

THE INFLUENCE OF CELL MEDIATED IMMUNE RESPONSE OF BRAHMAN
COWS ON CALVING INTERVAL, POST PARTUM INTERVAL, COLOSTRAL
IMMUNOGLOBULIN CONCENTRATION, SERUM IMMUNOGLOBULIN
CONCENTRATION, AND GROWTH OF THEIR CALVES

A Thesis

by

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ABSTRACT

The primary objective of this study was to determine relationships between cell-mediated immune response (CMIR) of cows and: calving interval; postpartum interval (PPI; calving to estrus); colostral immunoglobulins (IgG-1 and IgM); and calf growth. Multiparous Brahman cows (n=435) were previously evaluated for CMIR. In experiment 1- cows were classified into response groups based on mean and SD of CMIR. High responders were those with CMIR $\frac{1}{2}$ SD \geq the mean (CMIR H: ≥ 2.8), intermediate cows were within $\frac{1}{2}$ SD of the mean (I: 2.7-1.8), and cows $\frac{1}{2}$ SD \leq the mean were low (L: ≤ 1.7). Multiparous cows had records for six consecutive calving intervals and associated calf weaning weights. Cows with high CMIR had a greater stayability ($P < .0001$) over six calving intervals than cows in the low CMIR group. Cows in the high CMIR group had a tendency towards shorter overall calving interval than cows in the low CMIR group ($P = 0.06$). Calf weaning weight did not differ among CMIR groups ($P > 0.1$). In experiment 2, BW and BCS were recorded at 28-d intervals for 84 d before calving. On d0, d7, and d28 post-calving BCS and BW were recorded. If calves failed to nurse independently within 6 hr after birth, the calf was nursed to ensure colostrum consumption. To achieve milk letdown oxytocin was injected into a tail vessel 6-12 hours post-calving and the cow was milked by hand until the udder was depleted. Colostrum was analyzed for IgG-1 and IgM by ELISA. PROC MIXED and Chi Square procedures of SAS were used to analyze variables with a model including CMIR, calf sex, and their interaction as fixed effects. Cell-mediated immune response was not

related to PPI ($P=0.9$), cow BW ($P=0.9$) or BCS ($P=0.6$), calf birth weight ($P=0.6$), 28 d of age weight ($P=0.4$), or IgM colostrum concentration ($P=0.3$). An interaction between calf sex and CMIR was observed for both IgG-1 ($P=0.06$) and Total IG (IgG-1 + IgM; $P=0.06$) colostrum concentrations. Cow or calf performance was not related to CMIR class, but there was a tendency for an interaction between cow CMIR class and calf gender. Selection for high CMIR can result in selection of cows which are more likely to remain in the herd long enough to be profitable as they have a greater stayability and shorter calving intervals than their low CMIR herdmates.

DEDICATION

Dedicated to Mom, Dad, my army of siblings, Jake, my best friends, and the Dixie Chicken. Thank you for your support, encouragement, and motivation to accomplish this goal. I cannot wait to see what the future holds.

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Contributors

This work was a collaborative effort of the student, M. E. Mund, and the thesis advisory committee (Co-Chairs Dr. R.D. Randel, and T.H. Welsh, Jr. of the Department of Animal Science; Members Dr. J.P Banta and Dr. D.G. Riley of the Department of Animal Science).

The experiments were designed by R.D. Randel and T.H. Welsh, Jr. in consultation with M.E. Mund. Data collection and laboratory procedures conducted by M.E. Mund were supervised by T.H. Welsh, R.D. Randel, and C. Wellman. Technical assistance with data collection and laboratory procedures were provided by D. Nuendorff, D. Law, and L. Quail. Data analysis and interpretation were jointly accomplished by Mund, Randel, Welsh, Banta, and Riley. The thesis was written by Mund with editorial and interpretive assistance from the advisory committee. All other work conducted for the thesis was completed by the student independently.

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NOMENCLATURE

AMIR	Antibody mediated immune response
CMIR	Cell mediated immune response
DTH	Delayed-type hypersensitivity
EPD	Estimated progeny difference
PPI	Post partum interval
D	Day
CI	Calving interval
ADG	Average daily gain
GH	Growth hormone
IGF	Insulin-like growth factor
GLM	Generalized linear model

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1. INTRODUCTION AND LITERATURE REVIEW

Introduction

The objective of this research was to evaluate and study the immune system of Brahman cattle to determine how immune function is passed to the offspring through innate and adaptive immunity, passive immunity through colostrum, and the impact of cell-mediated immune response on calving interval and reproductive efficiency. Immune function is heritable and can be phenotypically selected for in bovine species (Cartwright et al., 2012). If producers were able to phenotypically select for immune function in their herds, cattle would be healthier and be less susceptible to illness and disease. As selection for greater immune function progresses, positive production aspects would increase and overall herd health would improve. With amplified immunological aptitude perhaps both reproductive performance of dams and growth and development of their calves would increase. When an animal is sick or undergoing an immune response to resist disease or illness, that individual is not performing at its genetic potential, therefore reducing the producer's income (UGA, 2001). When an animal is culled due to health reasons a negative expenditure results by having to replace that individual. If cattle are selected for a higher genetic immune functionality, the outcome will benefit the cow as well as future offspring, ultimately leading to increased income for the producer.

For an animal to be profitable it must be able to express its genetic potential, regardless of whether it is used for long-term breeding purposes or to be consumed as a

meat animal. In order to be successful in its overall purpose that animal must be able to gain weight, grow, and reproduce quickly and efficiently while adapting to and resisting many stressors, diseases and illnesses that they are exposed to. With reproduction being the primary goal of beef cattle production, it is important for producers to consider the level of immune function when selecting females. Calving interval is often used as an indicator of reproductive efficiency and might become a selection tool from an immunological standpoint. Research has shown that healthier females have shorter calving intervals due to shorter postpartum intervals. With shorter calving intervals, reproductive efficiency increases allowing cows that consistently produce a calf in a timely manner each year to remain in the breeding system long-term. Efficient breeders are more likely to remain in the herd past their economic break-even point and thus prove profitable for the producer.

One of the primary means of developing a strong immune system is acquiring it through passive immunity, via colostrum (Stelwagen et al., 2014). Cows that possess a strong immune system will be able to pass on that advantage to her offspring through contributing immune cells to the calf's innate immune system, as well as the immunoglobulins passed through colostrum consumption (Moraes et al., 2000). It is imperative for survival that calves are able to absorb immunoglobulins from colostrum as soon as possible after birth. Innate and adaptive immunity work together to combat illness and disease and are contributors to maintaining a homeostatic state in the body (Warrington et al., 2011).

Producers are constantly striving to increase production while reducing expenditures. Livestock of any kind would not be physically capable of fulfilling these kinds of producer expectations without possessing a strong immune system. An animal that is not using energy and resources to eliminate pathogens will perform better reproductively than those that are less immunologically competent. The improved calf performance will increase genetic potential for future breeding generations and those that make it to the feedlot will outperform their less competent counterparts.

Literature Review

Innate Immunity

Prior to the development of one's own immune facilitation the innate immunity of an animal is the host's first defender against harmful bacteria and pathogens (Guy et al., 1994). A collaboration of cellular components result in generation of both non-antigen and antigen-specific pathogenic agents, which interact with immune cells within the body (Warrington et al., 2011). Among these, phagocytes and natural killer cells are crucial in instigating an inflammatory response should the animal be affected by a pathogen. Pro-inflammatory cytokines congregate at the site of inflammation to attack and engulf the pathogen. Increased white blood cell production and elevation of body temperature ultimately kills the pathogen and prevents further production of the infectious agent (Carroll, 2014). The uniqueness of the innate immune system is that it does not possess a memory component, but responds the same way to any attack against the host. Because of this lack of memory, the innate immune response system is not as

fast-acting to repeated pathogen exposure as the adaptive immune system (Abbas and Lichtman, 2009).

Adaptive Immunity

As a calf develops and grows in size and strength during the first few months of life, its adaptive immune system also improves and intensifies. Upon external exposure to the environment, the adaptive immune response of calves become progressively initiated when receptors in the body recognize and react to lymphocyte action against foreign threats to the host (Abbas and Lichtman, 2009). A collaboration of both AMIR and CMIR makes up the functionality of the adaptive immune system, which involves numerous cells working in cooperation to aid in protection and functionality of the body system (Thompson-Crispi et al., 2012). The primary cells involved in adaptive immune defense are T cells, which develop from the thymus, CD4+ and CD8+ cells, naïve T cells, and naïve B cells. T cells are unique in that they do not recognize pathogens without antigen presenting cells (APC's) such as dendritic cells or macrophages, and a major histocompatibility complex I or II (MHC-I, MHC-II). Each of these cell types plays a role in antigen presentation to the lymphocytes, enhancement of the immune response by activating B cells, natural killer cells, and monocytes, and ultimately killing the pathogen. Following pathogen elimination, formation of a memory component for subsequent exposure occurs which is specific and fast acting (Abbas and Lichtman, 2009). The innate and adaptive immune systems work in concert to protect the host from

sickness and disease. Without one or the other the animal would lack the ability to protect itself from the pathogens that the environment presents on a daily basis.

Passive Immunity

Bovine species are born agammaglobulinemic, therefore the achievement of both a strong innate and adaptive immune system is dependent on passive immunity from the dam (Halleran et al., 2017; Burdick et al., 2009). The calf's first acquired immunity is gained through absorption of immunoglobulins in the dam's colostrum during the first 24 hr of life and is known as passive immunity. This passive immunity is temporary and eventually diminishes with age as the calf develops its own immune system through environmental exposure (Chase et al., 2008). Specifically focused on in this paper, IgG is the most abundant types of immunoglobulin in bovine colostrum. The immunoglobulins ingested by the calf are absorbed in the gut during the 24 hr after parturition. This initial time frame is critical because as time progresses the concentration of IgG decreases in the dams milk as well as the permeability of the gut to these immunoglobulins decreases (Stelwagen et al., 2014; Weaver et al., 2000). Perino (1993) investigated the result of complete failure of passive transfer, as well as partial failure of passive transfer, concluding that there are acceptable limits when determining if a dam has adequate concentrations of immunoglobulin's in the colostrum and these limits can help predict whether the calf will acquire a strong functioning immune system through passive transfer. According to a study done by Fleming et al. (2016), dams that possess a high functioning immune system have concentrations of IgG that have greater

than 80% IgG bioactive components in their colostrum. Sufficient IgG amounts indicate that the dam can pass on a strong antibody mediated immune system (AMIR) to the calf (Fleming et al., 2016; Wittum et al., 1995). The calf is dependent on this passive immune transfer because its own innate immune system is weak immediately following parturition. The only defense that the calf is equipped with after birth is its innate immune system. Unlike the adaptive immune system, the innate immune system is non-specific to pathogens and viruses. For this reason, it does not form memory cells when exposed to the same pathogen and therefore, does not recognize a pathogen upon subsequent exposure. However, it is valuable in its ability to respond quickly to immune challenges. Together the innate and adaptive immune systems work to amplify the overall immune function of the animal (Widmaier et al., 2008).

Stress Effects on Immunity

Smith and Vale (2006) noted that following the occurrence of high stress situations the brain initiates a response system to activate the hypothalamus and pituitary to release hormones. The two types of stress responses are known to be acute stress and chronic stress. An acute stress response occurs when the animal encounters a stressor for a short period of time and quickly recovers to a homeostatic state. Chronic stress is much more detrimental as it is the result of a long-term stress response that causes permanent damage to the endocrine, immune, and metabolic functions of the animal (Trevisi and Bertoni, 2009). The level of immune function that cattle possess could potentially affect their reaction to an acute stressor, making it advantageous to be a high immune

responder to increase productivity. It was reported by Aleri et al. (2015) that cattle who were in the higher immune response category had greater average daily gain and improved disease resistance which resulted in greater productivity. Correspondingly, these cattle also had higher basal serum cortisol concentrations. The cortisol measurements inferred that high responders had a better response to temporary, or acute stress during transportation and handling procedures, implying high basal cortisol concentrations are positively correlated to high immune function (Aleri et al., 2015). This acute stress response is beneficial to ensure that the animal handles stress appropriately by utilizing body resources to repel pathogens and return the body to a homeostatic state. However, when an animal does not have a strong immune system and is constantly eliminating pathogens, persistent high cortisol concentrations result in an increase in protein degradation and lipolysis, decreased reproductive performance, decreased digestion and food intake, increased respiration and cardiovascular rate, and ultimately, skeletal muscle protein catabolism (Curtis, 1983). Cortisol also interferes with growth hormone (GH) binding on the liver, which decreases insulin-like growth factor (IGF-1) production, thus interfering with growth. All of these consequences result in a suppression of the immune system, which makes cattle more susceptible to illness during high stress events and can impair the ability to mount a successful immune response, ultimately resulting in detrimental effects to production (Curtis, 1983; Lippolis, 2014). Various handling techniques and sanitary actions can be implemented when handling cattle; however, some stress is inevitable when many management

practices are implemented. Therefore, animals with high functioning immune systems will be able to better cope with the consequences of environmental bacterial challenges.

Cell Mediated Immunity

Cell mediated immune response (CMIR) is targeted towards intracellular pathogens, such as *Mycobacterium avium*, where AMIR acts on extracellular pathogens, such as *Escherichia coli* in the environment (Murphy et al., 2012). The CMIR is a protective immune process that involves the activation of specific cell types such as phagocytes and cytotoxic T cells and their secretory products (cytokines and chemokines). Cytokines and chemokines are released from cells to respond to and kill antigen-bearing cells. Cytotoxic T cells elicit an immune response by inducing apoptosis of host cells that have been infected by viruses, as well as host cells that contain intracellular bacteria (Warrington et al., 2011). Upon a cellular disturbance, macrophages are activated and work to destroy intracellular pathogens while stimulating cytokine production, mediating a strong immune response. T cells produce effector cells, such as cytotoxins, that are responsible for lysis of specific target cells, eliminating specific pathogens and retaining a memory for future pathogen exposure (Warrington et al., 2011). Indication of CMIR response in cattle has been measured by the delayed-type hypersensitivity (DTH) to the type 1 antigen and has been found to be moderately heritable in bovine species (Thompson-Crispi et al., 2012).

Antibody Mediated Immune Response

Antibody mediated immune response is facilitated by type 2 antigen macromolecules found in the extracellular fluid such as antibodies, complement proteins, and antimicrobial peptides. The B cells originate from stem cells in the bone marrow, which circulate through the blood or lymph and recognize antigens and pathogens by memory cells produced from exposure (Widmaier et al., 2008; Thompson-Crispi et al., 2012). Immunoglobulins produced by B cells play an important role in the production of many antibodies that identify and eliminate biological pathogens (Warrington et al., 2011). The B cells, particularly the memory B cells express antigen-binding receptors and are extremely effective in mounting a rapid response during a second exposure to an antigen (Warrington et al., 2011). When B cells are activated they prompt assistance from T cells, collaborating together to protect the body against sickness and disease. Environmental exposure and inevitable responses to pathogens will assist in the development and strengthening of the calves' active immune system by inducing both the CMIR and AMIR response systems. Ideally, an animal that possesses both a strong CMIR and AMIR would be more resistant to disease and illness, thus outperforming their lesser immune responder counterparts. However, several studies evaluating the relationship between CMIR and AMIR have concluded that the two tend to be negatively correlated (Hine et al., 2010; Martin et al., 2015). Thus, when an animal tests high for CMIR response, they concurrently test low for AMIR response, making genetic selection for immune response complicated. However, both aspects of these two immune systems are vital to the overall health and performance of the animal.

Heritability of Immunity

Heritability (h^2) is an estimate of variability in phenotypic traits and has been used as a selection tool for genetic improvement (Oldenbroek and Van Der Waaij, 2015). Heritability ranges from 0-1, with both genetic and environmental influence. The higher the heritability estimate, the more rapid genetic progress can be made. The heritability of immune system function through maternal transfer of immunity has not been studied as closely as passive immunity from colostrum. Recent studies have led scientists to believe that there is evidence to assume that the maternal immune cells can program cells of the fetus to attain a genetically acquired immune system (Cartwright et al., 2012). This would mean that dams with higher functioning immune systems would genetically pass on a more robust innate immune system to their offspring (Cartwright et al., 2012). As the calf matures and is exposed to environmental factors, its adaptive immune system develops and strengthens until it no longer relies on the antibodies received by its mother through colostrum (Chase et al., 2008). This maturing independence of the calf's adaptive immune system is essential, as maternal antibodies absorbed through colostrum intake begin to decay after 16-28 days post parturition (Chase et al., 2008). Several studies by Thompson-Crispi have reported the heritability of both CMIR and AMIR. Fluctuation of CMIR heritability occurs with factors such as age of the animal and stage of gestation, whereas AMIR heritability remained relatively steady with age and stage of gestation (Thompson-Crispi et al., 2012; 2014). With consideration of the heritability differences between CMIR and AMIR, selecting cattle based on their immune function could be incorporated as a selection tool for breeding

operations. Cattle that possess both higher innate and adaptive immune response systems would theoretically be able to pass on both systems in an enhanced form to their calves. With knowledge of immune response level of an animal, producers would be able to phenotypically select for such traits, much like selecting for any other EPD (expected progeny difference) trait. The ability to select individuals based on immune function would have a positive impact on production agriculture. Not only would average daily gain, weaning weight, and yearling weight increase in calves as a result of decreased illness and disease, the reproductive efficiency of breeding females would also increase.

Aspects of Immune Function During Pregnancy in Cattle

Reproduction is regulated by the nervous system and the endocrine system (Senger, 2005). These two systems collaborate together to fulfill the initial goal of conceiving and producing offspring. The hypothalamus is a cluster of nerve cells called hypothalamic nuclei, which produce gonadotropin releasing hormone (GnRH) after receiving signals from kisspeptin neurons. GnRH then travels through a portal system to the anterior pituitary to cause a release of follicle stimulating hormone (FSH) and luteinizing hormone (LH). Both of these hormones are crucial in their effect on the ovaries, causing them to aid in the development and maturation of dominant follicles, as well as, triggering ovulation. (Senger, 2003). Formation of the corpus luteum (CL) to produce progesterone occurs following ovulation- eventually leading to the lysing of the CL by PGF2alpha or maintaining the CL throughout pregnancy term (Senger, 2003). The achievement of estrous cyclicity is highly influenced by immune function. Lippolis

(2014) reported that corpus luteal regression is achieved by the assistance of cytokines and immune cells, which inhibit progesterone synthesis and cause apoptosis of the luteum cells. Immune cells have also been identified in the hypothalamus, pituitary gland, gonads and the uterus, affecting everything from hormonal release during estrous to maintaining pregnancy, and finally returning to estrous following parturition (Walusimbi and Pate, 2014). Steroid production to regulate reproductive actions is regulated by T lymphocytes, macrophages and monocytes, achieving both inhibitory and stimulatory effects (Walusimbi and Pate, 2014). Significant changes occur to the function of the immune system during the nine months of gestation, such as immune suppression during the first stages of gestation, followed by up-regulation of immunity causing an pro-inflammatory response crucial for the initiation of parturition (Mor et al., 2017). Immune cells interact with the reproductive tissues and play a vital role in the maintenance of pregnancy. One role of the immune cells is to cause a pro-inflammatory response during implantation and maturation. Throughout the second trimester of gestation a switch to an anti-inflammatory state occurs for fetal development. Near the completion of the third trimester, a pro-inflammatory response is again induced for the initiation of parturition (Mor et al., 2017). The anti-inflammatory stage during gestation can increase the probability of the mother developing a disease or illness, as fewer immune cells are active to combat invading bacteria (Lippolis, 2014) Unfortunately, increasing the activation of the immune system to combat the occurrence of developing a disease during pregnancy often results in spontaneous abortions because the immune cells view the fetus as a foreign invader. Therefore, the immune system is suppressed

during gestation to avoid death of the fetus (Lippolis, 2014). A strong innate immune system with functional neutrophils is key to mounting an initial immune response, should the dam be subjected to an illness or disease (Lippolis, 2014).

Uterine involution is one of the primary obstacles influencing the length of the post partum interval in breeding females. This process is recognized as the return to the pre-pregnancy state in order to resume estrous cycles and thus, prepare to conceive and support the next pregnancy (Kiracofe, 1980). Regression in uterine size, changes in vascularity, and tissue loss and regeneration all begin to occur following parturition (Kiracofe, 1980). The time necessary for these events to take place will determine how long the cow's postpartum interval will be, with evidence indicating that immune function and uterine involution are complimentary of one another (Watson, 2009). Immune cells are very important in regulating uterine involution while simultaneously protecting the dam from illness and disease while her body recovers from parturition (Watson, 2009). Following parturition, the immune system of the dam is compromised. Dietary energy intake is partitioned to lactation in lieu of maintenance of cow weight and body condition. Dams with lower immune systems will be more prone to endometritis, ketosis, and retained placenta, all of which lengthen the post partum interval, thus decreasing reproductive performance (Stoop et al., 2016; Thompson-Crips et al., 2014). With timely uterine involution, it has been observed that cows possessing overall higher immune response for both CMIR and AMIR exhibit a positive correlation between immune response and the number of services per conception (Thompson-Crispi et al., 2012). Cattle with stronger overall immune response will combat diseases more

efficiently, resulting in fewer services needed per conception, and therefore reproductively outperform less responsive individuals (Thompson-Crips et al., 2012).

Global Antimicrobial Resistance

The rise of antimicrobial resistance due to the continued use of antibiotics to medicate sick and diseased animals, as well as use for growth promotion, is quickly becoming problematic in the livestock production industry. The recent implementation of the National Action Plan has led to the elimination of the use of certain antibiotics used for growth promotion in food animals in hopes of mitigating the rising incidence of global antimicrobial resistance (NAP, 2015). An opinion paper on antimicrobial resistance stated that the occurrence of bacteria adapting and developing resistance against antibiotics is inevitable, and the continued overuse and misuse of antibiotics is largely to blame for this (Robinson et al., 2016). It has been estimated that the livestock industry alone utilized approximately 63,000 tons of antibiotics in 2010 alone (Robinson et al., 2016). Regardless if antibiotics are used for treating sick animals, growth promotion, or other reasons in animal agriculture, breeding strong immune systems into herds would assist in lowering the total use of antimicrobial drugs.

In conclusion, it is clear that essentially all aspects of beef cattle production can be influenced by the immune function of livestock. Resilient function of both the innate, passive, and adaptive immune systems is crucial to the performance of animals from birth to slaughter, or death. The idea that producers could select cattle based on

phenotypic immune function would greatly impact beef cattle production systems by assisting in mitigating disease, and illness.

Objectives

Based on this literature review, the objectives pursued in this study included the impact of cell-mediated immune response on cow calving interval and reproductive performance, postpartum interval, colostral immunoglobulin concentration, and the growth of their calves.

2. INFLUENCE OF CELL MEDIATED IMMUNE RESPONSE ON CALVING INTERVAL AND COW REPRODUCTIVE PERFORMANCE

Introduction

The beef industry in North America is dependent on productivity and performance of cattle in order to feed the ever-growing population of people. One of the determining factors in deciding which cow to keep or cull in a breeding herd is whether the cow successfully produces a live calf on a yearly basis. In Brahman cattle, age of puberty in heifers is around 19.4 mo., which is delayed when compared to their *Bos taurus* counterparts (Plasse et.al., 1968). The gestation length of Brahman cattle is approximately 291 d (Plasse et.al., 1968; Randel, 2005)- slightly longer than that for most *Bos taurus* cattle breeds. The delayed onset of puberty and extended gestational length of Brahman cattle leaves roughly 70 d for the 1st calf heifer or cow to return to estrus and conceive another pregnancy for the following year's calf crop. This presents producers with a special challenge in keeping a cow on a 365-d calving interval.

Dairy cattle that exhibit a greater cell-mediated immune response (CMIR) achieve uterine involution faster, return to estrus sooner, and rebreed more efficiently than cows that exhibit a lesser degree of response (Stoop et al., 2016). These cows also have a lower incidence of retained placenta and endometritis, making them more reproductively sound and efficient in a breeding herd. Furthermore, it has been discovered that CMIR is moderately heritable (Thompson-Crispi et al., 2012), making selection for immune response a potential tool to select cattle for the breeding herd. The

reduction of economically important diseases combined with robust reproductive efficiency would result in decreased maintenance cost and increased profitability for the producer. As a result of the heritability of immune response from the dam to her offspring, there is evidence of increased performance of calves born to high immune response females (Thompson-Crispi et al., 2012). Increased weaning and yearling weights, as well as a more robust response to vaccines have been observed in offspring from high immune response cows (Horn et al., 2014). The combination of lower cow maintenance cost with higher premiums for heavier calves at weaning increases net profit for the producer, thus encouraging the practicality of using immune function as a selection trait.

The objective of this study was to evaluate the relationship between CMIR of the cow and her reproductive performance measured by yearly calving intervals.

Materials and Methods

All experiments were approved by the Institutional Agricultural Animal Care and Use Committee (IAACUC) of the Texas A&M University System (AUP #2015-019A).

Cows

Primiparous and multiparous purebred Brahman cows (n=253) at the Texas A&M AgriLife Research Center in Overton (32.294N, -94.976W) were evaluated as follows. During the fall months, cows were maintained on rye and rye grass pastures, or fed free-choice coastal Bermuda grass hay, along with a daily supplement of corn and corn gluten meal (approximately 3.6 kg per head of a 3:1 ratio corn to corn gluten meal

concentrate). During the spring, cows grazed ryegrass and coastal Bermuda grass pastures with concentrate supplemented as needed. Calving records including breeding date, calving date and health records were kept on all cows for each year the cow remained in the study. The number of days from when the cow had a calf until she calved again determined the cows calving interval (CI) for each year she was in the breeding herd (Bourdon et al., 1982). The spring breeding season was divided into a 45 d artificial insemination (AI) season and a 45-d natural service season during the months of May to July each year. The fall breeding season was natural service only approximately 2 mo (Nov to Jan) each year. If a cow failed to conceive during one of the breeding seasons, she was moved into the following breeding season to attempt to achieve conception. If a cow failed to conceive after two spring breeding seasons she was culled from the herd.

CMIR Evaluation

All cows were evaluated for cell mediated immune response (CMIR; Cook, et al., 2017). Specifically, CMIR was evaluated by determining each cow's delayed-type hypersensitivity to *Candida albicans*. Each cow received in the left side of the neck a subcutaneous injection of 0.5 mg of killed *Candida albicans* and 0.75 mg Quil A adjuvant to initiate sensitivity to *Candida albicans* (D 0). Fourteen days (D 14) later tail skin fold thickness on the left side of the tail was measured for each cow by use of a spring-loaded Harpenden skinfold caliper (Creative Health Products Inc., Ann Arbor, MI). After the measurement was recorded, 0.1 mg killed *Candida albicans* was

subcutaneously injected to the cow's tail skin-fold. Twenty-four hr later (D 15) the thickness of the injection site was measured using a spring-loaded Harpenden skin fold caliper. Based on their CMIR results (i.e., degree of increased skin fold thickness in response to re-exposure to *Candida albicans*) the cows were labeled as members of the high, intermediate, or lowresponse groups. Specifically, cows that were $\frac{1}{2}$ standard deviation above the mean (mean= 2.25 mm) were classified as high responders (H: ≥ 2.8 mm), those that were between $\frac{1}{2}$ standard deviation above and belowthe mean were classified as intermediate responders (I: 2.7-1.8 mm), and cows that were $\frac{1}{2}$ standard deviation belowthe mean were classified as lowresponders (L: ≤ 1.8 mm).

Statistical Analysis

The data for this project were analyzed using statistical procedures of SAS specific for repeated measures where appropriate (SAS 9.4, SAS Institute, Cary, NC). Dependent variables included CI, pregnancy status, calving record, and calf adjusted 180-d weaning weight. Fixed variables included CMIR class of the cow, and calf sex. Random effects included cow sire. Calving intervals were calculated each year by measuring the amount of days between the day of parturition and the following years calving date for each individual animal. This measurement was repeated for every calf born from the animal during the 6 calving interval time frame of this experiment. The Proc Mixed procedures using SAS were applied to analyze the differences between high, intermediate, and low CMIR cows. Chi Square analysis was performed in SAS to analyze the relationship among high, intermediate, and low CMIR groups for each of the

6 calving intervals evaluated. The 180-d adjusted weaning weights were calculated by using BIF guidelines (2018) table for adjustment factors and the standard birth weight of 75 lb. for males and 70 lb. for females. Age of the dam was adjusted to account for differences in cow maturity during the 6 calving intervals assessed.

Results and Discussion

Calving interval was evaluated for each of the initial 7 life-time opportunities that mature cows and first calf heifers had to produce a calf. The primary objective of this portion of the study was to determine relationships between cell-mediated immune response (CMIR) of cows with 1) their calving interval and 2) the weaning weights of their calves.

In brief, cows that possess a High cell-mediated immune response have a greater stayability over six calving interval years than cows in the low CMIR response group. Relative to calving interval, cows in the High CMIR group had a tendency towards shorter overall calving interval compared to cows in the low CMIR group. Weaning weights for the calves did not differ among CMIR groups, however, there was a difference seen between calf sexes with bull calves weighing more at weaning than heifer calves.

Survival to Sixth Calving Interval

The longevity and survival of cows in the high CMIR group was evident compared to cows in the low CMIR group, greater proportions of high CMIR cows

survived to produce their sixth calf (Figure 1). Chi Square analysis was performed in SAS to test proportions of cows in each CMIR group with expected values for the 6 cumulative calving intervals. In the current study there were 158 cows that survived to have their sixth calf. The survivors included 96 high CMIR cows (60.76%), 38 intermediate cows (24.05%), and 24 low CMIR cows (15.19%). Chi Square analysis of these proportions indicated that cows possessing a more robust immune response were more likely to remain in the herd longer while producing a calf each year ($P < .0001$).

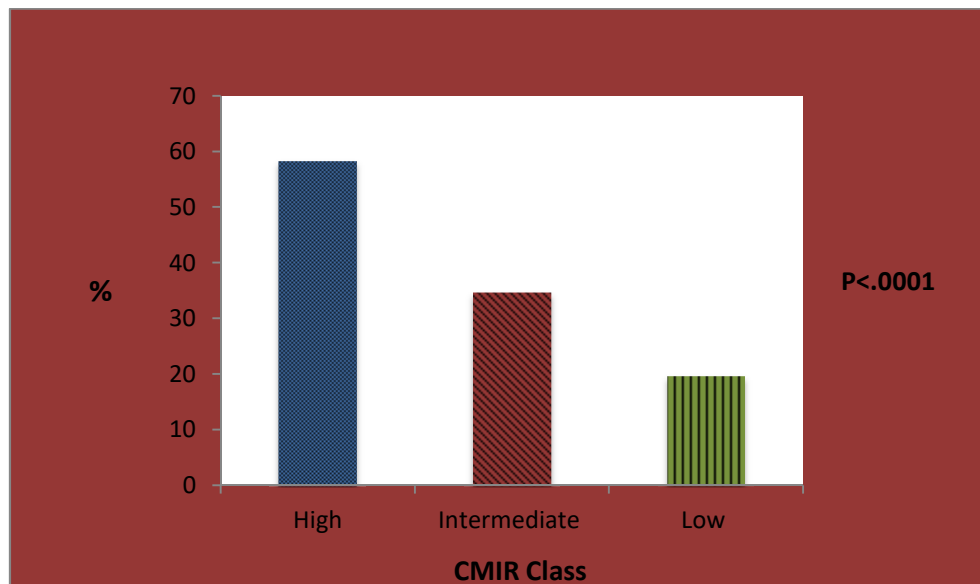


Figure 1. Percentage of cows in each CMIR group surviving to produce their 7th calf.

Profitability of a cattle herd is measured by weight of calf weaned per cow exposed (Herring, 2012). Profitability of an individual breeding female is often determined by her ability to become pregnant and produce a calf consistently each year (Dunn, 2002). Therefore, from an economic standpoint, cow reproductive performance

has been deemed the most important trait to select for in a cow herd. Calving interval is measured by the days from when an animal gives birth to when she gives birth again the following year. During the period of anestrus following parturition the reproductive tract undergoes uterine involution. Uterine involution is a physical repairing process that occurs in order to return the reproductive organs to the involuted state, capable of returning to estrus and conceiving another pregnancy (Kiracoff, 1980). A reproductively sound female will consistently give birth to a calf every 365 days. Bos indicus type breeds are known to have extended gestation length when compared to their Bos Taurus counterparts (Plasse et.al., 1968; Randel, 2005). On average, Brahman cattle have a gestation length of approximately 291 D, leaving roughly 70 days to return to estrus and become pregnant again following parturition (Randel, 2005).

Dairy cattle that possess a high immune response were reported to undergo uterine involution and return to estrus more efficiently than cows that had lower immune response systems (Thompson-Crips and Mallard, 2012). As a result of rapid uterine repair in these dairy cattle, high immune response females also had shorter CI, making them ideal females to remain in a breeding herd (Stoop et al., 2016; Thompson-Crips et al., 2014). In the current study, Brahman cows (n=) were evaluated previously for CMIR by tail skinfold thickness measurement after a subcutaneous injection of *Candida albicans* and were divided into 3 CMIR response groups (Cook et al., 2016). Specifically, the response groups were High (H), Intermediate (I) and Low(L). Yearly CI's were recorded on all Brahman females for six subsequent calving interval years. It was observed in the current study that cows in the High CMIR group had a greater

stayability than cows in the low CMIR group, as a greater percentage of High CMIR cows survived to wean their sixth calf. In order to turn a profit, cattle must wean a calf each year from the time she reaches two years old until she is five years old in order to cover her own maintenance cost. It is not until a cow reaches 6 years of age and weans her calf 4th calf that she potentially offsets her input cost and begins to return a profit in the breeding herd (Brigham, 2007).

Calving Interval by Year

Chi Square analysis was performed in SAS to test proportions of cows in each CMIR group with expected values for the 6 cumulative calving intervals. Between the first and second calf born (first calving interval), there was no statistical difference ($P > 0.10$) between CMIR classes for length of the calving interval. The average CI for low CMIR cows was 455.3 ± 22.89 days, intermediate CMIR cows was 490.36 ± 25.25 days, and high CMIR cows was 460.33 ± 22.16 days (Table 1).

Calving interval between the second and third calf born (second calving interval), differed ($P < 0.01$) among CMIR classes ($P = 0.01$; Table 1). Specifically, the second calving interval was 34 days shorter for cows in the high CMIR group than for cows in the Intermediate CMIR group ($P = 0.01$). Cows in the high CMIR group tended ($P = 0.09$) to have a shorter 2nd calving interval (by 26 days) than cows in the low CMIR group.

Between the third and fourth calf born (third calving interval), a tendency ($P = 0.07$) was observed among CMIR classes for the length of the calving interval. The average calving interval tended to be shorter in the high CMIR compared to the low

CMIR group as reflected by a difference of 27 fewer days for the high CMIR group ($P=0.07$) as shown in Table 1.

Between the fourth and fifth calf born (fourth calving interval), the length of the calving interval tended ($P = 0.08$) to differ between CMIR classes. Cows in the high CMIR group tended to have calving intervals shorter than low CMIR responders (Table 1). Mean calving interval lengths between CMIR groups appear to remain consistent with the third calving interval (Table 1).

Between the fifth and sixth calf born (fifth calving interval), length of the calving interval differed ($P<0.05$) among CMIR classes. During the fifth calving interval there tended to be a mean difference ($P = 0.09$) with cows in the high CMIR group being 26 days shorter than for cows in the intermediate CMIR group. However, as continued generations of calving intervals were observed, the number of cows consistently measured decreased due to younger cows not reaching the required age due to reproductive failure, culling, or death.

The influence of CMIR class on the overall length of all 6 calving intervals measured reveals that cows in the high CMIR response groups tend to have shorter calving intervals ($P = 0.06$) than cows in the low CMIR group. The results in Table 1 show a mean difference of 21 D in calving interval length, favoring high CMIR cows over low CMIR cows across the calving intervals measured in the current study.

Length of the calving interval was measured in days between high, intermediate, and low CMIR groups for each consecutive year that the cow had a calf. During the first calving interval measured, there was a tendency ($P<0.1$) for a difference in the length of

the calving interval between high and low CMIR groups, with low CMIR cows having a shorter first calving interval than high CMIR cows (Table 1). Chi Square results indicated that there were greater percentages of low CMIR cows with shorter calving intervals compared to high CMIR cows during this time (Figure 2). However, observation of the number of cows in each group reveals the possibility that this tendency could be the result of a much greater percentage of low cows evaluated than high cows. Continuing to the second calving interval, the results indicate a much greater number of High CMIR cows evaluated and a reduced number of low CMIR cows observed. These results of the second CI show that cows in the high CMIR group had shorter (420 d) calving interval than low CMIR cows (445d; $P=0.0125$). During the 3rd, 4th, and 5th CI, a tendency ($P<0.1$) was observed that favored high CMIR cows with shorter CI than cows in the low CMIR class. Figures of the cumulative calving intervals show the percentage difference between the number of cows in each CMIR group for the 3rd, 4th, and 5th CI, indicating that cows that possess a greater cell-mediated immune response are more likely to remain in a herd, producing and weaning a calf for each year they remain in the breeding program. Brigham (2007) demonstrates the economic importance that reproductive performance has on profitability, emphasizing that surviving to 6 years of age while producing a calf each year will deem the animal profitable. The results from the current study show the positive impact that a high cellular immune response system promotes a cow's probability of remaining in the herd for an economically relevant period of time, while simultaneously remaining reproductively profitable.

Table 1. Influence of CMIR class on Calving Interval (CI)

		High	Intermediate	Low	P-value
1 st CI	n=	64	55	134	
	Days	460.33 ± 22.16	490.36 ± 25.25	455.3 ± 22.89	>0.10
2 nd CI	n=	165	104	123	
	Days	420.24 ± 17 ^{a,x}	454.13 ± 18 ^{b,y}	445.85 ± 17 ^{ab,y}	0.0125
3 rd CI	n=	136	69	64	
	Days	426.09 ± 15.7 ^x	452.65 ± 16.5 ^y	446.41 ± 16 ^{xy}	0.0663
4 th CI	n=	120	58	38	
	Days	423.14 ± 15.5	447.84 ± 16.4	443.28 ± 15.7	0.0785
5 th CI	n=	96	38	24	
	Days	419.78 ± 15.3 ^x	445.79 ± 16.3 ^y	442.2 ± 15.6 ^{xy}	0.053
Overall CI	n=	133	110	192	
	Days	410.37 ± 16.07	432.83 ± 17.01	431.64 ± 16.53	0.0607

Data presented as Least Square Means ± SE.

Means with a different superscript differ within row.

Means with a common superscript do not differ within row (P>0.10).

^{ab}P<0.05

^{xy}P<0.1

Chi Square results for high VS low CMIR cows in each calving interval

As observed in Figure 2, there was no difference in the percentage of cows in the lower high CMIR group and the length of their 1st calving interval.

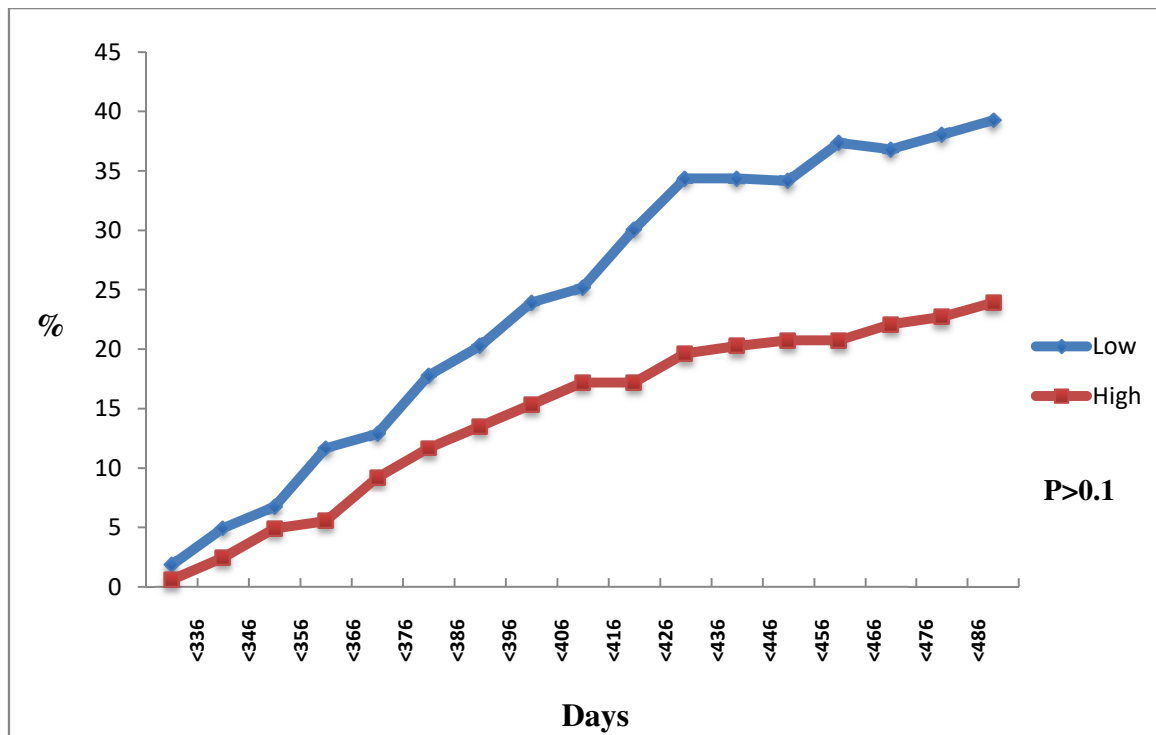


Figure 2. Cumulative calving interval between the first and second calf in high vs low CMIR cows. (n=L; 108, H; 55)

There were measurable statistical differences in the percentage of cows in the high and low CMIR classes and the length of their calving interval from the second to the third calf. Cows in the high CMIR class had a higher percentage of cows that had a calving interval less than 486 d during the interval from the second to the third calf.

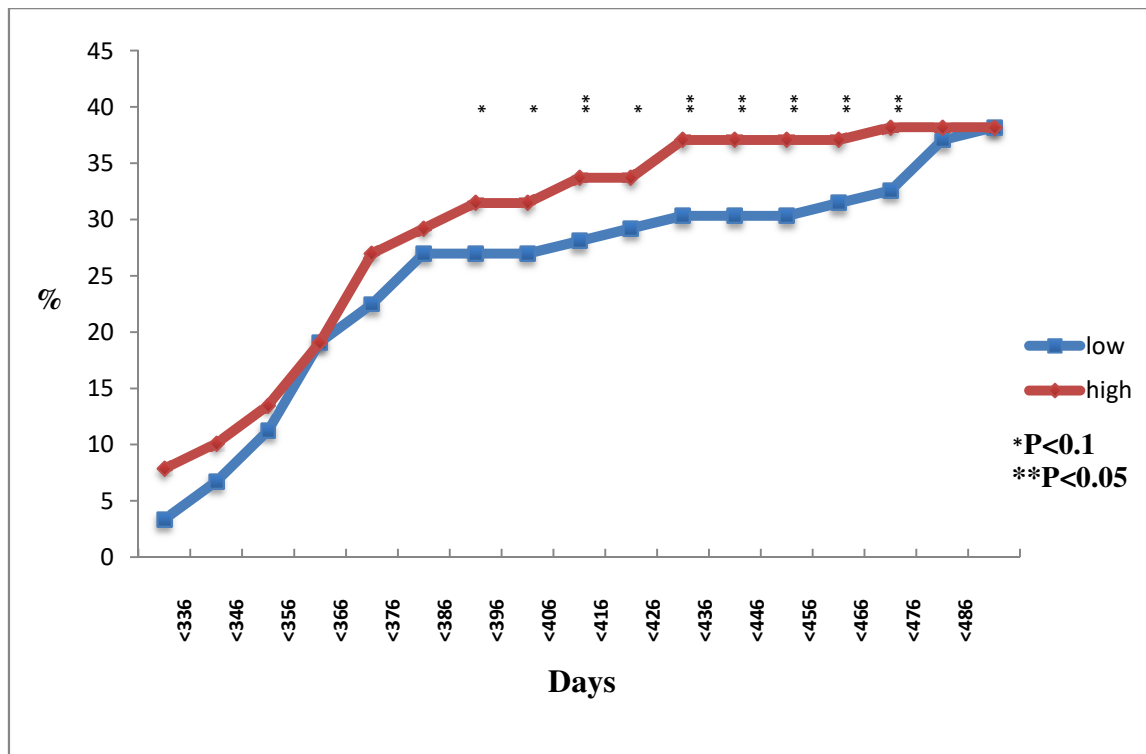


Figure 3. Cumulative calving interval between the second and third calf in high vs low CMIR cows. (n=L; 64, H; 46)

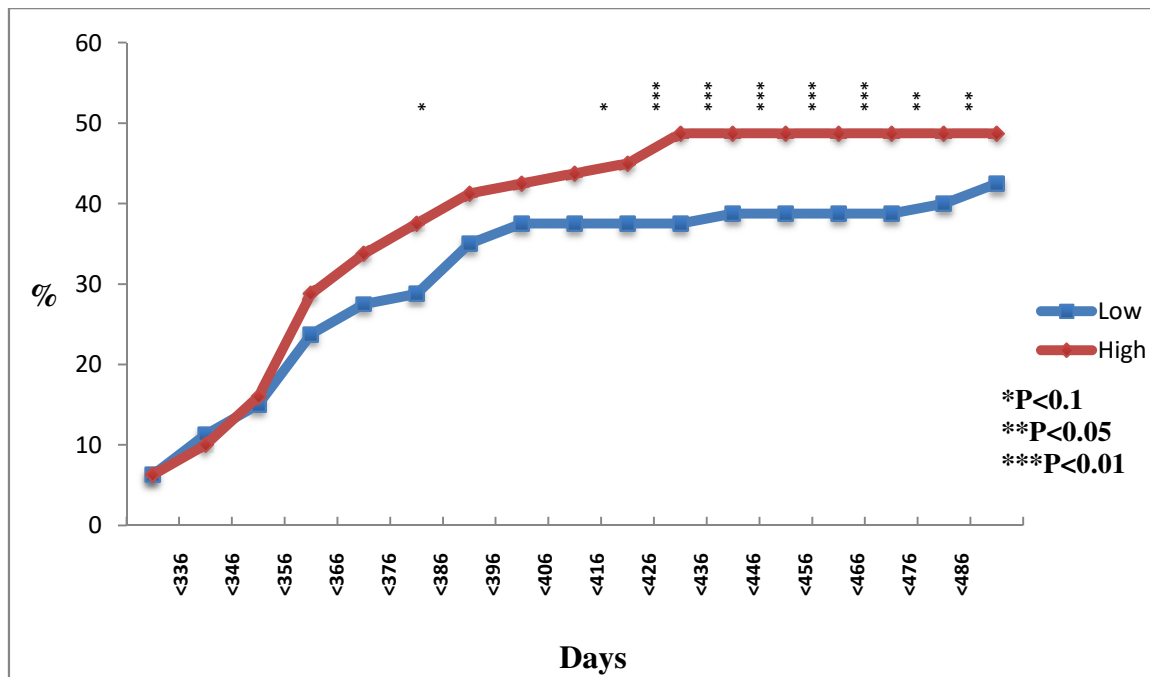


Figure 4. Cumulative calving interval between the third and fourth calf in High vs low CMIR cows. (N=L; 40, H; 40)

Figure 4 indicates the separation between the percentage of cows in the high CMIR class that achieved a shorter CI than cows in the low CMIR class during their 3-4 calving interval.

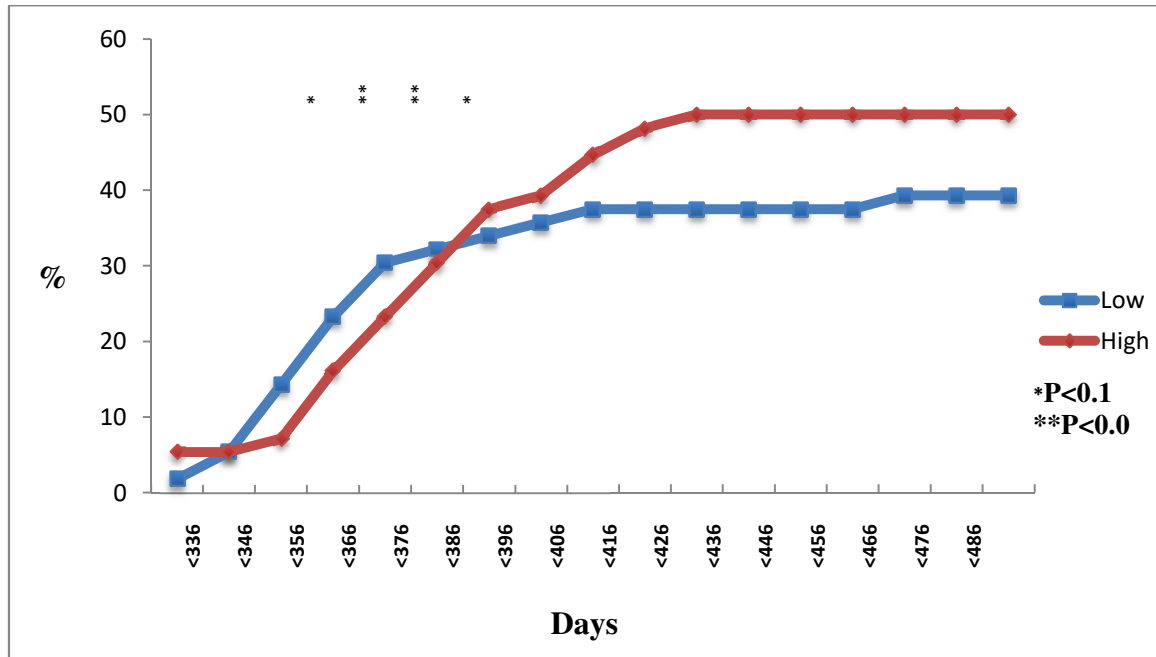


Figure 5. Cumulative calving interval between the fourth and fifth calf in high vs low CMIR cows. (n=L; 24, H; 32)

Results from Figure 5 show the statistical difference between high CMIR class cows and low CMIR class cows with respect to length of their 4th calving interval.

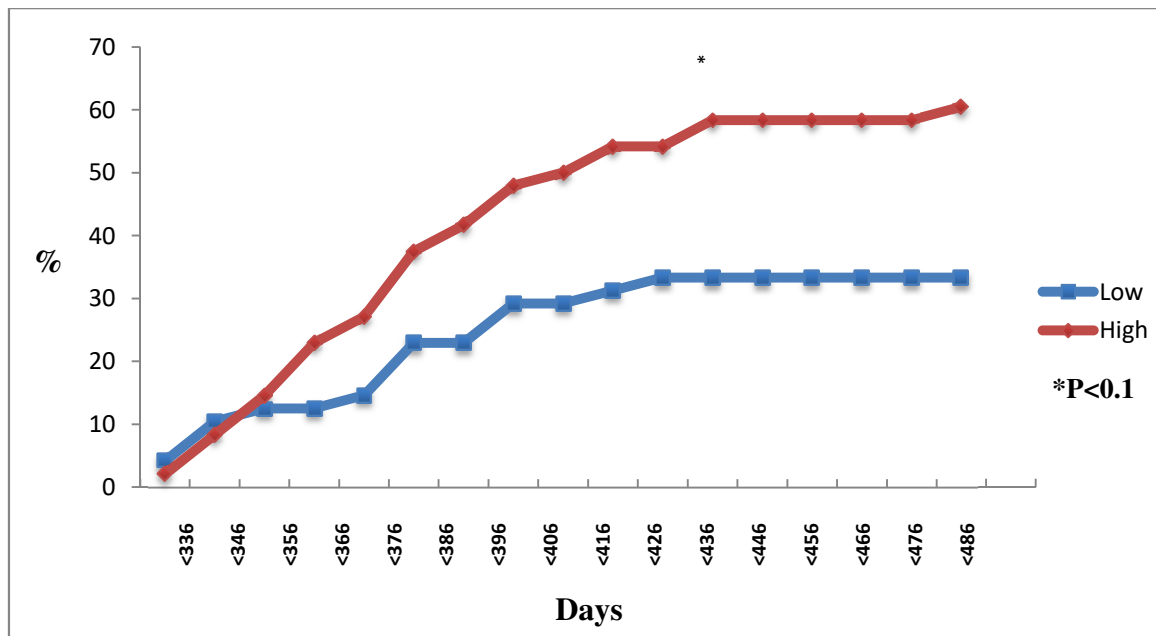


Figure 6. Cumulative calving interval between the fifth and sixth calf in high vs low CMIR cows, (n= L; 16, H; 32)

A tendency ($P < 0.1$) was observed during the interval between fifth and sixth calf between high and low CMIR cows. Results from Figure 6 shows a higher percentage of high CMIR cows achieving a calving interval less than 436 d than low CMIR cows.

180-D Adjusted Weaning Weight

The 180-d adjusted weaning weight (WW) did not differ among calves whose dams were classified as high, intermediate or low CMIR ($P = 0.80$, Table 2). There was a difference in calf sex, with bull calves weaning at heavier weights than heifer calves ($P = < .0001$; Table 3).

Table 2. Influence of CMIR class on calf 180-d adjusted weaning weight (WW)

	High	Intermediate	Low	P-value
<u>N</u>	133	110	192	
<u>kg</u>	178.83 ± 3.5	178.08 ± 3.82	180.73 ± 3.87	0.8008

Data presented as Least Square Means ± SE.

Absence of superscripts denotes no statistical significance (P>0.10).

Table 3. Influence of calf sex on 180-d adjusted weaning weight (WW)

	Male	Female	P-value
<u>kg</u>	185.38 ± 3.5	173.1 ± 3.22	0.0001

Data presented as Least Square Means ± SE.

Calf weaning weight is often an indication of how an animal will perform throughout its life and is routinely used as a selection trait through WW EPDs values. Producers use WW as a measure of profitability that evaluates not only the calf's growth performance, but also the cow's milking ability. There are statistical correlations between cow milk production and calf weaning weight, as one consequently influences the other (Funston et al., 2010). Disease, such as mastitis, affects colostrum and milk quality and quantity in the dam. Sufficient immunoglobulin and nutrient transfer to the calf through colostrum and milk consumption supports the healthy growth and development of the calf up until weaning (Banta et al., 2007). Mastitis and calf performance have been closely studied in dairy cattle and reports have indicated that

dairy cattle that possess a strong CMIR produce heavier, healthier calves than cows that have a weaker CMIR (Thompson-Crispi et al., 2012). In a related study, calves from higher immune responders had a more robust response to vaccines at weaning time than calves born from low immune responders, ultimately influencing their performance following weaning (Thompson-Crispi and Mallard, 2012).

In the current study evaluating Brahman calves born from high , intermediate, and low CMIR cows, there was no difference detected among the 3 CMIR classes and calf weaning weight. On average, weaning weights measured approximately 179 kg for calves born from high, intermediate, and low cows with no statistical difference among them. As predicted from previous research by Browning et al. (1997), there was a difference between the weaning weights of bull calves and heifer calves overall. On average, bull calves weaning 12 kg heavier than heifer calves. This difference was expected as bull calves are phenotypically heavier muscled and have larger frame sizes than heifers. The results from the current study indicated no difference among CMIR groups and calf weaning weight. There was evidence that bull calves were heavier at weaning than heifer calves in this current study.

Conclusion

From an economic standpoint, it is necessary for a cow to produce a calf each year for 4 consecutive years to reach her break-even point and pay off the cost of developing her as a production animal. Therefore, it is essential for cows to be reproductively sound and produce a calf each year until their 6th year of life. For an

animal to reproduce efficiently, she must have her nutritional needs met, avoid chronic stress situations, and be free from disease and illness. To accomplish this feat of animal well-being a cow must possess a strong immune system in order to achieve all the requirements asked of her as a production animal. The current study found that cattle that possess a High cell-mediated immune response had shorter calving intervals, and remained in the herd for 6 consecutive calving intervals. Not only did the High CMIR cows exhibit greater reproductive soundness, they also had a greater stayability in the herd by exceeding their break-even point for profitability. Cows in the low CMIR groups demonstrated longer calving intervals, which deemed them as less reproductively efficient. Many low CMIR cows also failed to remain in the herd to their 6th calving interval and therefore were a negative expenditure for the producer as they did not cover their own maintenance costs during their productive lifetime. Though phenotypically there is little difference between high and low CMIR groups, in terms of reproductive efficiency the productivity differences are clear.

The lack of statistical differences among CMIR groups in relation to calf weaning weight might lead the producer to believe that there is no benefit to selecting high CMIR cows for achieving greater weaning weight averages. However, we discussed that the current study reveals that the stayability for high CMIR cows is greater than low CMIR cows. Herring (2012) defined productivity as weight of calf weaned per cow exposed. Over 6 calving intervals, a greater proportion of High CMIR cows produced and weaned a calf each year, compared to low CMIR cows that did not survive to the 6th calving interval. Therefore, the overall weight of calf produced was

greater for high CMIR cows than low CMIR cows, making them more efficient in calf weaning weight in spite of the lack of statistical difference among CMIR classes.

In conclusion, is it economically efficient to have cows with a more robust immune response system to achieve both reproductive efficiency and profitability in a beef cattle production system.

3. INFLUENCE OF CELL MEDIATED IMMUNE RESPONSE OF BRAHMAN COWS ON POST PARTUM INTERVAL, COLOSTRAL IMMUNOGLOBULIN CONCENTRATION, AND GROWTH OF THEIR CALVES

Introduction

Antimicrobial resistance is becoming a threat to animal agriculture and the public wellbeing (NAP, 2015). By limiting the use of antibiotics used in beef production, there is a potential to decrease the risk of microbes developing antimicrobial resistance (AMR; Robinson et al., 2016). However, by indiscriminately restricting antibiotic usage, we may limit the productive capabilities of animals that do become exposed to pathogens because they will have to divert energy and protein resources to help them resist illness and disease. If producers were able to select cattle based on immune response they could breed hardier, healthier animals for production systems. The result would be fewer animals treated with antibiotics, more profitable production of livestock, and a potential reduction in risk of AMR for livestock and human populations.

Cell mediated immune response (CMIR) evaluation with a delayed-type hypersensitivity to *Candida albicans* has been used as a measure of immune competence in dairy cattle (Thompson-Crispi et al, 2012; Hernandez et al., 2005). Aleri et al, (2015) reported evidence that lactating dairy cattle that possessed high functioning immune systems exhibited improved average daily gain, decreased disease susceptibility, superior colostrum quality, as well as more robust response to vaccinations. Measurements of heritability for CMIR range between 0.19 and 0.49 in cattle depending

on the stage of production the animal is in upon testing, with the general heritability being 0.2 in dairy cattle (Thompson-Crispi et al., 2012). Glass et al., (2005) reported that *Bos indicus* breeds have a stronger cell mediated immune response than *Bos taurus* cattle, and therefore have increased resistance to viruses and parasites that could negatively impact their performance. Utilizing the moderate heritability of CMIR and its positive effects on production traits assures that selecting cattle based on immune response would be beneficial if *Bos indicus* breed types share the same positive influence from immune response traits as dairy cattle.

The objective of this study was to evaluate the relationships of the CMIR class of Brahman cows with their postpartum interval (PPI), colostral immunoglobulin concentration, and the subsequent growth of their calves.

Materials and Methods

All experiments were approved by the Institutional Agriculture Animal Care and Use Committee (IAACUC) of the Texas A&M University System (AUP #2014-003A).

Cows

Pregnant multiparous Brahman cows (n=52) at the Texas A&M AgriLife Research Center in Overton, TX (32.294N, -94.976W) were evaluated as follows. Health records were kept on all cattle throughout the project. All 52 Brahman cows were evaluated for cell mediated immune response (CMIR) prior to the start of this project (Cook et al., 2017). Specifically, CMIR was evaluated by determining each cow's delayed-type hypersensitivity to *Candida albicans*. Each cow received in the left side of their neck a subcutaneous injection of 0.5 mg of killed *Candida albicans* and 0.75 mg Quil A adjuvant to initiate sensitivity to *Candida albicans* (D 0). Fourteen days (D 14) later tail skin fold thickness on the left side of the tail was measured for each cow by use of a spring-loaded Harpenden skinfold caliper (Creative Health Products Inc., Ann Arbor, MI). After the measurement was recorded, 0.1 mg killed *Candida albicans* was subcutaneously injected into the cow's tail skin fold. Twenty-four hr later (D 15) the thickness of the injection site was measured using a spring-loaded Harpenden skin fold caliper. Based on their CMIR results (i.e., degree of increased skin fold thickness in response to re-exposure to *Candida albicans*) the cows were classified as members of the high, intermediate, or low response groups. Specifically, cows that were $\frac{1}{2}$ standard deviation above the mean (mean= 2.25 mm) were classified as high responders (H: ≥ 2.8

mm), those that were between $\frac{1}{2}$ standard deviation above and $\frac{1}{2}$ standard deviation below the mean were classified as intermediate responders (I: 2.7-1.8 mm), and cows that were $\frac{1}{2}$ standard deviation below the mean were classified as low responders (L: \leq 1.8 mm). Prior to calving, cows were maintained on rye and rye grass pastures, fed free-choice coastal Bermuda grass hay, along with a daily supplement of corn and corn gluten meal (approximately 3.6 kg per head of a 3:1 ratio corn to corn gluten meal concentrate). After calving, as the spring season progressed, cows grazed ryegrass and coastal Bermuda grass pastures with concentrate supplemented as needed. Cows were weighed using a calibrated scale and body condition (Mulliniks et al., 2016) was evaluated at 28-d intervals throughout the last 90 d of gestation. Blood samples were collected via puncture of a tail vessel 28 d prior to each cow's expected calving date. During a window of 6-12 hr after parturition blood samples (10 mL) were collected from the cows by puncture of a tail vessel and the cows were milked to obtain samples of colostrum. To achieve milk letdown, the cows received 1 ml of oxytocin (20 U.S.P. units, Valley Vet Supply, Maryville, KS) injected into a tail vessel and the colostrum was collected by milking the cow until her udder was depleted. The entire colostrum sample was mixed before samples were placed into centrifuge tubes. The colostrum samples were centrifuged to remove fat and the skim milk samples were sealed and stored at -20°C until analyzed for immunoglobulin content using double-antibody sandwich ELISA assays specific for bovine immunoglobulins (Gross et al., 2016). Colostrum samples were analyzed for IgG concentration using the Bovine IgG Quantitation Set ELISA (Bethyl Laboratories Inc. Montgomery, TX, USA). Blood samples (10 mL) were

collected via tail vessel puncture from the cows on days 7, 14 and 28 after calving. After D 28, weekly blood samples (10 mL) were collected from the tail vessel. Body weight and body condition score were also recorded on D 28. The length of the post partum interval (PPI) was determined as follows, estrus was detected using sterile bulls fitted with chin-ball markers and tail paint to detect cows in estrus. The time from calving to estrus was used to calculate PPI. All blood samples collected from the cows throughout the project were stored at room temperature until clotting occurred, and then centrifuged (Megafuge 40R, Global Industrial, Port Washington, NY, USA) at 1,764 x g for 30 min. Serum was pipetted into storage tubes and stored at -20C until analyzed for specific IgG and IgM by double sandwich, enzyme linked immunosorbent assay (Bovine Quantitation Set ELISA, Bethyl Laboratories Inc. Montgomery, TX, USA). To avoid the incidence of plate-to-plate variation with the IgG and IgM ELISA assays, all blood samples (D minus 28 through D 28) for each individual cow were analyzed on a single plate.

Calves

Calves born to the 52 Brahman cows were manually restrained in order to collect blood samples (5mL each) by jugular venipuncture once between 10 and 24 hr after parturition (D 0), after suckling to determine serum concentrations of immunoglobulins. At the time of blood collection, calves were weighed using a scale; received an identification tattoo inside their ear; and had identification tags placed in the middle one third of the left ear. On D 7, 14, and 28 blood samples (5 ml) were collected via jugular puncture; centrifuged at 1,764 x g for 30 minutes; and processed to yield serum and

stored at -20 °C until assayed for IgG and IgM concentrations by double sandwich, enzyme linked immunosorbent assay (Bovine Quantitation Set ELISA, Bethyl Laboratories Inc. Montgomery, TX, USA). Because of the incidence of plate-to-plate variation with the IgG and IgM ELISA assays, all blood samples (D 0 through D +28) for each individual calf were analyzed on a single plate. Calf samples were also organized by plate to intermingle calves born from each CMIR response class of dams. Body weight was recorded using a calibrated scale at each time of blood collection from the calves. Beginning on D 28 calves were assessed for temperament score by exit velocity (EV), which was the rate at which the calf exited the chute and traveled a distance of 1.83 meters. (Curley et al., 2006). Calves were also evaluated for pen score (PS) based on how they behave in a small group (n=5) while the handler moved about the pen (Curley et al., 2006). Both EV and PS were used to assign the calves an individual temperament score (TS). Twenty-eight days prior to weaning (D -28) calves had exit velocity, pen scores, body weights recorded, vaccinations, and had a blood sample (10 mL) collected via jugular puncture. Four different vaccines (n=4) were administered subcutaneously. The first vaccine included *L hardjo-bovis* and 5 strains of *leptospirosis*. The second vaccine included 7 way blackleg plus- *clostridium chauveoi*, *septicum*, *noryi*, *sordellii*, *perfringens* types C and D, and *haemolyticum*. The third vaccine included *IBR*, *BVD* types 1 and 2, *PI3*, and *BRSV*. Finally, an injection of *clostri-perfringens* type A toxoid was given to all of the calves. At the time of weaning, calves had a blood sample (10 mL) collected via the jugular vein, booster vaccines of the same injections given on D -28 administered, and were evaluated for body weight and

condition score as well as temperament score (mean of pen score and exit velocity).

Blood samples from the calves were processed and stored in the same manner as was done on D -28.

CMIR Evaluation of the Calves

Twenty-eight days after weaning the calves were evaluated for cell mediated immune response. The calves were restrained in a squeeze chute and a blood sample (10 mL) was collected by jugular venipuncture before vaccinations were administered. Blood samples collected from the calves were stored at room temperature until clotting occurred, and then centrifuged at 1,764 x g for 30 minutes. Serum was collected into storage tubes and stored at -20°C until analyzed for specific IgG and IgM by double sandwich, enzyme linked immunosorbent assay (Bovine Quantitation Set ELISA, Bethyl Laboratories Inc.). Following blood sampling the calves were vaccinated with a subcutaneous injection in the left side of the neck containing 0.5 mg of killed *Candida albicans* and 0.75 mg Quil A adjuvant to initiate sensitivity to *Candida albicans*. Forty-two days post-weaning animals had skinfold thickness measured using a spring-loaded Harpenden skinfold caliper and received an injection of 0.1 mg killed *Candida albicans* subcutaneously in the left side of the tail skin fold. Twenty-four hr later (D 43 post-weaning) the thickness of the injection site was measured using a spring-loaded Harpenden skin fold caliper. On D 43 a blood sample (10 mL) was collected via jugular puncture and processed to yield serum for immunoglobulin concentrations. Based on their CMIR results (i.e degree of increased skin fold thickness in response to re-exposure

to *Candida albicans*) the calves were labeled as members of the high , intermediate, or lowresponse groups.

IgG and IgM ELISA Procedures

1. Plate Coating

To determine IgG and IgM concentrations in the serum, 96-well R&D plates (IgG analysis) and Bethyl plates (IgM analysis) were coated with a dilution of 1 µl affinity purified antibody to 100 µl Coating Buffer (Bethyl E107/Sigma C3041) at pH 9.6. Then 100 µl of diluted antibody was added to each well. The plates were then incubated at room temperature (20-25°C) for 3-4 hr, or overnight in 2-8°C. After incubation, the antibody solution was aspirated from each well (dumping, suction or vacuum). A solution (TBST) consisting of 16.37g NaCl, 12.41g Tris Haze, and 1 mL Tween was combined to use as a blocking solution, sample conjugate dilution, and a plate washing solution and was made as needed. The plate was then washed five times as described in the Plate Washing section. Then, 300 µl of Blocking Solution (TBST) was added to each well on the plate and then incubated for 60 minutes at room temperature (20-25°C). Following incubation, the TBST was removed (dumping, suction or vacuum) and each well was washed five times as described in the Plate Washing section.

2. Plate Washing

Each well on the plate was filled with ELISA Wash Solution (300 μ l TBST) and removed by aspiration. This procedure was repeated four additional times for a total of five washes for each plate.

3. Standards and Samples

The standards were diluted in Sample/Conjugate Diluent (TBST) according to the recommendations of Bethyl Laboratories. The samples were diluted, based on the expected concentration of the analyte, to fall within the concentration range of the standards (1:400,000 IgG, 1:20,000 IgM). A 100 μ l aliquot of sample was transferred to each well from lowest concentration to highest concentration in order to avoid cross-contamination. The plates were then incubated for 60 minutes at room temperature (20-25°C). After incubation, the samples and standards were aspirated and the plate was washed five times.

4. HRP Detection Antibody

The HRP Detection Antibody was diluted in Sample/Conjugate Diluent (TBST). For 1:150,000 (IgG), 1 μ l of HRP Detection Antibody was added to 149 μ l of diluent (this was a 1:150 stock solution). Next, 1 μ l of 1:150 HRP stock was added to diluent for every mL needed of HRP 1:150,000 solution (eg. 8 μ l to 8 mL diluent). For 1:125,000 (IgM), 1 μ l of HRP Detection Antibody was added to 124 μ l of diluent. (this is a 1:125

stock solution). Then, 1 μ l of 1:125 HRP stock to diluent was added for every mL needed of HRP 1:125,000 solution (ex. 8 μ l to 8 mL diluent).

Then, 100 μ l of final HRP was transferred to each well, making sure to transfer IgG HRP to IgG wells, and IgM HRP to IgM wells. The plates were then incubated 60 min at room temperature (20-25°C). After incubation, HRP Detection Antibody was removed and the plate was washed five times. Fresh stock solution and a final dilution was made within 10 min of plating to help reduce the background.

5. TMB Substrate Incubation and Reaction Stop

The substrate solution was prepared according to the manufacturer's recommendation.

Using a sterile pipette, equal parts of Substrate A and B were transferred into separate, foil-wrapped tubes. Tubes were allowed time to reach room temperature away from light (in a drawer). Once substrates achieved room temperature, TMB was added to wells by combining A and B and inverting a few times to mix, then immediately poured into reservoir dish to start pipetting.

Next, 100 μ l of TMB Substrate Solution was added into each well. The enzymatic color reaction was allowed to develop at room temperature (20-25 °C) in the dark for 15 min. The substrate reaction yielded a blue solution. After 15 min to stop the reaction 100 μ l of ELISA Stop Solution (0.18 M H₂SO₄) was added to each well. The plate was tapped gently to mix. The solution in the wells changed from blue to yellow.

6. Absorbance Measurement

Evaluation of the plate was done within 30 min of stopping the reaction. The underside of wells was wiped with a lint-free tissue (Kimwipe), and the absorbance was measured on an ELISA plate reader with an endpoint protocol, set at 450 nm. A 4PL curve was used to evaluate the standard curve and unknown concentrations.

Statistical Analysis

The data for this project were analyzed using statistical procedures of SAS specific for repeated measures where appropriate (SAS 9.4, SAS Institute, Cary, NC). Cow response variables included: cow body change from D -28 to D +28, cow BCS change from D -28 to D +28, PPI, concentration of IgG and IgM in the colostrum, as well as the concentration of IgG and IgM in the serum. Calf response variables included: calf birth weight and average daily gain, adjusted 180-d weaning weight (BIF, 2018), concentration of IgG and IgM in the serum, pen score and exit velocity, and calf CMIR. Repeated measures included cow body weight, cow BCS, and calf weight. Fixed variables included cow CMIR class and calf sex. Cow sire was set as a random effect. Least Square Means were evaluated between CMIR class of the cow and calf sex. Evaluation and comparison of the calves was based on the CMIR classifications of their dams. The Proc Mixed procedures using SAS were applied to analyze the differences between high , intermediate, and low CMIR cows based on concentrations of IgG and IgM in the colostrum. Proc Mixed procedures in SAS were used to generate Least

Square Means to evaluate the influence of cow CMIR class on the dependent variables measured.

Results and Discussion

The primary objective of this study was to determine relationships between cell-mediated immune response (CMIR) of cows and postpartum interval (PPI; calving to estrus), colostral immunoglobulins (IgG-1 and IgM), serum immunoglobulins (IgG-1 and IgM), and calf growth.

In brief, there was no statistical relationship seen between CMIR class of the cow on cow body weight, cow body condition score, calf birth weight, calf D 28 weight, calf weaning weight, calf temperament score, and tail skinfold thickness of the calf following *Candida albicans* CMIR skinfold thickness test. However, there was a relationship found between cow CMIR class on colostral immunoglobulin concentration and calf sex (Table 9).

Cow Performance

Proc Mixed procedures in SAS were used to evaluate the relationship among CMIR class and cow BW and BCS. Body weight (BW) and body condition score (BCS) measurements began 28 D prior to expected calving date were subsequently measured on D 0, 7, and 28 post-calving, as well as 28 D prior to weaning (D-28), on the day of calf weaning (D0W), and 28 D after weaning (D+28). The results from Table 8 indicate that cell-mediated immune response group did not effect cow BW ($P = 0.9$) or BCS ($P =$

0.6). Cows were able to maintain a relatively constant body weight only losing an average of 8 kg of body weight from the final trimester of gestation (D -28 before calving) through the first month of the heavy lactation period following parturition (D +28 after calving) regardless of CMIR class.

Table 4. Influence of CMIR class on cow body weight change and BCS

	High	Intermediate	Low	P-value
<u>kg</u>	-8.82 ± 12.16	-2.53 ± 10.84	-5.45 ± 11.26	0.9
<u>BCS</u>	6.8 ± 0.2	7.3 ± 0.2	7.1 ± 0.2	0.6

Data presented as Least Square Means ± SE.

Absence of superscripts denotes no statistical significance (P>0.10).

Brahman cows in the current study were divided into three CMIR classes. Specifically, the 3 response groups were high (H), intermediate (I) and low (L) immune responders based on the tail skinfold thickness reaction to *Candida albicans* in a prior study (Cook et al, 2016). In the present study, there was no statistical relationship between CMIR class of the cow and cow body weight (BW) or cow body condition score (BCS) from pre-calving (D -28) through post calving (D 28). As expected, the cows lost weight from calving (D 0) to D 28 during the period of heavy lactation and increased nutrient requirements following parturition. Brahman cattle have been selected for hardiness and ability to thrive in harsh environmental conditions. Glass et al., (2005) reported that *Bos indicus* breeds have a stronger cell mediated immune response than

Bos taurus cattle, and therefore have increased resistance to viruses and parasites that have a profound impact on their ability to maintain body weight and condition.

Cow Post Partum Interval

Cell mediated immune response category did not influence PPI ($P = 0.9$). The average PPI for this group of cattle was 51 D. Gestation length of Brahman cattle is roughly 291 D, this gives cows approximately 70 D to return to estrus and rebreed to remain on a 365 D calving interval. Though there was no statistical difference in PPI between CMIR groups, these cattle are attaining their productive potential despite their differences in cell-mediated immune function.

There was no difference detected between the CMIR class of the cow and the length of the PPI. Uterine involution is a process of uterine repair that occurs following parturition in order to return the uterus to a state capable of supporting a subsequent pregnancy (Kiracofe, 1980). Uterine involution requires both a strong innate and adaptive immune system to return the uterus to the involuted state and ultimately achieve conception and produce a calf every year (Kiracofe, 1980). It has been reported that lactating dairy cattle that possess a strong antibody and cell mediated immune response complete uterine involution and return to estrus more efficiently than those with a lesser immune response (Stoop et al., 2016; Thompson-Crips et al., 2014). In the current study, there was no statistical significance observed among the 3 CMIR classes of the cows and its relationship to the length of the PPI. Historically, the gestation length of *Bos indicus* type cattle is approximately 291 D. Therefore, Brahman cattle have roughly

70 d to return to estrus and become pregnant again in order to stay on a 365-D calving interval. The average PPI for this group of cows was 51 D, regardless of CMIR class. The cows that repeatedly produce a calf every 365 D achieve profitability through reproductive efficiency. Open cows, for even one season, are likely costing the producer more in maintenance than she is worth, suggesting the potential economic advantage of selecting cows with shorter PPI (Forabosco et al., 2004).

Colostrum IgG-1 and IgM concentration

A tendency for an interaction between calf sex and CMIR class was observed for both IgG-1 ($P = 0.06$) and Total IG (IgG-1 + IgM; $P = 0.06$) colostrum concentrations (Table 9). Colostrum IgM concentration was not influenced by CMIR class of the cow ($P = 0.3$).

Table 5. CMIR class affecting colostrum immunoglobulin concentrations
IgG-1 colostrum (mg/mL)

	H	I	L
Female	90.4±39.6	208.2±51.2	51.0±56.1
Male	202.6±62.6	92.1±42.0	113.9±40.0

Total IG colostrum (mg/mL)

	H	I	L
Female	94.1±39.6	212.1±51.1	58.1±56.1
Male	205.8±62.6	95.4±41.7	118.5±39.6

Data presented as Least Square Means ± SE.

Absence of superscripts denotes no statistical significance ($P>0.10$).

In the present study, there was no difference detected between CMIR class of the cow and colostral immunoglobulin concentrations. Relationships were observed between the CMIR class of the cow and colostral IG concentration and calf sex. Calves, among other species, are born agammaglobulinemic and therefore rely on passive immune transfer from the dam to survive and thrive throughout their lives (Halleran et al., 2017). Flemming et al. (2016) reported that dairy cattle with a robust CMIR had higher concentrations of colostral immunoglobulins (IG) than those with lower CMIR. With greater concentrations of immunoglobulins, there is a better chance of calves ingesting adequate amounts of IGs for passive immune transfer, thus ensuring better survival and increased performance (Wittum, 1995). In this current study, there was no statistical evidence of influence of the CMIR of the cow ($n=49$) on colostral immunoglobulin concentrations. However, an interaction between calf sex and CMIR class was observed for both IgG-1, and Total IG colostrum concentrations (Table 1). Cows that were in the high (H) group for CMIR that gave birth to bull calves had higher concentrations of colostral IgG-1 and Total IG than those cows that gave birth to heifer calves. On the contrary, cows that were in the intermediate (I) group for CMIR that gave birth to heifer calves produced higher colostral IgG-1 and IG concentrations when compared to cows in the intermediate class that had bull calves. This evidence leads to the observation that there is a tendency towards statistical significance for the interaction between cow CMIR class and calf gender. In mice, there have been reports that estradiol and testosterone may have inhibitory effects on cell mediated immune response, therefore

leading to the idea that sex has an effect on the immune response of the animal (McCruden and Stimson, 1991; Wichmann et al., 1997). However, limited research has been done on the effects of sex on immune response in cattle and further investigation is warranted.

Cow performance (D 0 –Weaning)

Cell mediated immune response class did not affect cow weight gain or loss during the timeline from the day of calving to calf weaning ($P = .2366$). These results indicated that cow body weight maintenance was consistent with minimal weight loss from the period of heavy lactation following calving through calf weaning.

In the current study, there was no statistical evidence that CMIR class of the cow had an influence on calf birth weight, calf D 28 weight, or calf weaning weight. It has been reported that calves that acquire a strong CMIR from ingesting colostrum rich in immunoglobulins have increased performance than those calves that are born from lower response dams (Flemming et al., 2015). Routinely, dairy calves are removed from the dam shortly after birth and bottle-fed colostrum collected from the dam to ensure passive immune transfer. The initial stress this separation can cause on the calf could impact the calf's susceptibility to illness during this crucial life period, ultimately affecting their longtime performance (Soberon and Van Amburgh, 2013). It has been reported that dairy calves born from higher immune response dams have a more robust immune response system and are healthier than calves born from lower immune responders (Thompson-Crips et al., 2012). In the current study, there was no difference between

CMIR class of the cow and birth weight of her calf. The CMIR class of the cow did not impact calf body weight at 28 D of age. The average calf weight gain in this study from birth (D 0) to one month of age (D 28) was 25 kg. This leads to the assumption that the calves were meeting their nutritional requirements to remain on a positive growth curve for the first month of their life, regardless of which CMIR class their dams were in. There was also no difference seen among the 3 CMIR classes of the dams and the performance of the calves observed through weaning weights.

Cow Serum Immunoglobulin Concentration

Results from Table 10 and 11 indicated that cell-mediated immune response did not affect cow serum IgG (P = 0.4) or IgM (P = 0.2) concentrations during the sample timeline.

Table 6. Influence of cow CMIR class on cow serum IgG concentration (mg/mL) days relative to calving.

	D -28	D 0	D +7	D +28
<u>High (n=20)</u>	922.72 ±	927.96 ±	805.83 ±	694.20 ±
	241.17	241.17	241.17	241.17
<u>Intermediate (n=9)</u>	1573.7 ±	1048.90 ±	861.90 ±	1531.8 ±
	400.29	359.51	359.51	359.51
<u>Low(n=17)</u>	825.24 ±	1172.90 ±	1075.00 ±	520.83 ±
	261.58	261.58	261.58	268.35

Data presented as Least Square Means ± SE.

Absence of superscripts denotes no statistical significance (P>0.10).

Table 7. Influence of CMIR class on cow serum IgM concentration (mg/mL) by days relative to calving.

	D -28	D 0	D +7	D +28
<u>High (n=20)</u>	18.00 ± 4.29	23.66 ± 4.3	18.52 ± 4.29	15.46 ± 4.29
<u>Intermediate (n=9)</u>	23.74 ± 7.02	30.81 ± 6.44	25.05 ± 6.44	29.32 ± 6.44
<u>Low(n=17)</u>	13.06 ± 5.05	18.16 ± 5.06	19.72 ± 5.05	13.12 ± 5.05

Data presented as Least Square Means ± SE.

Absence of superscripts denotes no statistical significance ($P > 0.10$).

Calf Serum Immunoglobulin Concentration

Cow CMIR class did not influence serum IgG-1 ($P = 0.3$) or IgM ($P = 0.2$) concentration in the calves during the sample timeline. Statistical significance was observed for individual sample dates (Time) throughout the sampling timeline (IgG-1; $P = <.0001$, IgM; $P = <.0001$) indicating a change in serum immunoglobulin concentration as the calf matured.

Table 8. Influence of cow CMIR class on calf serum IgG concentration after birth (mg/mL).

	D 0	D 7	D 14	D 28
<u>High (n=20)</u>	375.19 ± 38.17	320.06 ± 38.17	259.2 ± 38.17	186.72 ± 38.67
<u>Intermediate (n=9)</u>	519.09 ± 56.90	379.17 ± 56.90	314.25 ± 56.90	244.75 ± 56.90
<u>Low(n=15)</u>	338.28 ± 44.07	283.76 ± 44.07	227.60 ± 44.07	176.39 ± 44.07

Data presented as Least Square Means ± SE.

*Individual classes did not differ (P>0.10).

P=0.1986

Table 9. Influence of cow CMIR class on calf serum IgG concentration by day relative to weaning (mg/mL).

	D-28	D0W	D+28
<u>High (n=20)</u>	218.16 ± 23.49	189.42 ± 23.49	244.62 ± 23.49
<u>Intermediate (n=9)</u>	219.46 ± 34.14	192.06 ± 34.14	327.26 ± 34.14
<u>Low(n=15)</u>	203.62 ± 26.44	221.89 ± 26.44	246.30 ± 27.06

Data presented as Least Square Means ± SE.

*Individual classes did not differ (P>0.10).

P=0.6946

Table 10. Influence of cow CMIR class on calf serum IgM concentration by day after birth (mg/mL).

	D 0	D 7	D 14	D 28
<u>High (n=20)</u>	9.54 ± 1.25	6.36 ± 1.25	5.05 ± 1.25	4.61 ± 1.27
<u>Intermediate (n=9)</u>	15.82 ± 1.86	7.56 ± 1.86	5.82 ± 1.86	6.14 ± 1.86
<u>Low(n=15)</u>	11.90 ± 1.49	5.83 ± 1.45	4.79 ± 1.45	4.61 ± 1.45

Data presented as Least Square Means ± SE.

*Individual classes did not differ (P>0.10).

P=0.3064

Table 11. Influence of cow CMIR class on calf serum IgM concentration by day relative to weaning (mg/mL).

	D-28	D0W	D+28
<u>High (n=20)</u>	15.66 ± 2.33	15.00 ± 2.30	23.26 ± 2.30
<u>Intermediate (n=9)</u>	20.63 ± 3.34	14.77 ± 3.34	23.79 ± 3.34
<u>Low(n=15)</u>	11.04 ± 2.60	14.30 ± 2.60	17.51 ± 2.60

Data presented as Least Square Means ± SE

*Individual classes did not differ (P>0.10)

P= 0.2560

Calf Performance

Cell-mediated immune response did not influence calf birth weight (P = 0.6) or body weight at 28 days of age (P = 0.4). The difference in birth weight due to calf sex that has been observed in beef cattle research was not observed in this sample group. This lack of difference could be due to the relatively small sample size and therefore a difference in birth weight between calf sexes was not observed.

The results from Table 16 indicate that calf weaning weight within CMIR class of the dam ($P = 0.7622$) did not differ among CMIR classes of the cow. Bull calves were heavier at weaning than heifer calves ($P = 0.0012$), as shown in Table 17. There was no difference in calf growth rates from birth to weaning ($P = 0.6065$). However, as predicted, there was a difference between calf growth rate between the sexes of calves, with bull calves having a higher weight gain from birth to weaning when compared to heifer calves ($P = .0007$).

Table 12. Influence of CMIR class on calf 180-d adjusted weaning weight.

	High	Intermediate	Low	P-value
<u>N</u>	14	16	15	
kg	199.69±4.59	197.75±6.71	203.49±5.04	0.7622

Table 13. Influence of calf sex on 180-d adjusted weaning weight.

	Male	Female	P-value
kg	211.53±4.53	189.10±4.49	0.0012

Data presented as Least Square Means \pm SE.

Statistical significance was seen between calf weaning weight and calf sex, with bull calves achieving heavier weaning weights than heifer calves in this study. There was no significance between CMIR class of the dam and the calf growth rate from birth (D 0) to weaning. However, there was a difference between the calf growth performance

and calf sex, with bull calves gaining weight more rapidly than heifer calves. The greater weight gain of bull calves compared to heifer calves is consistent with research findings by Browning et al. (1997).

Calf Temperament

Cell mediated immune response of the cow did not influence calf pen score (PS; $P = .7612$), calf exit velocity (EV; $P = .7896$) or temperament score ($P = .7742$).

In the current study, there was no statistical significance observed among CMIR classes of the cows affecting calf PS, calf EV, or calf Temperament. Temperament has been measured in calves by evaluating pen score (PS) and exit velocity (EV) (Curley et al., 2006). It has been reported that cattle with gentler temperament scores maintain weight and BCS better than cattle with more flighty temperaments (Curley et al., 2006; Hoppe et al., 2010; Voisinet et al., 1997). In this study, both PS and EV were measured to assign each calf with a temperament score. There was no statistical significance observed among the CMIR class of the dam and her calf's PS, calf EV, and calf temperament. The lack of statistical evidence might be due to the small sample size and low average temperament scores among the calf sample groups. Cattle at the Overton Research Station are handled regularly and are used to human interaction, therefore were not overly excitable during sampling days.

Calf CMIR

Evaluation of CMIR class of the calf was evaluated by skinfold thickness measurement following injection of *Candida albicans* identical to the CMIR evaluation

of their dams. The results from this study indicated that cow CMIR class did not influence skinfold thickness difference (SFT) of the calves at weaning ($P = .6866$).

Tail skinfold thickness measurement after a subcutaneous injection of *Candida albicans* has been used to evaluate CMIR in dairy cattle (Thompson-Crispi and Mallard, 2012). Previous studies that evaluated CMIR response in dairy cattle found that those that possessed a higher immune response were less susceptible to pathogens that cause economically important diseases, such as mastitis, endometritis, ketosis, and retained placenta (Thompson-Crispi et al., 2012). It was also observed in a related study that CMIR response was estimated to be heritable (h^2) by 0.17 in dairy cattle (Thompson-Crispi et al., 2012). Selection of dairy cows based on their immune response has allowed for the generation of more productive calves in terms of their future reproductive performance and milk production (Hine et al., 2010; Martin et al., 2015).

Calf Serum Immunoglobulin Correlations

PROC CORR procedures in SAS were used to generate the correlation data in this study. A positive correlation between IgG-1 serum concentration on D 14 and calf weight at D 28 ($P = 0.07$; $r = -0.28302$) and weaning ($P = 0.009$; $r = -0.3898$) was observed, as well as a positive correlation between birth weight ($P = 0.03$; $r = 0.32488$) and D 28 IgM serum concentration in the calves. This indicates that higher serum immunoglobulin concentration post parturition positively impacts calf growth.

In the present study, there was no statistical difference between the CMIR classes of the cows on the CMIR of the calves at weaning. The CMIR response of the cow has

been shown to be moderately heritable in several studies and can potentially be used as a selection tool to cull cattle that are less immune competent and outperformed by higher immune response cattle (Thompson-Crips et al., 2012). The current study evaluated CMIR response in calves that were born to cows that had been previously evaluated for CMIR. There was no statistical difference seen among the CMIR of the calves at weaning and the CMIR class of the dam. Perhaps this was due to the small sample size of $n=49$ and the lack of disease among the sample group. However, there was a correlation seen between colostral IgM concentration and CMIR of the calf. This correlation indicates that a higher concentration of IgM in the colostrum may result in a more robust CMIR response in the calf.

In conclusion, there was no statistical evidence that CMIR class of the cow influenced cow postpartum interval, colostral immunoglobulin concentration, cow performance, or calf performance. The evidence of CMIR influencing dairy cattle performance in the supporting literature was not reflected in the current study using Brahman cattle. However, the correlation between calf sex and colostral immunoglobulin concentration of the cow warrants further investigation. Possible genetic differences exist between CMIR class of the cow and the colostral IG concentration based on the sex of the calf. Furthermore, the correlation between colostral IgM concentration and CMIR supports previous research reports of improved immune response as a result of high quality colostrum intake.

Conclusion

It is logical to believe that cows that have a better immune system will outperform their less immune competent counterparts. Research in dairy cattle has shown this phenomenon to be true and evidence to support the idea of selection for high immune responders has been published. However, when comparing the dairy cattle studies with the current Brahman cattle study, it must be remembered that these are two very different animals with vastly different requirements. As beef cattle in East Texas, the stress that the Brahman cattle faced was light in comparison to the physiological challenge that dairy cattle encounter daily. As there were few stressors affecting the Brahman cattle, the purpose of this study was to evaluate the functional differences of CMIR on the cow's body condition and weight maintenance, colostral immunoglobulin concentration, and reproductive efficiency.

The results indicated no difference among CMIR classes for any of the measurements in the cows. Within the calf population, evaluation of their performance in terms of weight gain and temperament was measured and compared to their own CMIR class, which could be inherited from their dam by innate and passive transfer. However, no statistical difference was observed between calf performances, and there was no relation of their own immune class associated to the CMIR class of their dam. Furthermore, concentrations of immunoglobulin were not different among CMIR classes. These results could be the outcome of a small sample size combined with the lack of a stressor to test their immune systems. Based on previous research and the results from chapter 2 of this thesis, it is believed that there are benefits in terms of long-

term reproductive performance when selecting for higher immune responders within the herd.

In conclusion, CMIR class of the dam was not statistically conclusive to support selection of high immune responders to improve cow metabolic performance, PPI, or colostral immunoglobulin concentration. However, benefits of stayability and overall number of calves weaned in a cow's lifetime were seen to favor cows that possessed a high CMIR.

4. GENERAL CONCLUSIONS

The purpose of this study was to gain a better understanding of the immune function in Brahman cows by specifically evaluating cell-mediated immune response and its effects on production traits in beef cattle. These production traits included cow CMIR class, calving intervals, postpartum interval, body weight and condition maintenance, serum immunoglobulin concentration, and colostral immunoglobulin concentrations. The calves were evaluated for birth weight, growth through weaning, temperament, serum immunoglobulin concentration, and CMIR class. The findings from this research study concluded that there are economic benefits of selecting higher immune response cattle in the herd compared to the potential financial loss caused by lower immune response cattle. It was demonstrated that cattle that possess a higher immune response system are more capable to remain in the herd as a more reproductively efficient animal and achieve greater profitability. These high response cattle also would achieve a greater number of pounds of calf weaned than low response cows, as the low responders did not produce as many calves in their lifetime. These results indicate that immune function is important to consider when making selection within the herd. The benefits of keeping an older cow that produces a calf every year is more economically efficient than purchasing or developing a replacement heifer.

REFERENCES

- Abbas, A. K., and A. H. Lichtman. 2009. Basic immunology: functions and disorders of the immune system. Philadelphia, PA 19103-2899.
- Aleri, J. W., B. C. Hine, M. F. Pyman, P. D. Mansell, W. J. Wales, B. Mallard, and A. D. Fisher. 2015. Assessing adaptive immune response phenotypes in Australia Holstein-Friesian heifers in a pasture-based production system. *J. Anim. Sci.* 93:3713-3721. doi: 10.2527/jas2015-9078
- Banta, J. P., N. C. Burdick, J. C. White, D. A. Neuendorff, A. W. Lewis, J. C. Laurenz, T. H. Welsh, and R. D. Randel. 2007. The use of neonatal blood parameters to predict preweaning weight gain of Brahman calves. The Agriculture Program-Texas A&M University System.
- Brigham, B.W., S.E. Speidel, R.M. Enns, and D. J. Garrick. 2007. Stayability to alternate ages. *Dep. Anim. Sci. Colorado State University, Ft. Collins, CO* 80523.
- Browning, R., B. G. Warrington, J. W. Holloway, and R. D. Randel. 1997. Testicular size at weaning in tropically-adapted beef bulls as influenced by breed of sire and dam. *Theriogenology* 48: 257-265.

Burdick, N. C., J. P. Banta, D. A. Neuendorff, J. C. White, R. C. Vann, J. C. Laurenz, T.

H. Welsh, R. D. Randel. 2009. Interrelationships among growth, endocrine, immune, and temperament variables in neonatal Brahman calves. *J. Ani. Sci.* 87:3202-3210. doi: 10.2527/jas.2009-1931.

Bourdon, R. M., and J. S. Brinks. 1982. Genetic, environmental and phenotypic relationships among gestation length, birth weight, growth traits and age at first calving in beef cattle. *J. Anim. Sci.* 55: 543-553.

Brigham, B. W., S. E. Speidel, R. M. Enns, and D. J. Garrick. 2007. Stayability to alternate ages. *Dep. Anim. Sci. Colorado State University, Fort Collins, CO* 80523.

Carroll, J. A. 2014. Bidirectional communication: Growth and immunity in domestic livestock. *J. Anim. Sci.* 86:E126-E137. doi: 10.2527/jas.2007/0480.

Cartwright, S. L., L. R. Schaeffer, E. B. Burnside, and B. A. Mallard. 2012. Adaptive immune response, survival, and somatic cell score between postpartum Holstein and Norwegian Red x Holstein first-calf heifers. *J. Anim. Sci.* 90:2970-2978. doi: 10.2527/jas2011-4233.

- Chase, C. L., D. J. Hurley, A. J. Reber. 2008. Neonatal immune development in the calf and its impact on vaccine response. *Vet. Clin. Food Anim.* 24:87-104. doi: 10.1016/j.cvfa.2007.11.001
- Cook, C. L., T. H. Welsh, T. J. Garcia, D. G. Riley, W. Mwangi, J. Bray, A. W. Lewis, D. A. Neuendorff, and R. D. Randel. 2017. Cellular and antibody mediated immune responses are influenced by sex and pregnancy status in mature Brahman cattle. *J. Anim. Sci.* 95(Suppl. 4):219-220.
- Curley, K. O., J. C. Paschal, T. H. Welsh, and R. D. Randel. 2006. Exit velocity as a measure of cattle temperament is repeatable and associated with serum concentration of cortisol in Brahman bulls. *J. Anim. Sci.* 84:3100-3103. doi: 10.2527/jas.2006-055
- Curtis, S. E. 1983. Environmental management in animal agriculture. Iowa State University Press. p. 3-22.
- Dunn, B.H. "Measuring Cow–Calf Profitability and Financial Efficiency." *Proceedings, 34th Annual Beef Improvement Federation Research Symposium and Meeting*. Omaha, NE, July 2002.

Fleming, K., K. A. Thompson-Crispi, D. C. Hodgins, F. Miglior, M. Corredig, and B. A. Mallard. 2016. Short communication: variation of total immunoglobulin G and B-lactoglobulin concentrations in colostrum and milk from Canadian Holsteins classified as high , average, or low immune responders. *J. Dairy Sci.* 99:2358-2363. doi: 10.3168/jds.2015-9707

Forabosco, F., A. F. Groen, R. Bozzi, J. A. M. Van Arendonk, F. Filippini, P. Boettchers, and P. Bijima. 2004. Phenotypic relationships between longevity, type traits, and production in Chianina beef cattle. *J. Anim. Sci.* 82:1572-1580.

Funston, R. N., D. M. Larson, and K. A. Vonnahme. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: Implications for beef cattle production. *J. Anim. Sci.* 88: E205-E215. doi: 10.2527/jas.2009-2351.

Glass, E. J., P. M. Preston, A. Springbett, S. Craigmile, E. Kirvar, and G. Wilkie. 2005. *Bos taurus* and *Bos indicus* (Sahiwal) calves respond differently to infection with *Theileria annulata* and produce markedly different levels of acute phase proteins. *Int. J. Parasitol.* 35:337-347.

Gross, J. J., E. C. Kessler, and R. M. Bruckmaier. 2016. Quarter vs. colostrum composition assessed by Brix Refractometry, specific gravity and visual color

appearance in primiparous and multiparous dairy cows. *Tran. Anim. Sci.* 1:26-35. doi: 10.2527/tas2016.0001

Guy, M. A., T. B. McFadden, D. C. Cockrell, and T. E. Besser. 1994. Regulation of colostrum formation in beef and dairy cows. *J. Dairy. Sci.* 77:3002-3007. doi: 10.3168/jds. 50022-0302(94)777241-6.

Halleran, J., H. J. Sylvester, and D. M. Foster. 2017. Short Communication: Apparent efficiency of colostral immunoglobulin G absorption in Holstein heifers. *J. Dairy. Sci.* 100:3282-3286. doi: 10.3168/jds.2016-11904.

Hernandez, A., N. Karrow, B. A. Mallard. 2003. Evaluation of immune response of cattle as a means to identify high or lowresponders and use microarray to differentiate gene expression. *Genet. Sel. Evol.* 35:S67-81. doi: 10.1051/gse:2003017.

Herring, A. D. 2012. Beef cattle production systems. Department of Animal. Science, Texas A&M University, College Station, Texas, USA. doi: 10.1079/9781780645070.

- Hine, B. C., S. L. Cartwright, B. A. Mallard. 2010. Effects of age on pregnancy status on adaptive immune responses of Canadian Holstein replacement heifers. *J. Dairy Sci.* 94:981-991. doi: 10.3168/jds.2010-3329.
- Hoppe, S., H. R. Brandt, S. König, G. Erhardt, and M. Gauly. 2010. Temperament traits of beef calves measured under field conditions and their relationships to performance. *J. Anim. Sci.* 88:1982–1989.
- Horn, M. J., M. L. Van Emon, P. J. Gunn, S. D. Eicher, R. P. Lemenager, J. Burgess, N. Pyatt, and S. L. Lake. 2014. Effects of maternal natural (*RRR* α -tocopherol acetate) or synthetic (all-*rac* α -tocopherol acetate) vitamin E supplementation on suckling calf performance, colostrum immunoglobulin G, and immune function. *J. Anim. Sci.* 88:3128-3135. doi: 10.2527/jas.2009-2035.
- Kiracofe, G. H. 1980. Uterine Involution Its Role In Regulating Postpartum Intervals. *J. Anim. Sci.* 51:16-28. doi: 10.2527/1980.51Supplement_II16x
- Lippolis, J. D. 2014. Immunological signaling networks: Integrating the body's immune response. *J. Anim, Sci.* 86:E53-E63. doi: 10.2527/jas.2007-0620
- Martin, C. E., M. A. Paibomesai, S. M. Emam, J. Gallienne, B. C. Hine, K. A. Thompson-Crispi, B. A. Mallard. 2015. Short communication: cytokine profiles

from blood mononuclear cells of dairy cows with divergent immune response phenotypes. *J. Dairy Sci.* 99:2364-2371. doi: 10.3168/jds.2015-9449.

McCruden, A. B. and W. H. Stimson. 1991. Sex hormones and immune function. *Psychoneuroimmunology* (2nd ed.): 475–493.

Mor, G., P. Aldo, A. B. Alvero. 2017. The unique immunological and microbial aspects of pregnancy. Yale University School of Medicine. Doi: 10.1038/nri.2017.64.

Moraes, M. P., R. Weiblen, M. C. Rebelatto, A. M. Da Silva. 2000. Relationship between passive immunity and morbidity and weight gain in dairy cattle. *Cienc. Rural* [online]. 30: 299-304. doi. Org/10.1590/S0103-84782000000200017.

Mulliniks, J. T., E. R. Cope, Z. D. McFarlane, J. D. Hobbs, and R. C. Waterman. 2016. How physiology metabolism, experience and adaptability influence productivity. *J. Anim. Sci.* 2016.94(S6):111-119. doi: 10.2527/jas2015-0711.

Murphy, K., P. Travers, M. Walport, and C. Janeway. 2012. Janeway's immunobiology (8th ed.). New York: Garland Science.

NAP. 2015. National action plan for combating antibiotic-resistant bacteria. Extension available at:

https://obamawhitehouse.archives.gov/sites/default/files/docs/national_action_plan_for_combating_antibiotic-resistant_bacteria.pdf (Accessed March 22, 2017)

Oldenbroek, K., and L van der Waaij. 2015. Animal Breeding and Genetics. Centre for Genetic Resources The Netherlands and Animal Breeding and Genomics Centre. Groen Kennisnet: <https://wiki.groenkennisnet.nl/display/TAB/>

Parish, J. 2018. BIF Guidelines. NMREC Prairie Research Unit, PO Box 60, Prairie, MS 39756.

Perino, L. J., Sutherland, R. L., Woollen, N. E. 1993. Serum #-glutamyltransferase activity and protein concentration at birth and after suckling in calves with adequate and inadequate passive transfer of immunoglobulin G. Am. J. Vet. Res. 54:56-59

Plasse, D., A. C. Warnick and M. Koger. 1968a. Reproductive behavior of *Bos indicus* females in a subtropical environment. I. Puberty and ovulation frequency in Brahman and Brahman x British heifers. J. Anim. Sci. 27:94.

Randel, R. D. 2005. Reproduction of *Bos indicus* breeds and crosses. Texas A&M University, College Station. Proceedings, Applied Reproductive Strategies in Beef Cattle.

- Robinson, T. P., D. P. Bu, J. Carrique-Mas, E. M. Fevre, M. Gilbert, D. Grace, S. I. Hay, J. Jiwakanon, M. Kakkar, S. Kariuki, R. Laxminarayan, J. Lubroth, U. Magnusson, P. Thi Ngoc, T. P. Van Boeckle, and M. E. Woolhouse. 2016. Opinion paper: Antibiotic resistance: mitigation opportunities in livestock sector development. *Animal*. 2016. doi: 10.1017/S1751731116001828
- Senger, P. L. 2005. Pathways to pregnancy and parturition. Innovative technologies. Redmon, OR 97756. ISBN:0-9657648-3-4.
- Smith, S.M., W.W. Vale. 2006. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. *Dialogues in Clinical Neuroscience*. 2006;8(4):383-395.
- Soberon, F. and M. E. Van Amburgh. 2013. The effects of nutrient intake from milk or milk replacer of preweaned dairy calves on lactation milk yield as adults: a meta-analysis of current data. *J. Anim. Sci.* 91:706-712. doi: 10.2527/jas2012-5834.
- Stelwagen, K., E. Carpenter, B. Haigh, A. Hodgkinson, and T. T. Wheeler. 2014. Immune components of bovine colostrum and milk. *J. Anim. Sci.* 87:3-9. doi: 10.2527/jas.2008-1377

Stoop, C. L., K. A. Thompson-Crispi, S. L. Cartwright, and B. A. Mallard. 2016. Short communication: variation in production parameters among Canadian Holstein cows classified as high , average, and lowimmune responders. *J. Dairy Sci.* 99:4870-4874. doi: 10.3168/jds.2015-10145

Thompson-Crispi, K. A., A. Sewalem, F. Miglior, and B. A. Mallard. 2012. Genetic parameters of adaptive immune response traits in Canadian Holsteins. *J. Dairy Sci.* 95:401-409. doi:10.3168/jds.2011-4452

Thompson-Crispi, K. A., and B. A. Mallard. 2012. Type 1 and type 2 response profiles of commercial dairy cows in 4 regions across Canada. *Can. J. Vet. Res.* 76:120-8.

Thompson-Crispi, K. A., H. Atalla, F. Miglior, B. A. Mallard. 2014. Bovine mastitis. *Front. Immunol.* 5:493. doi: 10.3389/fimmu.2014.00493.

Trevisi, E., and G. Bertoni. 2009. Some physiological and biochemical methods for acute and chronic stress evaluation in dairy cows. *Ital. J. Anim. Sci.* 8:sup1, 265-286. doi: 10.4081/ijas.2009.s1.265

UGA. 2001. Profitable Cattle Marketing for the Cow-Calf Producer. (Published June 1, 2001) UGA Extension. Available at:
<http://extension.uga.edu/publications/detail.cfm?number=B1078>. Accessed

March 15, 2017

Voisinet, B. D., T. Grandin, T. D. Tatum, S. F. O'Connor, and J. J. Struthers. 1997.

Feedlot cattle with calm temperaments have greater average daily gains than cattle with excitable temperaments. *J. Anim. Sci.* 75:892–896.

Walusimbi, S. S., and J. L. Pate. 2014. Physiology and endocrinology symposium: Role

of immune cells in the corpus luteum. *J. Anim. Sci.* 2013.91:1650-1659. doi: 10.2527/jas2012-6179.

Warrington, R., W. Watson, H. L. Kim, and F. R. Antonetti. 2011. An Introduction to

immunology and immunopathology. *Allergy, Asthma & Clinical Immunology.* 7:S1. doi: 10.1186/1710-1492-7-S1-S1.

Watson, C. J. 2009. Immune cell regulators in mouse mammary development and

involution. *J. Anim. Sci.* 87:35-42. doi:10.2527/jas.2008-1333.

Weaver, D. M., J. W. Tyler, D. C. VanMetre, D. E. Hostetler, and G. M. Barrington.

2000. Passive transfer of colostral immunoglobulins in calves. *J. Vet. Intern. Med.* 14:569-577. doi: 0891-6640/00/1406-0002.

Wichmann M. W., A. Ayala, and I. H. Chaudry. 1997. Male sex steroids are responsible

for depressing macrophage immune function after trauma-hemorrhage. *Amer. J. Cell Physiol.* 273:1335–1340.

Widmaier, E. P., H. Raff, K. T. Strang. 2008. *Vander's Human Physiology: The Mechanisms Of Body Function*. Boston: McGraw-Hill Higher Education, 2008. Print.

Wittum, T. E., and L. J. Perino. 1995. Passive immune status at postpartum hour 24 and long-term health and performance of calves. *Am. J. Vet. Res.* 56: 1149-1154.