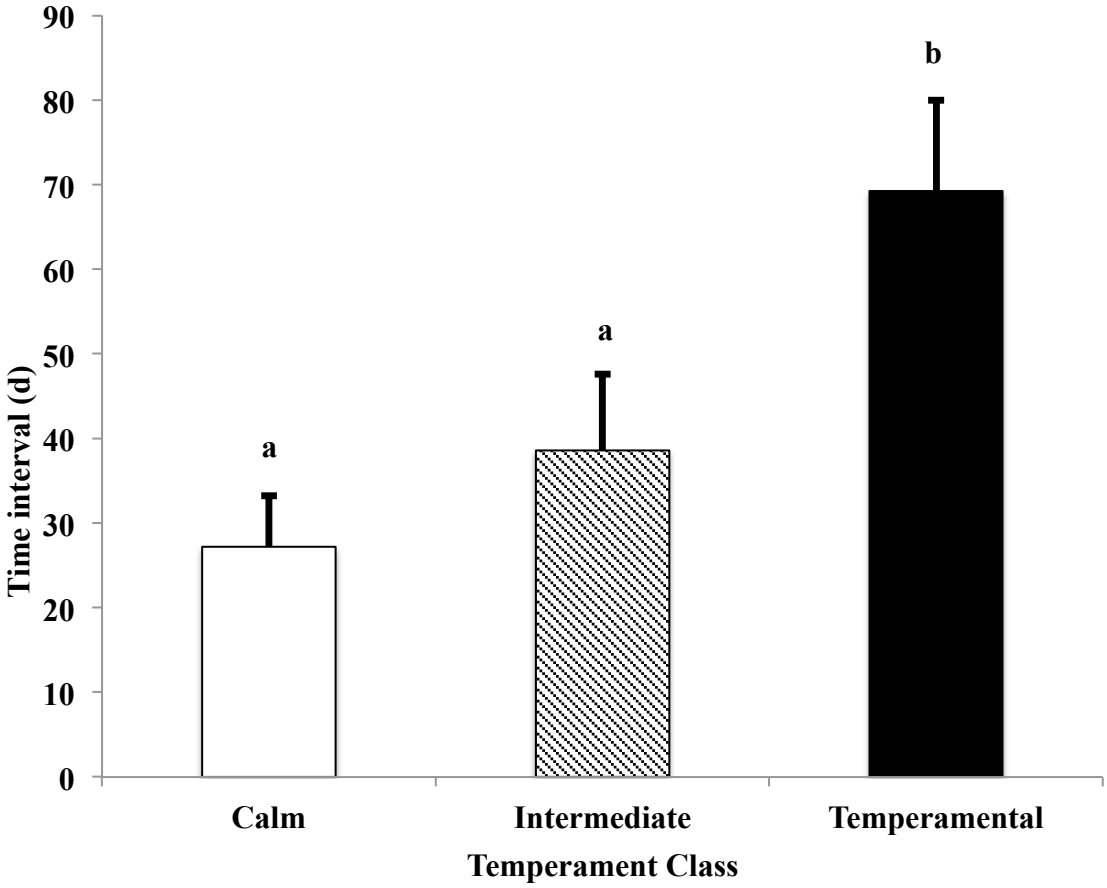

Figure 7. The effect of temperament class[1] on the time interval between puberty[2] and sexual maturity[3] in yearling Brahman bulls, (P = 0.0077). [1]Temperament class was based on temperament scores, calm (< 1.78), intermediate (1.78-2.90) and temperamental (> 2.90).

Discussion

Age, body weight, SC and PTV were similar between treatment groups at puberty and sexual maturity; however, body weight was significantly different at first sperm with PNS bulls being heavier than control bulls. Testicular weight has been closely correlated with semen production in breeding bulls (Coulter and Foote, 1979). Additionally, body weight was negatively correlated with age at puberty and sexual maturity and body weight was positively correlated with paired-testes weight and seminiferous tubule volume (Brito et al., 2012). In the current study body weight was affected at first sperm but the age at first sperm was not different for PNS bulls. The PNS bulls had to reach a greater weight in order to reach first sperm. However, this effect disappeared at puberty and sexual maturity where the treatment groups were similar for all variables.

Age at first sperm, puberty and sexual maturity did not differ between prenatal treatment groups, which was not expected. Brito et al. (2004) found that age and weight were important for sexual development. In *Bos indicus* and *Bos indicus* crosses, bulls that reached puberty earlier were heavier than bulls that reached puberty later, they also had increased scrotal circumference. Although blood was not analyzed for serum cortisol and testosterone in this study, previous research has shown that prenatal exposure to stress can alter the HPA axis of the progeny that in turn can result in increased basal concentrations of cortisol (Price et al., 2013). Cortisol alters the production of testosterone by causing gonadal tissue to become resistant to hormones such as testosterone as well as causing interference at the anterior pituitary and the

hypothalamus leading to disruption in gonadal utility and CRH suppressed GnRH secretion therefore affecting sperm production (Borg et al, 1991; Tsigos and Chrousos, 2002: Welsh et al., 1982). It could be hypothesized that due to the lack of a significant age difference between control and PNS bulls at first sperm, puberty and sexual maturity, cortisol and testosterone concentrations of these bulls may not be significantly different.

Age and weight of the bulls at all ages of interest were similar between the control and PNS bulls in this study; however, these values are slightly numerically lower than other studies using spring-born *Bos indicus* bulls (Fields et al., 1982; Tatman et al., 2004). The difference in scrotal circumference between first sperm and puberty and puberty and sexual maturity was no greater than 1 cm on average; however, in previous research this difference was much greater. In spring-born Brahman bulls scrotal circumference increased on average 3 cm between puberty and sexual maturity whereas in the current study the difference between puberty and sexual maturity for scrotal circumference was 1 cm (Tatman et al., 2004). The reason for the lack of difference is unclear.

Scrotal circumference has been used as a measure of reproductive capacity for bulls (Burrow, 2001; Coulter et al., 1987). Scrotal circumference per 100 kg BW or PTV per 100 kg BW is not a common measurement; however, in this study it demonstrates that even though PNS bulls had a greater BW than control bulls, they were delayed in testicular development relative to BW. Makarechian et al. (1985) suggested that using SC per 100 kg BW could be a useful measurement because it could remove variation in

SC due to BW when comparing bulls of the same age. This difference in yearling bulls based on SC per 100 kg BW; however, was not significant at 2 years of age when comparing various breeds. There is evidence that preweaning gain has a greater affect on SC (R^2= 0.68) than post-weaning or feedlot weight gain when comparing beef breeds.

Previous studies of cattle, rodents and humans have concluded that temperament was affected by prenatal stress (Buitelaar et al., 2003; Littlejohn et al., 2013; Weinstock, 2008). Therefore it could be speculated that PNS affected the temperament of these bulls, which in turn affected the sexual development, such as scrotal circumference and PTV growth.

Temperament also significantly affected scrotal circumference at first sperm and tended to affect PTV at first sperm and sexual maturity. Temperamental bulls had a greater scrotal circumference and PTV than calm or intermediate bulls at all of these developmental time points. Therefore, temperamental bulls had an extended time between puberty and sexual maturity and they tended to have a larger PTV at sexual maturity. In previous studies, temperament was negatively correlated with scrotal circumference, weaning weight and yearling weight. In the study by Burrow (2001), scrotal circumference was only measured at weaning, 12 and 18 mo of age, instead of every two wk until sexual maturity was achieved. In the current study temperamental bulls had a greater scrotal circumference and PTV at first sperm and a greater PTV at sexual maturity, which would indicate that a calmer temperament was beneficial for reproductive performance. Time interval between puberty and sexual maturity was significantly greater in temperamental bulls indicating a delay in sexual development.

Sant'Anna et al. (2012) demonstrated negative genetic correlations between flight speed and weaning weight and flight speed and scrotal circumference, suggesting that temperament measured by flight speed should be part of the selection criteria for a breeding program. Scrotal circumference for this study was only measured at 550 d of age and not at first sperm, puberty or sexual maturity specifically (Sant'Anna et al., 2012).

All bulls on this study presumably had the same nutritional treatments. This is important because previous studies have shown that pre-weaning growth and nutrition is very important when analyzing reproductive parameters later in life. A study with Holstein bulls that were fed three different diets during the pre-weaning period, 60-75, 100 and 140-160% of NRC requirements for 80 wk revealed that, high intake diet bulls were heavier and reached puberty sooner than the medium and low intake diets (Bratton et al., 1956; Barth et al., 2008). Additionally, a study by Nolan et al. (1990), revealed that high gain intake diet bulls compared to moderate gain intake diet bulls had a greater scrotal circumference, hip height, serum testosterone concentration and body weight at first sperm.

Prenatally stressed bulls had delayed testes development relative to their body weight. Age, scrotal circumference and PTV were not significantly different between prenatal treatment groups at either first sperm, puberty, and sexual maturity between prenatal treatment groups. Temperament did not affect age and body weight at first sperm, puberty or sexual maturity; however, scrotal circumference and PTV were affected by temperament at first sperm and sexual maturity. Specifically, temperamental

bulls had a greater scrotal circumference and PTV than calm or intermediate bulls at all stages of development. The interval from first sperm to puberty was not affected by PNS or temperament; however, the interval between puberty and sexual maturity was affected by temperament with temperamental bulls requiring a greater amount of time than calm or intermediate bulls, indicating that there was a significant delay in sexual development. The results demonstrated a suppression of testicular development due to increased cortisol concentrations as shown by an increase in scrotal circumference per 100 kg of BW in control bulls. These results and previous research lead to the conclusion that both prenatal stress and temperament negatively affect sexual development of Brahman bulls.

CHAPTER V

SUMMARY/CONCLUSION

Yeast cell wall has been suggested as a beneficial alternative to antibiotic growth promoters and as a mediator for stress in weaned or transported animals. Very few studies have analyzed the impact of calf health when yeast cell wall is fed via maternal supplementation. Previous studies had proposed that yeast cell wall and other yeast additives benefit the rumen by improving the viability of the rumen microbes. This would cause increased feed intake, fiber digestion and microbial protein, which would all lead to improved weight gain. Our current study revealed that yeast cell wall did not improve performance in the mother. Prepartum and postpartum weight gains did not differ between prenatally supplemented or control cows, additionally the postpartum interval and pregnancy rates were not affected by prenatally supplementation.

Breed, calf age, gender and health of the animal can affect immune cells. Examination of the calves revealed that prenatal supplementation did not affect the total lymphocyte, monocyte, segmented and banded neutrophil counts as a percentage of total white blood cells. There was a tendency for a treatment by sex interaction in which the control females had a greater percentage of monocytes than the control males, yeast females and males. Temperament has been shown to decrease ADG, increased basal concentrations of cortisol and overall decreased performance in cattle. In this study, temperament had a tendency to affect monocytes with temperamental calves having a greater percentage of monocytes than calm or intermediate calves.

Due to the lack of difference in cow weight it was expected that prenatal supplementation of yeast cell wall would not affect calf weights at 24 h and 14 d of age; however, there was a tendency for control calves to be heavier than yeast calves. While the birth weight and weaning weight of the calves are comparable to previous reports, the cause for control bull calves to be heavier than yeast bull calves and both groups of heifers is unknown.

Cortisol on 28 d of age was negatively correlated with lymphocytes at 28 d and ADG for all contemporary groups except temperamental calves; the cause of this is unknown since temperament did not significantly affect cortisol in this study. Cortisol at 14 d; however, did not show a strong correlation with lymphocyte percentages at 28 d, which means that 14 d cortisol might not be a good indicator of future lymphocyte progression.

While prenatal yeast cell wall supplementation did not improve cow or calf performance, it did not have a deleterious effect on the cow or calf. Postpartum interval and pregnancy rates were similar between treatment groups as well as prepartum and postpartum BCS and BW. Calf weights and leukocytes did not differ by treatment but there was some sexual dimorphism in which females had a greater percentage of monocytes than males. These results suggest that prenatal yeast cell wall supplementation does not benefit cow or calf performance under the conditions of this study, with healthy cows that did not receive a pathogen or nutritional challenge.

The second study was to determine if prenatal stress affected the feeding behavior of yearling Brahman bulls. Routinely, feeding behavior studies allowed for an

adjustment period to the feeding system, such as the GrowSafe system. However, we wanted to analyze the adjustment period to determine if there were differences between prenatally stressed and/or temperament groups during this period.

Prenatal stress can increase temperamental behavior and an altered stress response such as increased cortisol, increased heart rate and decreased clearance rate of plasma cortisol. Temperamental cattle have reduced average daily gain in a feedlot system and decreased body weight at weaning. Temperament of feedlot cattle has been shown by the negative correlation between flight speed and dry matter intake ($r = -0.34$) and head-down duration (min/d) ($r = -0.56 \pm 0.38$). This demonstrates that temperamental cattle spend less time feeding than calmer cattle.

We revealed that temperament, rather than prenatal stress, had a greater impact on feeding behavior. The number of visits, meal events, head-down time and head-down time per meal were all significantly greater for temperamental than calm or intermediate bulls. Prenatal stress did have an affect in which, PNS bulls did demonstrate a greater head-down time per meal and average meal size than control animals. In previous studies, temperament was negatively correlated with head-down time, which differs from the current study's findings. For the current study, head-down time was defined as when an animal's transponder was detected to start and end with the last two readings greater than 300 s. Whereas, head-down time in other studies has been defined as the number of times the transponder was detected multiplied by 5.7 s, which was the systems scanning time. The GrowSafe system was used for both types of studies. The difference in head-down time between previous and current studies could be due to

temperamental bulls leaving and returning to the bunk within the same meal event causing the head-down time to be extended. Temperamental bulls had a greater head-down time and head-down time per meal but the average meal size was not affected. It could then be suggested that temperamental animals are less efficient.

The final study was to determine if prenatal stress and/or postnatal temperament has an affect on age, body weight, scrotal circumference and paired-testes volume at first sperm, puberty and sexual maturity. Age, scrotal circumference and PTV were not significantly different between prenatal treatment groups at either first sperm, puberty, and sexual maturity between prenatal treatment groups. Temperament did not affect age and body weight at first sperm, puberty and sexual maturity; however, temperamental bulls had a greater scrotal circumference and PTV than calm or intermediate bulls. The interval from first sperm to puberty was not affected by PNS or temperament. The time interval between puberty and sexual maturity; however, was affected by temperament with temperamental bulls requiring a greater amount of time than calm or intermediate bulls, indicating that there was a significant delay in sexual development from puberty to sexual maturity. Scrotal circumference per 100 kg BW or PTV per 100 kg BW is not a common measurement but it has been suggested that SC per 100 kg BW could be a useful measurement because it could remove variation in SC due to BW when comparing bulls of the same age. In the current study it demonstrates that even though PNS bulls had a greater BW than control bulls, they were delayed in testicular development relative to BW. These results and previous research demonstrated that both prenatal stress and temperament affect sexual development of Brahman bulls.

From the experiments carried out we conclude that prenatal supplementation of yeast cell wall to the mother, and prenatal stress and postnatal temperament on feeding behavior and sexual development in bulls:

1. Yeast cell wall supplementation of late gestating and early lactating cows did not affect cow or calf performance.
2. Temperament can affect the feeding behavior of bulls in a bunk feed system; therefore, temperament needs to be considered in the design of future feeding studies.
3. Prenatal stress and postnatal temperament cause delays in sexual development in bulls; therefore, prenatal conditions need to be evaluated and considered when determining potential future reproductive performance.

NOMENCLATURE

ABP	Androgen binding protein
ACTH	Adrenocorticotropin-releasing hormone
ADG	Average daily gain
AGP	Antibiotic growth promoters
AMS	Average meal size
AvCort	Average cortisol
BCS	Body condition score
BW	Body weight
C	Control
cm	centimeter(s)
Cort	Cortisol
CRH	Corticotropin-releasing hormone
CRL:AGD	Crown-rump length: anogenital distance
d	day(s)
DMI	Dry matter intake
FR	Feeding rate
FSH	Follicle stimulating hormone
g	Gram(s)
GnRH	Gonadotropin releasing hormone
HCW	Hot carcass weight
HDT	Head down time

HDTM	Head down time per meal
HPA	Hypothalamic-pituitary-adrenal axis
h	hour(s)
IgA	Immunoglobulin A
IgG	Immunoglobulin G
kg	kilogram(s)
LH	Luteinizing hormone
LPS	Lipopolysaccharide
m	Meters
ME	Meal events
min	minute(s)
mo	month(s)
NRC	National research council
NV	Number of visits
PNS	Prenatal Stress
PPI	Postpartum interval
PTV	Paired-testes volume
RFI	Residual feed intake
SC	Scrotal circumference
WBC	White blood cells
wk	week(s)
YCW	Yeast cell wall

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