ANTIBODY MEDIATED IMMUNE RESPONSE AND CELLULAR MEDIATED IMMUNE RESPONSE CHARACTERIZATION IN BRAHMAN CATTLE

A Thesis

By

CHRISTIAN L COOK

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Chair of Committee,	Thomas H. Welsh, Jr.
Co-Chair of Committee,	Ronald D. Randel
Committee Members,	Luc R. Berghman
	David G. Riley
Head of Department,	G. Cliff Lamb

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ABSTRACT

The studies described herein were designed to characterize measures of immune responsiveness in weaned Brahman calves, breeding bulls and cows. Experiment 1 included 55 weaned bull and 57 weaned heifer Brahman calves. Experiment 2 included 84 sexually mature, non-pregnant Brahman cows, 33 cows in early stages of pregnancy (d1-97), 60 cows in mid-pregnancy (d98-194), 71 cows in late pregnancy (d195-292), and 25 fertile bulls. Antibody mediated immune response (AMIR) was determined by a vaccine specific IgG, enzyme linked immunosorbent assay (ELISA) in response to cattle receiving *Salmonella* Newport Extract vaccine. Cell mediated immune response (CMIR) was determined by a subcutaneous (neck) sensitization dose of $25x10^3$ protein nitrogen units (PNU) *Candida albicans* with 750 µg Quil-A adjuvant on day 0. On day 14 caudal skin fold thickness (SFT) was measured using Harpenden calipers prior to intradermal injection of $5x10^3$ PNU CA into the skin fold and on day 15 the injection site SFT was measured again. Response was determined by the difference in SFT from day 15 (post-injection) and day 14 (pre-injection).

In Experiment 1 with immature cattle, the CMIR was greater ($P < 0.05$) in bulls than heifers; however, AMIR did not differ between bulls and heifers. In weaned Brahman calves AMIR was not influenced by sex; however, there was sexual dimorphism associated with CMIR, in that bull calves had a greater response than heifers ($P < 0.05$).

In mature Brahman cattle, the mean CMIR was lowest in non-pregnant cows relative to pregnant cows (early, middle, and late) and bulls. Stage of pregnancy did not

affect CMIR nor did fertile bulls and pregnant cows differ in CMIR. Regarding AMIR, fertile bulls and non-pregnant cows did not differ. Although AMIR did not differ between non-pregnant and early pregnant cows, stage of pregnancy was a factor as AMIR was least in the middle and late pregnant cows. Physiological status, stage of pregnancy, and sex should be considered when evaluating either cellular or antibody mediated immune response in mature Brahman cattle. Selection of high immune responding animals could lead to improved health, productivity, and a decreased reliance on antimicrobials.

DEDICATION

Dedicated to Eric, Kody, Boomer, and Drake. Thank you for the support and constant motivation to be the best that I can be. I am excited for all the adventures the future holds.

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The experiments were designed by T.H. Welsh and R.D. Randel in consultation with C.L. Cook. Data collection and laboratory procedures conducted by C.L. Cook were supervised by T.H. Welsh, R.D. Randel, and W. Mwangi. Technical assistance with data collection and laboratory procedures were provided by J. Bray, D. Neuendorff, T. Garcia, and A. Lewis. Data analyses and interpretation were jointly accomplished by Cook, Welsh, Randel, Riley, and Berghman. The thesis was written by Cook 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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CHAPTER I

INTRODUCTION

Immune responsiveness may become a criterion for the selection of breeding bulls and cows because immune response has been associated with improvements in many production traits such as colostrum quality, vaccine-induced antibody production, average daily gain, feed efficiency, and herd health. Low immune responding animals can pose economic problems for the livestock industry due to increased costs to producers. These animals can have an increase in stress hormones which inhibit immune function as well as an overall increased susceptibility to disease and infections. Once ill, producers need to use antibiotics to treat these animals thus posing the concern about antimicrobials being in consumer products. However, high immune responding cattle tend to have increased production traits that would be beneficial to the beef industry. Their increased growth rates, disease resistance, and increased response to vaccination make them ideal breeding and production animals. Through an increased understanding of how to select for high immune responding animals, factors influencing immune response including sex, pregnancy, and physiological status, animal management practices can be modified to reduce the negative impacts of low immune responding animals.

CHAPTER II

LITERATURE REVIEW

IMMUNE SYSTEM

Immunity is defined as resistance to disease, specifically infectious disease (Abbas et al., 2014). All parts of the body that mediate resistance to disease comprise the immune system. Reactions by cells, tissues, and organs due to the presence of an infectious agent initiate an immune response. To keep an animal healthy, it is necessary for the immune system to be able to recognize many agents including: antigens, parasitic worms, bacteria, and viruses. This is of utmost importance as pathogens have the potential to rapidly adapt and mutate. The immune system is developed throughout fetal life and is partially functional by approximately 30 days of gestation. However, the immune system is not fully functional at birth and the newborn must rely on maternal antibodies transferred by consumption of colostrum and requires time to become fully functional. Beginning with jawed vertebrates, the immune system can be described as having two branches known as the innate and adaptive immune systems.

The innate immune system is the body's natural and immediate protection from pathogenic invaders. The adaptive part of the immune system develops slowly and over time; however, it provides stronger and more specific protection against pathogenic invaders. The adaptive immune system is comprised of humoral and cell-mediated immunity. The humoral aspect is mediated by antibodies which are produced by B lymphocytes. The cell mediated aspect of the adaptive immune system is meditated by T lymphocytes.

Immunity may be induced in an individual by infection, vaccination (active immunity), or by transfer of antibodies or lymphocytes from an actively immunized individual (passive immunity) (Abbas et al., 2014). Passive immunity is of vital importance to newborn animals whose immune systems are not mature enough to protect themselves against pathogens. Passive immunity can also be useful in providing immediate protection to unimmunized individuals; such as those who have a viral infection or are bitten by a poisonous animal. Immunologic memory is established when one is first exposed to an antigen and a primary immune response is initiated from naïve lymphocytes. When the individual is exposed to the same antigen later, secondary immune responses are initiated that provide faster, longer and better immune responses from memory B cells. Strength of an immune response depends on the size, stability, foreignness, and complexity of the antigen (Tizard, 2004). Larger, more complex molecules make better antigens and mount larger immune responses. Organs are more likely to be rejected if they are from a different species or a different blood group.

While the adaptive immune system gets stronger over time with repeated exposure, the innate immune system responds to an antigen the same way regardless of the number of times of exposure to the antigen. In the innate immune system, the epithelium provides protection between the body and the external environment. One of the principal reactions if the protective barriers fail is inflammation. Major cells recruited to sites of infection and inflammation include neutrophils and macrophages. Neutrophils are the most abundant leukocyte in blood and are stimulated by cytokines. They are the first cells to respond in the event of inflammation but do not provide long

term protection because they have a lifespan of only a few hours. Macrophages serve several important roles in host defense: they produce cytokines that induce and regulate inflammation, they ingest and destroy microbes, and they clear dead tissues and initiate the process of tissue repair (Abbas et al., 2014). Another important function of macrophages is their ability to secrete proteins and cytokines to control immune responses. These pro-inflammatory cytokines include IL-1 that turns on cells of the adaptive immune system and stimulates antibody production, IL-6 that promotes antibody formation and inflammatory responses that cause sickness, IL-12 that activates cell mediated immunity, and IL-18 that promotes interferon gamma (Tizard, 2004). The immune system is very complex and along with protecting the body from infection, the major cells also offer protection against cancer, tumors, and repair tissues.

ADAPTIVE IMMUNITY

The adaptive immune system provides a stronger and more specific response to antigens after being initiated by cells of the innate immune system. The major cells of the adaptive immune system are B cells and T cells. The B cells are derived from bursa in avian species and the bone marrow of others, while T cells are derived from the thymus. The T cells are part of the cell mediated aspect of adaptive immunity while B cells are part of the humoral response and produce antibodies. When the antibody mediated branch of the immune system mitigates a humoral response, pro-inflammatory reactions are initiated.

Species differences are responsible for some of the variability in antibodies and their abundance in serum, colostrum, and milk. Primates and rodents have hemochorial placentas, which enable transfer of immunoglobulin G (IgG) freely across the placenta. Dogs and cats have endotheliochorial placentas which allow some IgG to pass through but more than 90% of IgG is transferred in colostrum. In contrast, large farm animals such as horses and cattle do not absorb antibodies until after the animal is born and has ingested colostrum. These animals obtain immediate protection through colostrum by the passive transfer of antibodies from the dam. Failure of the animal to suckle colostrum within the first twelve hours postpartum can have devastating effects on the newborn including severe illness or even death. It is also imperative that the dam produces quality colostrum with high concentrations of antibodies to provide the newborn with significant protection from antigens (Tizard, 2004).

One of the antibodies produced by B cells that is of vital importance in all species is IgG because it generates long lasting immunity while also regulating antigens. In most mammals, IgG is the predominant immunoglobulin in colostrum and IgA is predominant in milk. In cattle IgG1 is the predominant immunoglobulin in milk. IgG is mostly derived from blood while IgA is locally synthesized in the mammary gland. This results in animals having high IgG concentrations at birth which decline and are catabolized as the animal ages and forms its own antibodies. Animals will also make specific antibodies in response to vaccination that will decline over time and require boosters. This happens when B cells recognize their target antigen in the spleen or lymph nodes and become activated, initiating a variety of reactions including germinal

center formation and class switch recombination resulting in the production of antigen-specific antibodies (Wardemann et al., 2007).

The presence of maternal antibodies plays a major role in when to vaccinate as maternal antibodies prevent newborns from mounting their own specific immune responses. As the newborn matures and passive immunity from maternal sources wanes, the ability of the animal to respond to a vaccine increases. The ideal timing for vaccination can be determined by measuring the antibody titers of the mother: the higher the titer the longer vaccination should be delayed (Tizard, 2004). Other factors such as age at vaccination, route of administration, presence or absence of a vaccine adjuvant, and type of vaccine could affect immune responses of animals vaccinated when maternal antibodies are present (Chamorro et al., 2016). Adjuvants are substances that when combined with specific vaccine antigens accelerate and enhance the immune response. Influence of these factors means that even animals vaccinated at their universally accepted age could not be receiving optimum protection.

Temperament is also under evaluation for its role in immunity and performance traits in cattle. Voisinet et al. (1997) found that Brahman cattle with an excitable temperament were negatively affected while in the feedlot as shown by decreased average daily gains. Oliphint et al. (2006) and Duff and Galyean (2007) evaluated the effects of vaccination on temperamental calves and determined that antibody responses were decreased 3-fold for temperamental calves and they also had a decreased average daily gain. Arthington et al. (2013) evaluated the effects of vaccination on performance of beef calves and determined that within two weeks post vaccination beef calves have

decreased average daily gain and feed efficiency due to an acute phase protein response. Acute phase protein responses have systemic and local effects and are mainly synthesized in the liver. Once acute phase proteins are secreted inflammation can occur emphasizing the importance of high immune responding cattle that require less antimicrobials and vaccinations.

Selection for cattle with high antibody mediated immune responses has been evaluated in several dairy cattle experiments. In a study by Wagter et al. in 2003 it was shown that selection for high immune responding cattle by antibody mediated immune response (AMIR) could be beneficial to herd life by maintaining optimal milk yield, yet minimizing the occurrences of diseases such as mastitis in Holstein cattle. This study was constructed using data from a previous experiment by Mallard et al. in 1997 where it was determined that AMIR could be measured in cows, that cows with high AMIR had the greatest responses when immunized, and cows with high AMIR had the lowest instances of diseases. Antibody responses were evaluated before and after parturition and showed that not every cow had a depressed antibody response peripartum. This information also suggested that cows with high antibody responses would have higher concentrations of antibodies in colostrum (Wagter et al., 2003). This emphasizes the importance of having good vaccination protocols for pregnant cattle to ensure optimal passive transfer of antibodies to the calf as well as the benefits of selecting for cattle based on their AMIR.

Purebred Holstein cattle have seen increased unwanted instances of inbreeding and disease due to the intensive genetic selection focused on increased milk yield. A

study by Thomson-Crispi et al. in 2012 evaluated the genetic parameters of adaptive immune response traits in Holstein cattle in Canada. It was found that some immune traits were moderately heritable, do not have negative effects on production traits, and that cattle can be selected to have high AMIR (Thomson-Crispi et al., 2012). Heritability is defined as the proportion of total variation between individuals in a given population due to genetic variation (Wray and Visscher, 2008). Heritability can range from 0 (no genetic contribution) to 1 (all differences on a trait reflect genetic variation). The heritability of AMIR has been studied in several species including mice, chickens, and pigs. In mice AMIR was found to have a heritability between 0.18-0.36 when the mice were immunized with sheep erythrocytes after being genetically selected (Feingold et al., 1976). Feingold et al. (1976) calculated heritability as $h^2 = R/S$ with S being the selection differential and R being the response to the selection. When selected for high AMIR, chickens had a heritability of 0.61 when selecting for high IgM and 0.52 for high IgG. The female chickens had higher serum concentrations and antibody titers than the males (Sarker et al., 1999). This suggests that maternal influence is important for AMIR selection which has been reported in studies in humans and mice. It is likely that females have a greater immune response than males because of the stress that reproduction and raising offspring has on the immune system (Grossman et al., 1989).

In Yorkshire pigs, selection for high AMIR led to an increase in production traits important to the swine industry such as increased weight gain and generation of more antibodies in response to vaccination (Wilkie et al., 1999). It is important from a producer standpoint that selection for high AMIR does not have negative effects on other

areas of production. As crossbreeding becomes more prevalent especially in cattle, it has become important to see if immune response is affected. A study in crossbred calves showed that crossbred calves had greater primary antibody responses to several antigens when compared with purebred calves (Cartwright et al., 2011). Cartwright et al. (2011) used 140 purebred Canadian Holstein and 142 crossbred (Canadian Holstein x Norwegian Red) calves at an average age of four months. *Candida albicans* was used to induce a type 1, cell mediated immune response (CMIR) and hen egg white lysozyme (HEWL) was used to induce a type 2, antibody mediated immune response (AMIR). CMIR was determined by taking the average of triplicate skin fold measurements that were taken on days 21 and 23. AMIR was determined by ELISA run on sera samples obtained on days 0, 14, and 21 (Cartwright et al., 2011). The optical density values of samples were corrected to positive controls that were on each plate to compare samples run on different plates and different days (Cartwright et al., 2011). These results suggest that crossbreeding could have various benefits to a cattle operation including reduction of disease and need for antibiotics due to crossbred calves having higher AMIR and CMIR responses.

Lymphocyte response has been used to determine if cell mediated immunity (CMIR) influenced the length and severity of infections. In a study by Davies et al. (1973) cattle were infected with infectious bovine rhinotracheitis and treated three months later with a synthetic corticosteroid. The results of this study showed that CMIR was increased toward the end of virus recovery and resolution of clinical signs. This demonstrates the significance of the cell mediated aspect of the immune system and its

importance in viral infections. The antibody mediated aspect of the immune system would not work as efficiently without the aid of T cells directly targeting or killing cells infected with viruses and activating B cells to secrete antibodies and macrophages to destroy microbial invaders.

The effect that stress and behavior have on CMIR has also been under evaluation. Hessing et al. (1995) considered differences in cell mediated immunity in pigs based on their behavior. The pigs were labeled as aggressive or non-aggressive determined by their interactions with other pigs and as resistant or nonresistant when restrained on their back. The results of this study showed that aggressive and resistant pigs had an increased CMIR when evaluated in vivo and in vitro using specific and nonspecific antigens. This experiment also showed that when stressed, the aggressive and resistant pigs had a decreased CMIR when compared to the non-aggressive and non-resistant pigs. This suggests that stress can have major effects on the immune status of an animal and put them at an increased risk of developing disease. A major cause of stress in production animals is weaning. Earlier weaning days may be profitable for producers from the standpoint of a smaller postpartum interval and earlier time to market, but it can come at an immunological cost. It was shown that pigs that were weaned before they were five weeks old had suppressed CMIR both in vivo and in vitro (Blecha et al., 1983). This emphasizes the importance of reducing stress on young animals and providing them ample time to develop their immune system before introducing many stressors. The benefits of a stronger immune system could result in

improved feed efficiency, improved vaccination response, and less instances of illness and disease.

INNATE IMMUNITY

The innate immune system protects the body from infection by activating the adaptive immune system and attacking foreign molecules. The innate system can protect the body from attacking foreign molecules by physical barriers including skin, the gut, and mucosal tissues. Cells of the innate immune system such as neutrophils, natural killer cells, macrophages, and dendritic cells also aid in protection of the body. The innate immune system triggers a nonspecific response that usually results in inflammation around the site of the pathogen. The cells of the innate system can recognize and eliminate pathogens by using pattern recognition receptors (PRRs) that are able to identify pathogen associated molecular patterns (PAMPs). Macrophages and neutrophils initiate the inflammatory response of the immune system by activating proinflammatory cytokines (TNF-alpha, IL-1, and IL-6). Neutrophils are eventually removed from the site of inflammation because they undergo apoptosis and the macrophages phagocytize the neutrophils. To promote wound healing after inflammation the body initiates acute phase responses (APR) that can result in physiological responses such as fever, along with pain and swelling at the inflammation site.

An increase in asthma and allergies has led many to believe the hygiene hypothesis that states that decreases in immunity are a result of increased cleanliness and

hygienic techniques. This is believed to have caused an overall decrease in the innate immunity of individuals. A study of Amish children found that Amish children have less instances of asthma and allergies (Stein et al., 2016). These children had their IgE concentrations, cytokine responses, gene expression, and blood leukocytes measured to determine the strength of their innate immune response. It was concluded that the lifestyle of the Amish and their children's exposures to farming practices, animals, and dust was able to strengthen their immune response from birth.

Bovine viral diarrhea virus (BVDV) and bovine herpesvirus 1 (BHV-1) require a robust innate immune system to provide protection from viral and bacterial pathogens. Stressors such as weaning, transportation, change in social groups, climate and environmental changes, as well as a low body condition can put calves at a higher risk to contract disease (Babcock et al., 2013; Duff and Galyean, 2007). Cattle with compromised immune systems will also not eat as much, resulting in reduced weight gains and decreased carcass quality (Galyean, 1999). Th17 cells are important to the innate immune system due to their recruitment of neutrophils and macrophages to infection sites. Th17 is considered pro-inflammatory and produces IL-17A, IL-17F, and IL-23 to provide protection to mucosal surfaces such as the bovine trachea (Caswell, 2013). This suggests that if the innate immune system of cattle produces a Th1 (response against intracellular parasites, bacteria, or viruses) or Th2 (response against extracellular parasites) response over a Th17 response to fight respiratory infection it will be more susceptible to respiratory disease (Caswell, 2013).

HIGH IMMUNE RESPONDERS

The selection for ‘general’ immune responsiveness is an idea that was first investigated in pigs by Wilkie and Mallard in 1999. Their approach involved identification and selection of animals with an enhanced general immune response, that was assessed by combining measures of the animal's antibody- and cell-mediated adaptive immune responses. This was a move from the previous focus that had involved selecting animals that merely had immunity from one kind of disease or illness. The impact that selecting animals with immunity against a multitude of diseases could be useful in decreasing the use of antibiotics in consumable animals. This would be of vital importance because of the link between antibiotic use on the farm and the increase in antibiotic resistance in pathogens affecting humans. Levy et al. (1976) conducted a study where animals and workers were observed after a tetracycline-supplemented feed was introduced. It took only two weeks for the animals’ gut microbiota to almost all become tetracycline resistant and in six months the workers stool contained more than eighty percent tetracycline resistant bacteria (compared to just seven percent in non-exposed workers). The other interesting finding was that when tetracycline-supplemented feed was removed, the workers’ tetracycline resistant bacteria were almost eliminated from their intestinal tract within a six-month period. A discussion paper by Spellberg et al. (2016) summed up this situation with two facts: adding antibiotics to animals’ feed and water contributed to the spread of antibiotic-resistant bacteria to human beings; and many parties promote the routine use of therapeutic antibiotics in livestock specifically because they perceive (possibly incorrectly) that it enables the

meat, poultry, and drug industries to maximize production and profits. This suggests that there needs to be a move away from such antibiotic reliance and that genetic selection might be the answer.

Inclusion of traits such as AMIR and CMIR into selection indexes have the potential to aid the industry in various ways besides improved immunity and less dependence on antibiotics. Genetic approaches have been shown to work well in combination with other preventive approaches, including vaccination, and may in fact enhance other traits, such as reproduction, feed efficiency and growth (Wilkie and Mallard, 1999; Wagter et al., 2003; Mallard and Wilkie, 2007; Mallard et al., 2014; Aleri et al., 2015). Early studies in pigs noted that high immune responding pigs consistently reached market weight of 100 kg ten to twelve days before low immune responders (Mallard and Wilkie, 2007). Another recent study of Australian Holstein heifer calves showed that high immune responding calves had greater average daily weight gains than low responders (Aleri et al., 2015). These studies show the great impact that the immune response has on production traits. If focus for genetic selection was based on AMIR and CMIR there could potentially be improvements in other important areas such as growth for market. In dairy cattle, utilizing this approach resulted in reduced mastitis in high immune responders, as well as improved response to vaccination and colostrum quality (Wagter et al., 2000; Thompson-Crispi et al., 2012b; ThompsonCrispi et al., 2013). A similar response to what was seen in dairy cattle would be important to the beef industry. An improved response to vaccination could lead to less of a reliance and need for booster vaccinations as well as decreases in illnesses. Improved colostrum

quality would also be important because it would allow newborn calves to receive more IgG in their mother's colostrum. Receiving more antibodies from the colostrum would be another way to potentially improve herds by having more high immune responding individuals during the critical neonatal period.

Active immunity stimulated by vaccination has been another area of study. In 2014, a study was done to determine whether vaccinating cows before parturition with commercially available *Salmonella* bacterial extract would produce *Salmonella*-specific IgG antibodies in the colostrum and transfer the colostral antibodies to the calf (Smith et al., 2014). In this study, thirty Holstein cows were vaccinated with *Salmonella enterica* serovar Newport bacterial extract and received a booster vaccination four weeks later. Another thirty cows received only saline. The fifty-nine calves were fed fresh colostrum from their dams within four hours post-partum and had blood samples taken twenty four hours later (Smith et al., 2014). The results of this study showed that vaccinated cattle had higher *Salmonella* antibody titers at calving ($P = 0.01$) and in their colostrum ($P = 0.011$). The calves that received colostrum from a vaccinated dam had increased *Salmonella* antibodies (1.04 ± 0.03) when compared to the calves that were born to unvaccinated dams (0.30 ± 0.02) (Smith et al., 2014). The results of this study indicated that *Salmonella* vaccine can stimulate antibodies and that these IgGs can be passed to the calf via milk. However, there are still more studies that need to be done regarding immune response including if increased antibodies will provide benefits to the animal when met with a bacterial challenge.

The heritability of high immune responders has also been shown in several studies. Mallard et al. (2014) reported that daughters of high immune responding sires were at a lower risk for disease as well as more productive than daughters of low immune responding sires. The knowledge that a high immune responding sire will pass on his genes is important from a producer standpoint because it means that the dam does not necessarily have to be a high immune responder to have offspring, chiefly female, with those traits. This suggests AMIR and CMIR selection does not seem to have adverse effects on production or reproduction, which enhances the benefits of utilizing it as a selection tool. A study on the heritability of immune response was also performed in lactating Holsteins in Canada (Thompson-Crispi et al., 2012). In this study the objectives were to estimate genetic parameters of cell-mediated (CMIR) and antibody-mediated immune response (AMIR) as well as the combined immune response (IR) traits of dairy cattle on a national scale and to associate estimated breeding values of CMIR, AMIR, and overall IR with routinely evaluated traits in Canada. The cattle in this study were observed with a delayed-type hypersensitivity to *Candida albicans*, a type-1 test antigen, as an indicator of CMIR (Hernandez et al., 2005). Primary and secondary immune responses to a type-2 test antigen, hen egg white lysozyme (HEWL), was used as an indicator of AMIR (Heriazon et al., 2009b). The results of this study showed that mean AMIR response was higher at day 21 than day 14 for both IgG1 and IgG2. This showed the immunization regimen was efficacious, and the immunizations induced measurable primary and secondary antibody responses (Thompson-Crispi et al., 2012). The results of this study along with several others have shown that CMIR and

AMIR are low to moderately heritable (Thompson-Crispi et al., 2012; Abdel-Azim et al., 2005; Wagter et al., 2000; Hernández et al., 2006). Heritability of the secondary antibody response (day 21) was higher than primary (day 14) for both IgG1 (0.34 vs 0.29) and IgG2 (0.41 vs 0.16). The genetic correlation between CMIR and AMIR was negative as observed by Thompson-Crispi et al. (2012). A negative correlation between CMIR and AMIR has been observed in several other species including mice and chickens, emphasizing the importance of selecting cattle for both traits to provide broad-based disease resistance to a multitude of organisms (Thompson-Crispi et al., 2012; Biozzi et al. 1979; Mouton et al., 1984; Sarker et al., 2000).

High immune responding dairy cattle have previously been associated with decreased occurrence of disease and improved response to vaccination. The low to moderate heritability of these traits suggests that it would be possible to breed cattle for improved immunity to minimize the impact of disease and improve overall health. Both the innate and adaptive aspects of the immune system play direct roles in an animal's protection against disease and effectiveness of vaccination so it is important to evaluate both when determining an overall high immune responding animal. With AMIR and CMIR being heritable and having positive effects on production traits it is of vital importance to investigate utilizing AMIR and CMIR as selection tools for breeding animals.

IMMUNE RESPONSE DURING PREGNANCY

Additional research is needed to better understand alterations in the immune system during pregnancy. One area of discussion has been on whether pregnancy causes a state of immunosuppression and susceptibility to disease. The presence of natural killer cells, dendritic cells, and macrophages at the implantation site facilitate and protect the fetus and mother during pregnancy, showing that the immune system at the implantation site is not suppressed, but instead is active, functional and regulated (Mor et al., 2010; Shimada et al., 2006; Ashkar et al., 2000). While pregnancy does make changes in the body it does not appear to be immunosuppressive but rather protective of the fetus. With the fetus being considered a semi-allograft it has been studied why the mother does not reject it as a foreign body. Down regulation of Th1, elimination of activated T cells, and the placenta's production of IL-4, IL-10, prostaglandin, and progesterone help to facilitate acceptance of the fetus by the mother (Roth et al., 1996; Hilkens et al., 1995; Szekeres-Bartho et al., 1996; Holt et al., 2000; Guller et al., 1999; Hammer et al., 1999).

It was previously thought that pregnancy put the mother's immune system into an anti-inflammatory state, but it has since been found that there is variation based on the stage of pregnancy (Mor, 2006, 2007). While the first trimester is considered pro-inflammatory, the second trimester is anti-inflammatory as the fetus undergoes rapid growth and development (Romero et al., 2006). As the mother nears the end of pregnancy the immune system reverts back to a pro-inflammatory response because parturition requires an influx of immune cells to the myometrium (Romero et al., 2006).

There is also evidence to support that allergy sensitivity and innate immunity can be developed and influenced during pregnancy, as children born to mothers that were very active in farming were less likely to develop allergies than those from mothers who were not actively involved in farming activities. It has been noted that people in rural areas are more exposed to endotoxins than those in urban areas. These endotoxins are linked to factors that are associated with counteracting allergies which stimulates the secretions of IL-12 and interferon gamma. This could help explain why there is a lower frequency of asthma, hay fever, and atopic sensitization in children growing up on a farm (Riedler et al., 2001). Another interesting discovery is that children that are exposed to farm animals and pets that do not live on a farm are also less likely to develop allergies or asthma. This is promising that there are ways that we can expose children to necessary pathogens and can improve the immune system responses even before birth.

In cattle, there is limited information on how immune status changes throughout pregnancy. However, a study by Hine et al. (2011) evaluated the immune responses of twenty Canadian Holstein cows in early pregnancy (< 100 days) and twenty-three cows in mid pregnancy (100-200 days). The results of this study showed that pregnancy status did not influence AMIR but age did, with younger cows having a reduced response (Hine et al., 2011). When examining CMIR, results showed that cows in early pregnancy had an increased CMIR which increased as the cows got older. A decrease in lymphocytes and neutrophils was found immediately after calving but an increase

directly prior to calving has been reported in a few cattle studies (Saad et al., 1988 and Nagahata et al., 1992).

The influence that short term energy restriction in late gestation of beef cows has on their calf's humoral immune response to vaccination was evaluated by Moriel et al. (2016). While short term energy restriction did not have negative effects on growth, serum IgG concentrations, or plasma cortisol concentrations, calves born to these dams did experience suppressed vaccination responses shown by decreased titers to BVDV-1 than those born to energy unrestricted dams (Moriel et al. 2016). This emphasizes the importance of maintaining energy intake throughout pregnancy and that the immune system can be primed throughout gestation.

Because of the importance of a robust immune system, these experiments sought to investigate selection tools to determine high immune responding individuals. Specifically, these studies were designed to determine factors influencing antibody and cellular mediated immune responses in Brahman cattle. The goals of this study were to:

1. Examine the influence of temperament traits on antibody and cellular mediated immunity in weaned Brahman calves;
2. Characterize antibody and cellular mediated responses in heifer calves compared to bull calves;
3. Examine the influence of sex on antibody and cellular mediated immune responses in mature Brahman cattle; and,
4. Examine the effects of pregnancy status on antibody and cellular mediated immune responses in Brahman cows.

CHAPTER III

FACTORS AFFECTING ANTIBODY MEDIATED IMMUNE RESPONSE (AMIR) AND CELLULAR MEDIATED IMMUNE RESPONSE (CMIR) IN WEANED BRAHMAN CALVES

INTRODUCTION

Immune function could be a tool to select healthier cattle. The immune system is divided into two categories: the innate immune system and the adaptive immune system. Innate immunity is the body's nonspecific defense mechanism triggered when an antigen appears, while adaptive immunity utilizes specific antigen recognition systems and creates immunological memory after initial exposure. Cell mediated immunity includes activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of cytokines in response to an antigen. Antibody mediated immunity utilizes the production of antibodies by B-lymphocytes to bind to specific antigens.

High immune responders have been reported to have increased feed efficiency and average daily gains in cattle and swine (Aleri et al., 2015 and ThompsonCrispi et al., 2013). High immune responders also have improved response to vaccination, higher colostrum quality, and increased disease resistance in cows (ThompsonCrispi et al., 2013). Aleri et al. in 2015 found that animals with high CMIR and AMIR tended to have significantly higher cortisol concentrations suggesting that high immune responding animals have a higher stress response or a higher basal serum concentration. However, cortisol concentrations in the same group of cattle were lower during the

second testing period suggesting that handling stress influences cortisol concentrations. Several studies have shown that cortisol concentration can vary, with mild stressful conditions such as exercise and handling enhancing the immune system and its response, while long term and chronic stressors suppress the immune response (Hines et al., 1996).

Temperament has also been evaluated regarding the immune system and is defined as the reactivity, or fear response, to humans (Fordyce et al., 1988). Temperament has been shown to have effects on the immune system, cortisol concentrations, carcass quality and average daily gain of cattle. Fell et al. in 1999 found that calves with nervous temperaments had higher cortisol concentrations at weaning, lower average daily gains, and increased morbidity when compared with calmer calves. Serum concentrations of IgM were also lower in the calmer calves (Fell et al. 1999).

The objective of this study was to determine if sex, body weight, body condition score, or weaning temperament influenced the AMIR and CMIR of weaned Brahman calves. AMIR evaluation was determined by response to *Salmonella* Newport extract vaccine as it was a novel vaccine to the herd. To evaluate CMIR *C. albicans* with Quil-A was used as an adjuvant because of its effectiveness in inducing an inflammatory response (Cartwright et al., 2012). Caudal tail fold thickness following a local cellular immune challenge was used because it is a quantitative characteristic for analyzing CMIR (Hernandez et al., 2005).

MATERIALS AND METHODS

Experimental Design

All experimental procedures were in compliance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching and approved by the Institutional Animal Care and Use Committee of Texas A&M AgriLife Research. Fifty-five bull calves and fifty-seven heifer calves (264 d of age) from the Texas A&M AgriLife Research Center's purebred Brahman herd in Overton, TX were selected for use in this study. Temperament score (Curley et al., 2006a; King et al., 2006) was an average of exit velocity (EV) and pen score (PS). Exit velocity is an objective measurement that records the rate (m/s) at which cattle exit a working chute (Burrow et al., 1988; Curley et al., 2006a). Pen score (Hammond et al., 1996) is a subjective measurement in which cattle are separated into small groups of three to five and their reactivity to a human observer scored on a scale of 1 (calm, docile, approachable) to 5 (aggressive, volatile, crazy).

On day 0 whole blood samples (2 x 10 mL) were collected via jugular venipuncture in VACUTAINER® tubes (BD, Franklin Lakes, NJ) and serum isolated for determination of cortisol and IgG concentrations before receiving a subcutaneous (neck) sensitization dose of $25x10^3$ protein nitrogen units (PNU) *Candida albicans* (CA; Greer Labs, Lenoir) with 750 µg Quil-A adjuvant (InvivoGen, San Diego). Body weight and body condition score were recorded prior to injection. On day 14 caudal skin fold thickness (SFT) was measured using Harpenden calipers prior to intradermal injection of $5x10^3$ PNU CA into the skin fold. On day 15 injection site SFT was measured and blood

serum samples were collected. Response was determined by the difference in SFT from day 15 (post-injection) and day 14 (pre-injection).

Cortisol

Serum concentrations of cortisol were determined using a single antibody radioimmunoassay (DSL-2100; Diagnostic Systems Labs, Webster, TX) utilizing rabbit anti-cortisol antiserum coated tubes according to the manufacturer's directions.

Evaluation of AMIR

Sera were obtained from blood samples collected on day 0 and day 15 that were allowed to clot overnight at 4^0C before centrifugation for 30 minutes at 3000 rpm. Harvested serum samples were stored at -20^0C until analyzed for vaccine specific IgG by a double sandwich, enzyme linked immunosorbent assay. Ninety-six well plates were coated with *Salmonella* Newport Extract vaccine (Zoetis, Florham Park, NJ) dissolved in carbonate buffer 1:4 dilution and all plates were blocked with 3% Tween 20 in PBS. Sera samples and controls, were diluted to 1:700. Positive and negative controls were obtained by pooling sera samples pre-immunization (negative control) and sera samples obtained on d 15 (positive control). All controls and samples were added to the plate in triplicate, allowing 15 animals (day 0 and day 15) to be run per plate. Sheep anti-bovine IgG was used at the secondary antibody and was diluted to 1:8000. Absorbance was read at 450 nm using an automated microplate reader. To calculate AMIR the following equation was used: (Day 15 average / Positive control average) – (Day 0 average /

Positive control average). Calves were then categorized into the following classes: Low- Mean minus ½ SD; Intermediate- Within ½ SD of the mean; High- Mean plus ½ SD.

Evaluation of CMIR

Response was determined by the difference in SFT from day 15 (post-injection) and day 14 (pre-injection). Response class is divided into low, intermediate, and high determined by ½ standard deviation from SFT response means within sex.

Statistical Analysis

Data were analyzed using the general linear model procedure of SAS (SAS Inst., Inc., Cary, NC) to evaluate the effects of calf sex, sire, weaning temperament and their interactions on CMIR, CMIR class, AMIR and AMIR class. Specific comparisons were made using Fisher's Protected Least Significant Difference, with $P < 0.05$ considered significant. Pearson's correlation coefficients were also calculated for the following variables: weight, temperament, AMIR response class, and CMIR response class.

RESULTS

Cortisol

There were no significant effects of cortisol on AMIR or CMIR, temperament, growth traits, or response classes ($P > 0.05$).

AMIR

Body weight was greater in bulls than in heifers on day 0 ($P < 0.01$) (Fig. 1) and day 15 ($P < 0.001$) (Fig. 2). Both Pen Score and Temperament Score were greater in heifers than bulls ($P < 0.001$) (Fig. 3). The AMIR was not different between bulls and heifers ($P > 0.8$) (Fig. 4). In response class data (High, Intermediate, Low) there were no significant effects on growth or temperament traits regarding AMIR (Fig. 5-8).

CMIR

The CMIR was greater in bulls compared to heifers ($P < 0.02$) (Fig. 9). Growth and temperament traits did not differ ($P = 0.5$) among CMIR classes (Fig. 10-13).

There was no correlation between AMIR or CMIR among each other or among temperament or growth traits, nor within response classes ($P > 0.05$). Sire variance was > 0.

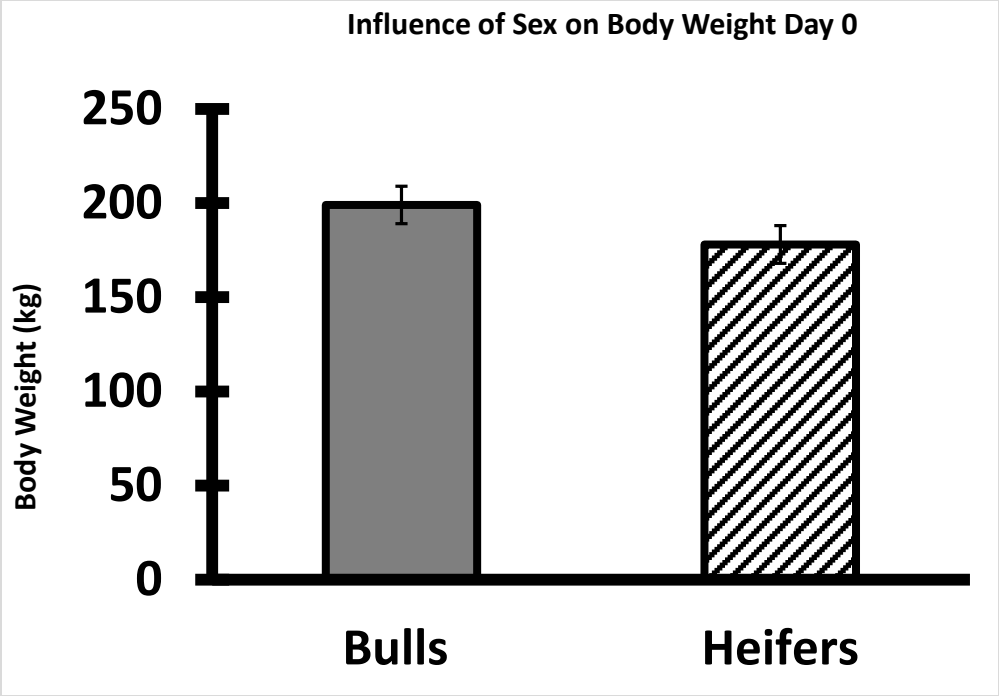

Figure 1. Influence of sex on body weight of weaned Brahman bull and heifer calves on day 0. Weaned bull calves had greater body weights on day 0 than the heifer calves ($P < 0.01$; SEM = ± 10).

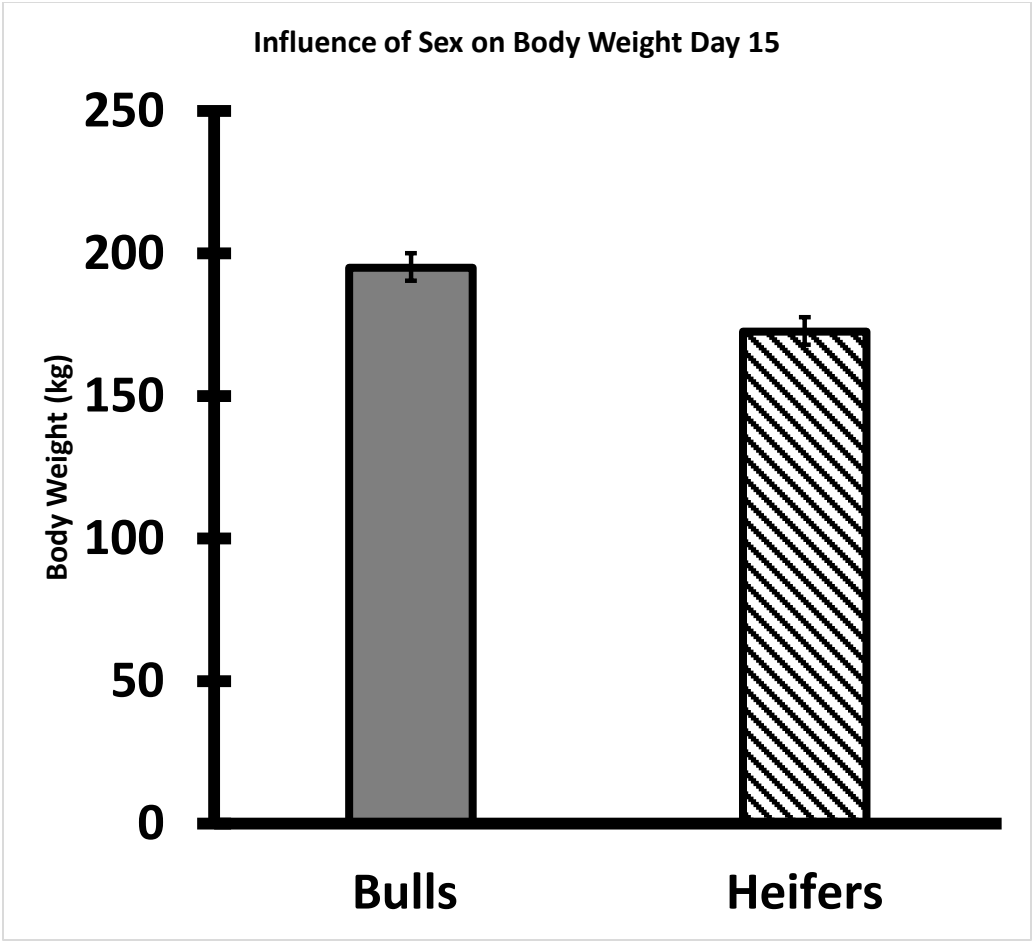

Figure 2. Influence of sex on body weight of weaned Brahman bull and heifer calves on day 15. Weaned bull calves had greater body weights on day 15 than the heifer calves ($P < 0.001$; SEM ±5)

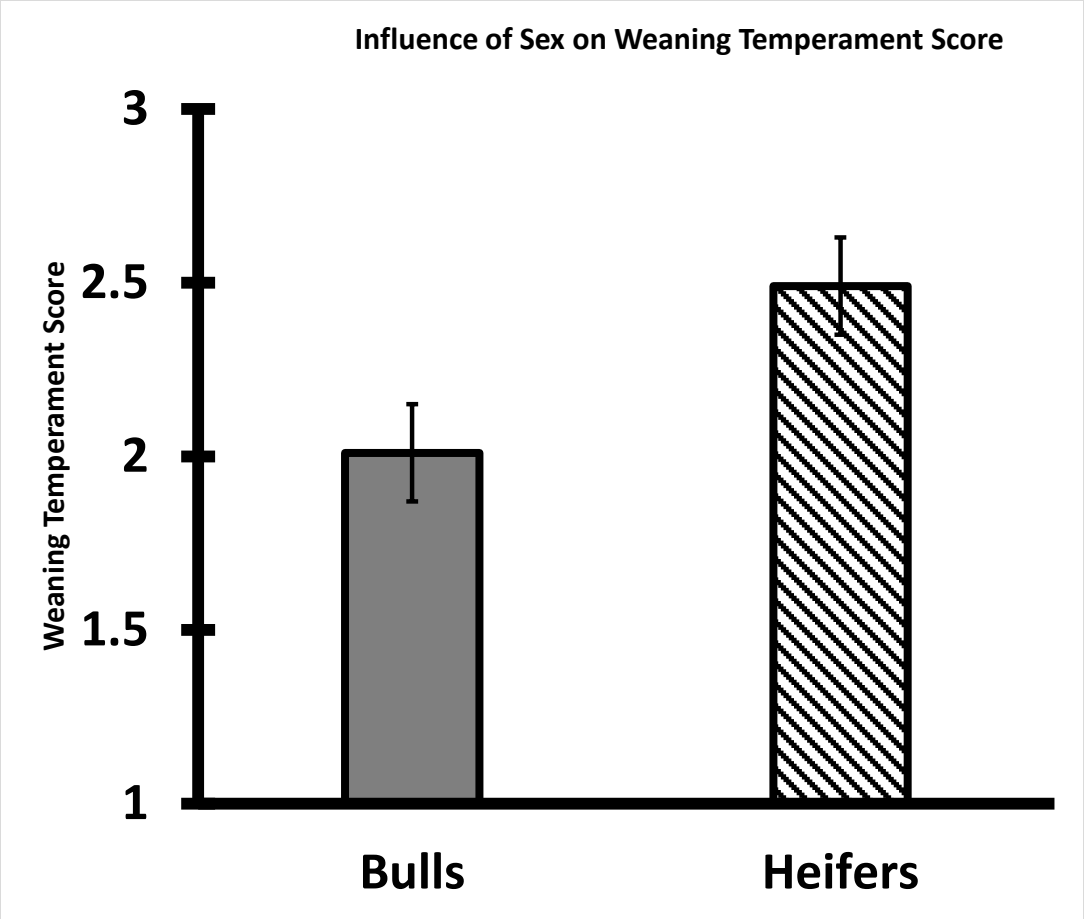

Figure 3. Influence of sex on weaning temperament score in weaned Brahman bull and heifer calves. Heifer calves had a higher weaning temperament score than the bull calves ($P < 0.01$; SEM = ± .14).

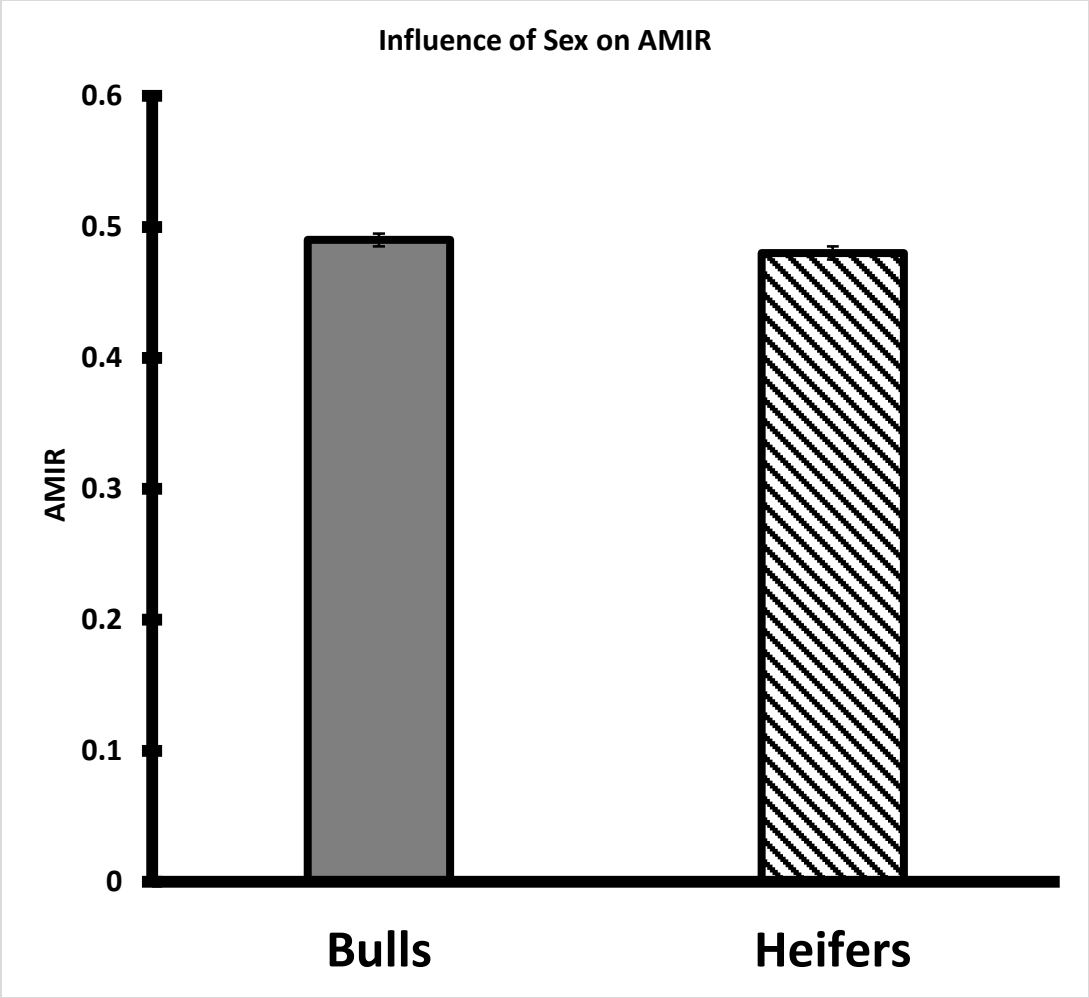

Figure 4. Influence of sex on antibody mediated immune response (AMIR) in weaned Brahman calves. AMIR was not different between bull and heifer calves ($P > 0.05$; SEM = ± 0.05)

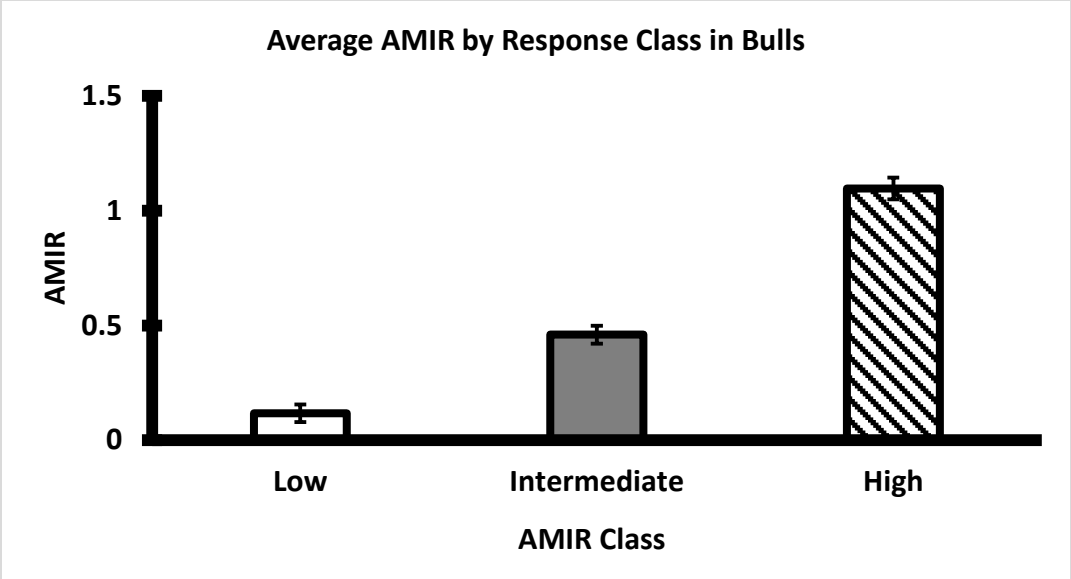

Figure 5. Average AMIR of weaned Brahman bull calves by AMIR response class (n = 22 Low, 17 Intermediate and 16 High bulls). Error bars represent +/- ½ SD from the mean.

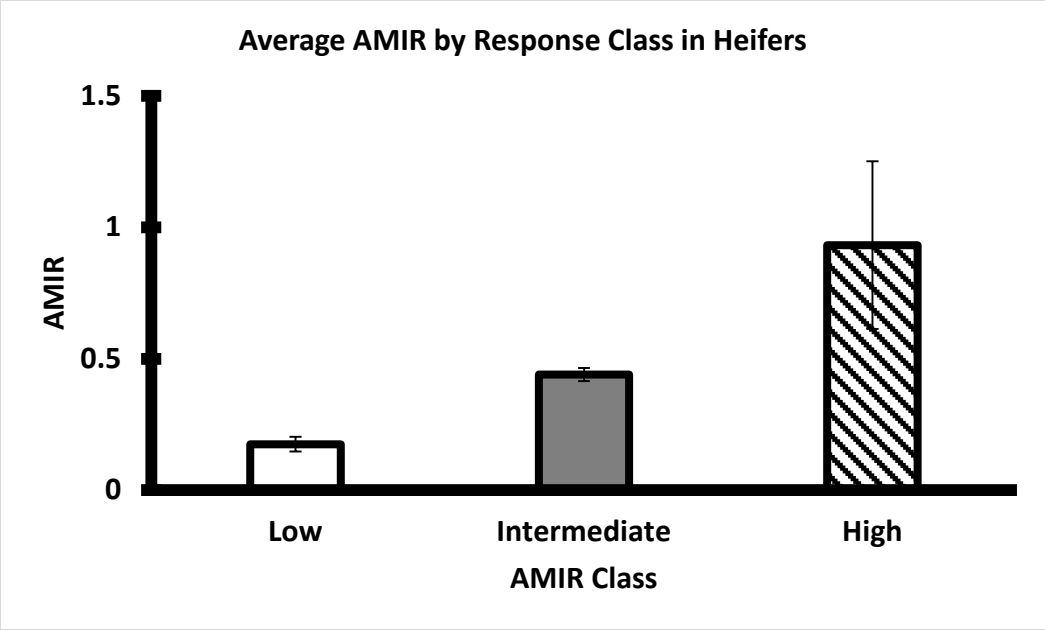

Figure 6. Average AMIR of weaned Brahman heifer calves by AMIR response class (n = 17 Low, 26 Intermediate and 14 High heifers). Error bars represent +/- ½ SD from the mean.

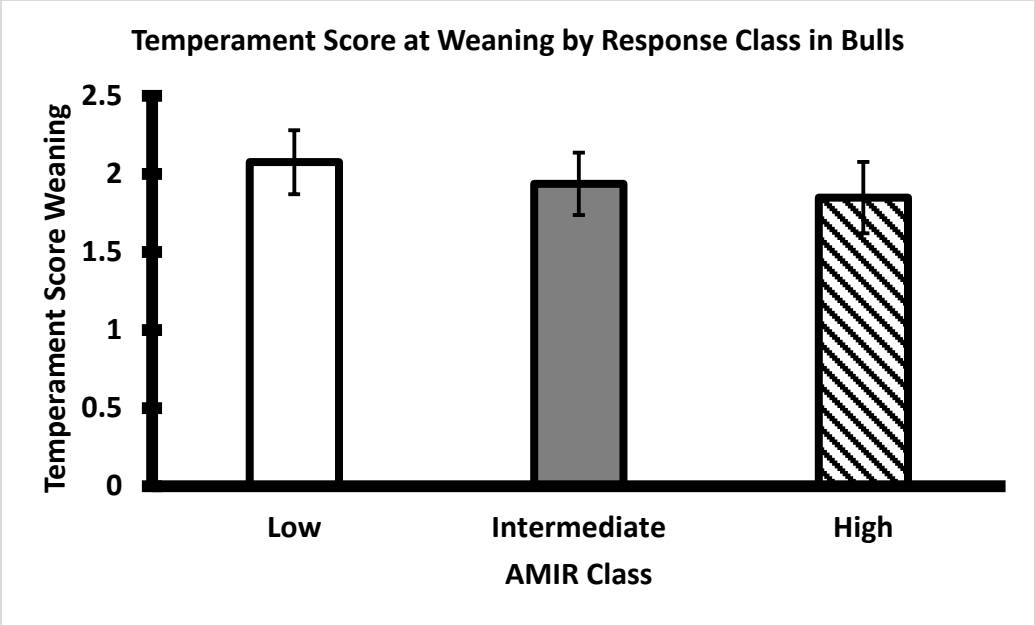

Figure 7. Average temperament score by AMIR response class of weaned Brahman bull calves (n = 22 Low, 17 Intermediate and 16 High bulls). P > 0.05

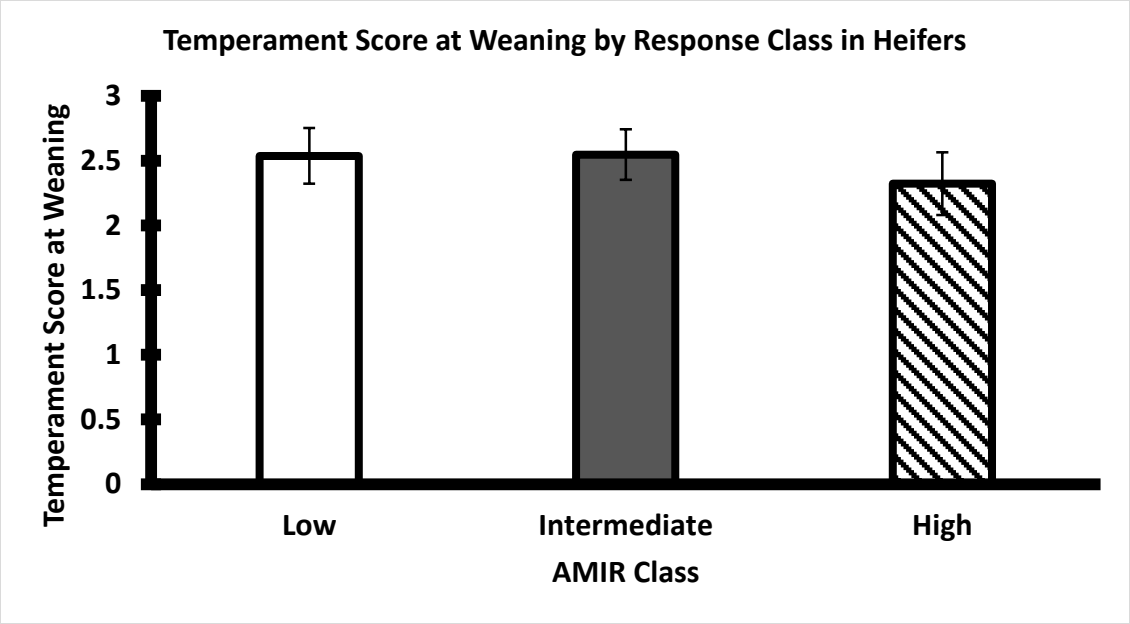

Figure 8. Average temperament score of weaned Brahman heifer calves by AMIR response class (n = 17 Low, 26 Intermediate and 14 High heifers; $P > 0.05$).

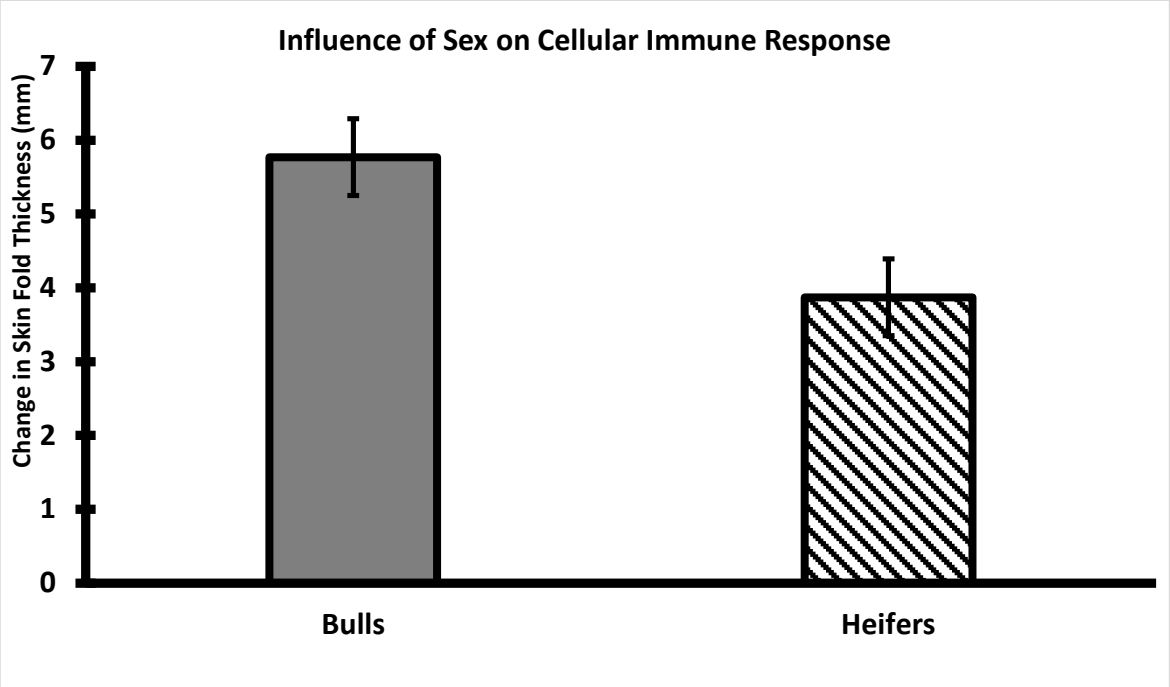

Figure 9. The influence of sex on cell mediated immune response (CMIR) in weaned Brahman calves. CMIR was greater in bulls than in heifer calves ($P < 0.05$).

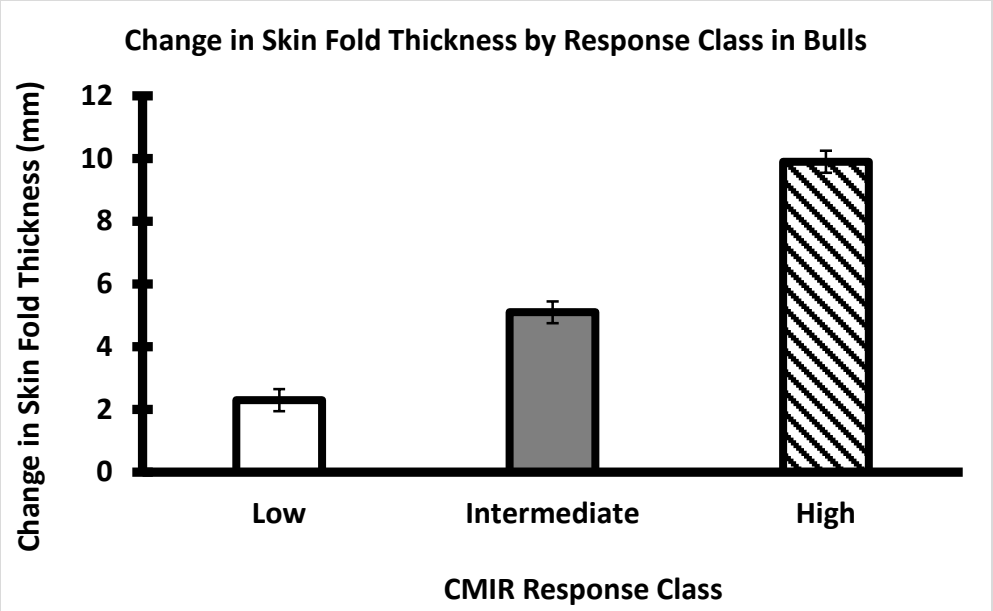

Figure 10. Average CMIR of weaned Brahman bull calves by CMIR response class (n = 19 Low, 19 Intermediate and 17 High bulls). Error bars represent +/- ½ SD from the mean.

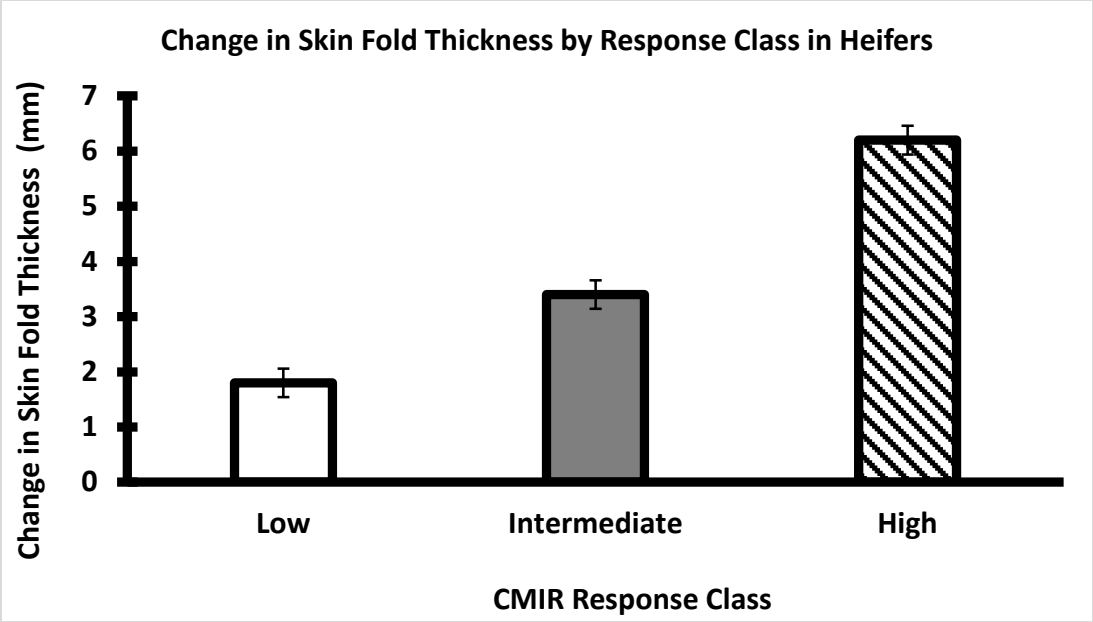

Figure 11. Average CMIR of weaned Brahman heifer calves by CMIR response class (n = 20 Low, 16 Intermediate and 21 High heifers). Error bars represent +/- ½ SD from the mean.

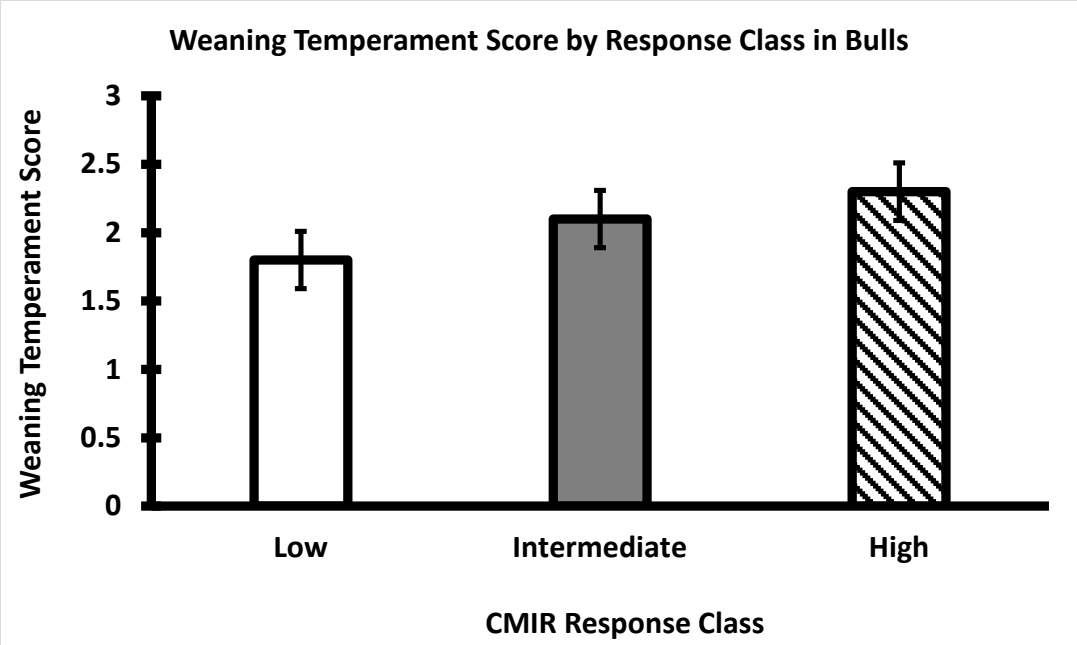

Figure 12. Average temperament score of weaned Brahman bull calves by CMIR response class (n = 19 Low, 19 Intermediate and 17 High bulls). $P > 0.05$.

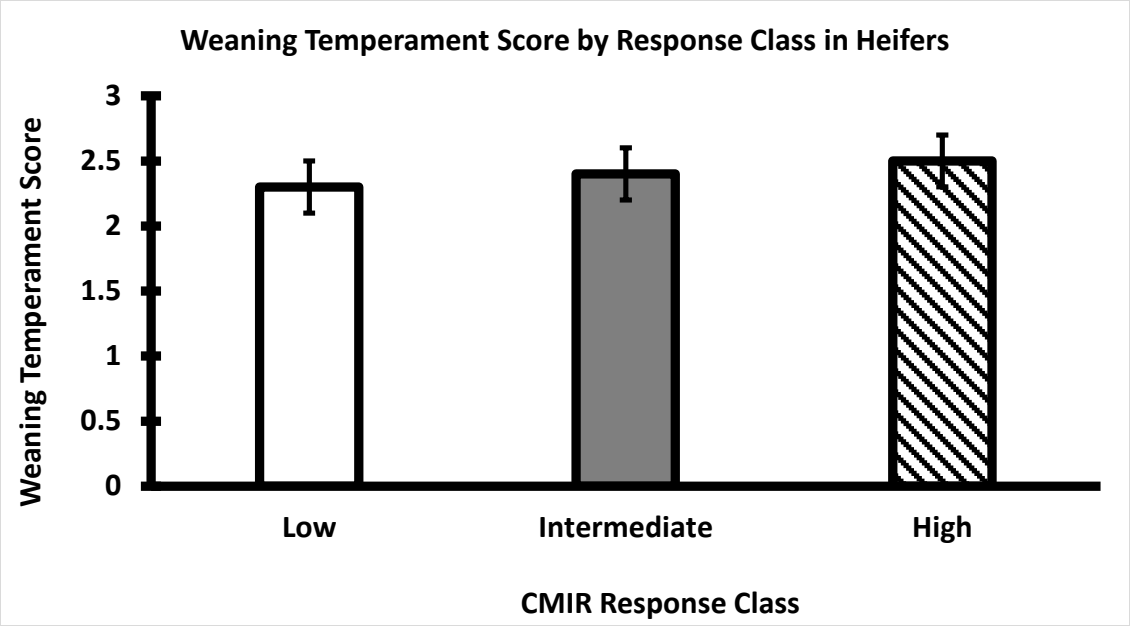

Figure 13. Average temperament score of weaned Brahman heifer calves by CMIR response class (n = 20 Low, 16 Intermediate and 21 High heifers). $P > 0.05$

DISCUSSION

This study investigated factors affecting the AMIR and CMIR of weaned Brahman calves. There was a sexual dimorphism associated with CMIR, in that bulls had a greater response than heifers (Fig. 9). For AMIR, there was no difference between bull and heifer calves (Fig. 4). The weaning temperament score of heifer calves was greater than that of the bull calves (Fig. 3). Body weight was greater on day 0 (Fig. 1) and day 15 (Fig. 2) in bull calves.

In this study, the heifer calves were more temperamental than the bull calves. *Bos indicus* cattle breeds have been reported to be more temperamental than *Bos taurus* breeds with females being more temperamental than males (Hoppe et al., 2010; Voisinet et al., 1997). Body weight of the bull calves was higher than that of the heifer calves, which is consistent with findings by Browning et al. (1999). Bulls having a greater CMIR could be due to females having a decreased response to vaccination related to their increased temperament scores. It could also be due to males having increased IL-12 and Th-1 responses (Ruggeri et al., 2016 and Duff and Galyean 2007).

The selection for 'general' immune responsiveness was first investigated in pigs by Wilkie and Mallard in 1999. Their approach involved identification and selection of animals with an enhanced general immune response, that was assessed by combining measures of the animal's antibody- and cell-mediated adaptive immune responses. To test CMIR, as in our study, *Candida albicans* was used to induce a type 1 immune response bias. Romani in 2000 and Herazion et al. in 2009 determined that *Candida albicans* with Quil-A and hen-egg white lysosome were antigen/adjuvant combinations

capable of inducing CMIR and AMIR, respectively, without interfering with diagnostic tests in cattle species. The tail skin fold was determined as the ideal injection site for CMIR evaluation because the neck was significantly more sensitive (Herazion et al., 2009). In our study, *Salmonella* Newport Extract vaccine was used for determination of AMIR because it was a novel vaccine for the herd. *Salmonella* can be a devastating problem to dairy and beef industries and is a significant foodborne pathogen that has been reported to be increasing in incidence by the USDA. One of the best prevention methods for the control of *Salmonella* is vaccination. Herd benefits of *Salmonella* Newport Extract vaccine include increased milk yield and increased protection from *Salmonella* infection (Hermesch et al., 2008).

The ability to separate bull and heifer calves based on their adaptive immune response can be supported by several other studies. However, factors such calf age at vaccination, maternal antibodies of the dam, type of vaccine, adjuvants or lack thereof, and location of the vaccination play a role in an individual calf's response to the vaccine (Chamorro et al., 2016). This indicates that differences in antibody response after vaccination would be expected as calves can range from having high to low antibody titers regardless of the sex of the calf (Kirkpatrick et al., 2001; Chamorro et al., 2016). Studies by Aleri et al. (2015) and Wagter et al. (2000) in dairy cattle agree with the results of our study in that high and low AMIR and CMIR animals can be identified.

Negative correlations have been shown between AMIR and CMIR in several species, which is similar to our results in weaned Brahman heifer calves. The genetic correlation between AMIR and CMIR being negative could be due to the cytokines

promoting CMIR tending to inhibit AMIR, and *vice versa*. This suggests the importance of selecting cattle for both traits to provide broad-based disease resistance to a multitude of organisms (Thompson-Crispi et al., 2012; Biozzi et al. 1979; Mouton et al., 1984; Sarker et al., 2000). The higher correlation in bull calves could be related to the sexual dimorphism seen in CMIR in the bull calves (Table1.).

Table 1. Correlations between AMIR and CMIR in weaned Brahman calves.

Species	Correlation	Standard Error	P-value	Author
Cattle (Brahman)	0.18 (bull and heifer calves) 0.01 (heifer calves) 0.3 (bull calves)	0.22 0.23 0.22	> 0.05	Cook et al., 2017 unpublished data
Cattle (Holstein)	-0.13 (cows)	0.37	> 0.05	Thompson-Crispi et al., 2012

The benefits of selecting for high immune responding cattle have been evaluated in various studies. One of the advantages of selecting for AMIR and CMIR is the low to moderate heritability associated with these traits (Thompson-Crispi et al., 2012; Abdel-Azim et al., 2005; Wagter et al., 2000; Hernández et al., 2006). Another benefit of high immune responding animals that has been seen in dairy cattle is an association of decreased occurrence of disease and improved response to vaccination. Thompson-

Crispi et al. (2012) reported that cows that had high AMIR and CMIR had decreased instances of mastitis, metritis, and other illnesses. The improvement seen in production and reproductive aspects of dairy cattle by selecting for enhanced immunity is something that needs further elucidation in beef cattle. Our data should encourage further consideration and study of AMIR and CMIR for use as selection tools in Brahman cattle.

CHAPTER IV

CELLULAR AND ANTIBODY MEDIATED IMMUNE RESPONSES ARE INFLUENCED BY SEX AND PREGNANCY STATUS IN BRAHMAN CATTLE

INTRODUCTION

Immune function could be a tool to select healthier cattle. The immune system is divided into two categories: the innate immune system and the adaptive immune system. Innate immunity is the body's nonspecific defense mechanism triggered when an antigen appears while adaptive immunity utilizes specific antigen recognition systems and creates immunological memory after initial exposure. Cell mediated immunity includes activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of cytokines in response to an antigen. Antibody mediated immunity utilizes the production of antibodies by B-lymphocytes to bind to specific antigens.

High immune responders have been reported to have increased feed efficiency and average daily gains in cattle and swine (Aleri et al., 2015 and ThompsonCrispi et al., 2013). High immune responders also have improved responses to vaccination, higher colostrum quality, and increased disease resistance in cows (ThompsonCrispi et al., 2013). Aleri et al. in 2015 found that animals with high CMIR (cell mediated immune response) and AMIR (antibody mediated immune response) tended to have significantly higher cortisol concentrations suggesting that high immune responding animals have a higher stress response or a higher basal serum concentration. However, cortisol concentrations in the same group of cattle were lower during the second testing period

suggesting that handling stress influences cortisol concentrations. Several studies have shown that cortisol concentration can vary, with mild stressful conditions such as exercise and handling enhancing the immune system and its response while long term and chronic stressors suppress the immune response (Hines et al., 1996).

In cattle, there is limited information on how immune status changes throughout pregnancy. However, a study by Hine et al. evaluated the immune responses of twenty Canadian Holstein cows in early pregnancy (< 100 days) and twenty-three cows in mid pregnancy (100-200 days). The results of this study showed that pregnancy stage did not influence AMIR but age did, with younger cows having a reduced response (Hine et al., 2011). Cows in early pregnancy had higher CMIR which increased as the cows got older. A decrease in lymphocytes and neutrophils immediately after calving but an increase directly prior to parturition has also been observed (Saad et al., 1988 and Nagahata et al., 1992). However, there is overall little information about the influence pregnancy stage and sex have on the immune system, especially in beef cattle.

Therefore, our study was designed to determine if cellular and antibody mediated immune responses are influenced by sex and/or pregnancy stage in Brahman cattle.

MATERIALS AND METHODS

Experimental Design

All experimental procedures were in compliance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching and approved by the Institutional Animal Care and Use Committee of Texas A&M AgriLife Research. Eighty-four non-

pregnant cows, thirty-three cows in an early stage of pregnancy (d1-97), sixty cows in mid-pregnancy (d98-194), seventy-one cows in late pregnancy (d195-292), and twenty-five fertile bulls from the Texas A&M AgriLife Research Center's purebred Brahman herd in Overton, TX were selected for use in this study.

On day 0 whole blood samples (2 x 10 mL) were collected via jugular venipuncture in VACUTAINER® tubes (BD, Franklin Lakes, NJ) and serum isolated for determination of cortisol and IgG concentrations before receiving a subcutaneous (neck) sensitization dose of $25x10^3$ protein nitrogen units (PNU) *Candida albicans* (CA; Greer Labs, Lenoir) with 750 µg Quil-A adjuvant (InvivoGen, San Diego). On day 14 caudal skin fold thickness (SFT) was measured using Harpenden calipers prior to intradermal injection of $5x10^3$ PNU CA into skin fold. On day 15 injection site SFT was measured and whole blood samples were taken. Response was determined by the difference in SFT from day 15 (post-injection) and day 14 (pre-injection).

Cortisol

Serum concentrations of cortisol were determined using a single antibody radioimmunoassay (DSL-2100; Diagnostic Systems Labs, Webster, TX) utilizing rabbit anti-cortisol antiserum coated tubes according to the manufacturer's directions.

Evaluation of AMIR

Sera were obtained from blood samples collected on day 0 and day 15 that were allowed to clot overnight at 4 degrees C before centrifugation for 30 minutes at 3000

rpm. Harvested serum samples were stored at -20 degrees C until analyzed for vaccine specific IgG by a double sandwich, enzyme linked immunosorbent assay. Ninety-six well plates were coated with *Salmonella* Newport Extract vaccine (Zoetis, Florham Park, NJ) dissolved in carbonate buffer and all plates were blocked with 3% Tween 20 in PBS. Sera samples and controls, were diluted to 1:700. Positive and negative controls were obtained by pooling sera samples pre-immunization (negative control) and sera samples obtained on d 15 (positive control). All controls and samples were added to the plate in triplicate, allowing 15 animals (day 0 and day 15) to be run per plate. Sheep anti-bovine IgG was used as the secondary antibody and was diluted to 1:8000. Absorbance was read at 450 nm using an automated microplate reader. To calculate AMIR the following equation was used: (Day 15 average / Positive control average) – (Day 0 average / Positive control average).

Evaluation of CMIR

Response was determined by the difference in SFT from day 15 (post-injection) and day 14 (pre-injection).

Statistical Analysis

Data were analyzed using general model procedures of JMP (SAS Inst., Inc., Cary, NC) to evaluate the effects of sex and pregnancy status and their interactions on AMIR and CMIR response. Specific comparisons were made using Fisher's Protected

Least Significant Difference, with $P < 0.05$ considered significant. Pearson's correlation coefficients were also calculated for AMIR and CMIR.

RESULTS

AMIR

Fertile bulls and non-pregnant cows did not differ in AMIR ($P > 0.05$) (Fig. 15). Although AMIR did not differ between non-pregnant and early pregnant cows, ($P > 0.05$) stage of pregnancy was a factor as AMIR was least in the middle and late pregnant cows ($P < 0.05$) (Fig. 15).

CMIR

Mean CMIR was least ($P < 0.05$) in non-pregnant cows relative to pregnant cows (early, middle, and late) and bulls (Fig. 14). Stage of pregnancy did not affect CMIR ($P > 0.05$) nor did fertile bulls and pregnant cows differ in CMIR ($P > 0.05$) (Fig. 14).

There was a negative correlation between AMIR and CMIR in bulls and cows in middle and late pregnancy ($P > 0.05$). There was no correlation between AMIR and CMIR in non-pregnant cows and cows in early pregnancy ($P > 0.05$) Table 2.

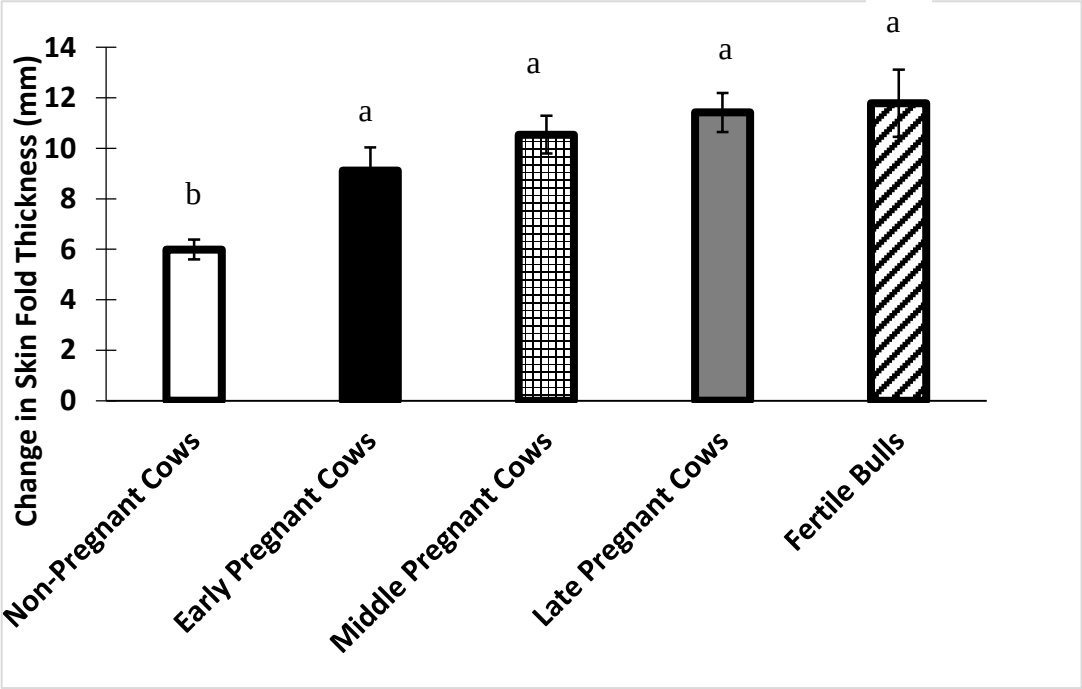

Figure 14. Comparison of CMIR among mature Brahman cattle. Columns bearing different letters (a,b) differ at $P < 0.05$.

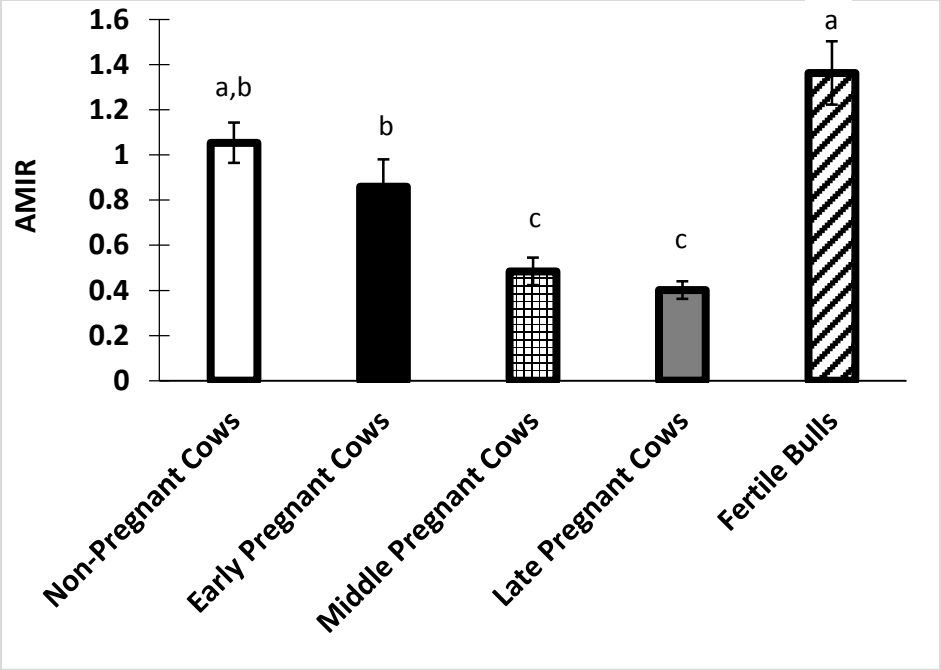

Figure 15. Comparison of AMIR among mature Brahman cattle. Columns bearing different letters (a,b) differ at $P < 0.05$.

DISCUSSION

This study investigated if sex and pregnancy status affected AMIR and/ or CMIR in mature Brahman cattle. Mean CMIR was least in non-pregnant cows relative to pregnant cows (early, middle, and late) and bulls (Fig. 14). Stage of pregnancy did not affect CMIR nor did fertile bulls and pregnant cows differ in CMIR (Fig. 14). Regarding AMIR, fertile bulls and non-pregnant cows did not differ (Fig. 15). Although AMIR did not differ between non-pregnant and early pregnant cows, stage of pregnancy was a factor as AMIR was least in the middle and late pregnant cows (Fig 15). This should encourage further consideration and study of AMIR and CMIR for use as selection tools in Brahman cattle, but sex and pregnancy status must be taken into consideration.

Hine et al. (2011) also reported that AMIR does not differ between non-pregnant and early pregnant dairy cows. B-cell production has been shown to be decreased in dairy cattle in late stages of pregnancy suggesting a lower AMIR and increased susceptibility to infection in agreement with our results (Nagahata et al., 1992). However, our results differed from others regarding the AMIR between early pregnant and cows in middle pregnancy. This could be due to physiological differences between dairy and beef cattle, other studies using diverse ages of cattle, and sample size differences. Our study used mature Brahman cows with an average age of eight years and had a much larger sample size per status group than many other studies. The importance of pregnant cows having a high AMIR has been reported by others including Paré et al. in 1997. In their study, it was found that pregnant cows infected with

Nespora caninum were less likely to abort their calves if they had high AMIR. This was especially important during late gestation when maternal antibodies are decreased. However, future studies would be needed to determine maternal versus fetal influence on immune response during pregnancy as close to term the fetus not only has a functional immune system of its own but has IgM and IgG producing cells (Schultz et al. 1973).

Results from our study differed from others regarding CMIR in cattle. A study by Hine et al. (2011) found that CMIR was the highest in dairy cattle in the early stages of pregnancy. While we did not see these differences among pregnant Brahman cattle this could be due to several factors including age, sample size, and physiological differences. The cattle in other studies were twenty-four months and younger in age which could make them difficult to compare to the older, more mature cattle that our study utilized. Studies have shown that CMIR increases with age which supports our findings (Hine et al. 2011; Rossi et al. 1981; and Begley et al. 2009). We also had a large sample size per status group which could cause differences in results from previous studies. There are very limited data on the immune system and its responses during pregnancy in beef cattle. Future research should focus on the physiological differences between dairy and beef cattle breeds as well as differences between *Bos indicus* and *Bos taurus* cattle.

Bos indicus cattle have an increased resistance to viruses which would suggest they have stronger cell mediated immunity than *Bos taurus* cattle (Glass et al. 2005). This has been shown in their good performance traits in various environments, tick resistance, and ability to fight viral infections (Glass et al. 2005). A study by Rangappa

et al. in 1995 presented data on Bos indicus cattle that showed they have more responsive T cells and more IL-1 production from macrophages than *Bos taurus* cattle. This indicates that *Bos indicus* cattle have a more vigorous immune response to antigens than that of *Bos taurus* cattle.

There is contradictory research regarding the influence of sex on the immune system, and there is very limited information on this area in cattle. In mice, it has been reported that estradiol and testosterone have inhibitory effects on CMIR and that AMIR is enhanced by estradiol and decreased by testosterone (McCruden et al. 1991 and Wichmann et al. 1997). A study by Bilbo et al. (2000) found that sex steroid hormones enhance the immune function of Siberian hamsters suggesting that immune function can be enhanced in animals during the breeding season to protect from parasites as well as benefit reproduction. More research needs to be done in this area regarding cattle but this could explain bulls having higher AMIR and CMIR in our study.

The selection for 'general' immune responsiveness was first investigated in pigs by Wilkie and Mallard in 1999. Their approach involved the identification and selection of animals with an enhanced general immune response, that was assessed by combining measures of the animal's antibody- and cell-mediated adaptive immune responses. To test CMIR, as in our study, *Candida albicans* was used to induce a type 1 immune response bias. Romani in 2000 and Herazion et al. in 2009 determined that *Candida albicans* with Quil-A and hen-egg white lysosome were antigen/adjuvant combinations capable of inducing CMIR and AMIR, respectively, without interfering with diagnostic tests in cattle species. The tail skin fold was determined as the ideal injection site for

CMIR evaluation because the neck was significantly more sensitive (Herazion et al., 2009). In our study, *Salmonella* Newport Extract vaccine was used for determination of AMIR because it was a novel vaccine for the herd. *Salmonella* can be a devastating problem to the dairy and beef industries and is a significant foodborne pathogen that has been reported to be increasing in instances by the USDA. One of the best prevention methods for the control of *Salmonella* is vaccination. Herd benefits of *Salmonella* Newport Extract vaccine include increased milk yield and increased protection from *Salmonella* infection (Hermesch et al., 2008).

Negative correlations have been shown between AMIR and CMIR in several species, which agrees with our results in mature cattle with correlations ranging from negative to 0 (Table 2.). The genetic correlation between AMIR and CMIR being negative could be due to the cytokines promoting CMIR tending to inhibit AMIR, and vice versa. This emphasizes the importance of selecting cattle for both traits to provide broad-based disease resistance to a multitude of organisms (Thompson-Crispi et al., 2012; Biozzi et al. 1979; Mouton et al., 1984; Sarker et al., 2000).

Table 2. Correlations between AMIR and CMIR in mature Brahman cattle.

Species	Correlation	Standard Error	P value	Author
Brahman Bulls	-0.09	6.81	> 0.05	Cook et al., 2017 unpublished data
Brahman Cows (non-pregnant)	0.08	3.65	> 0.05	Cook et al., 2017 unpublished data

Table 2 continued. Correlations between AMIR and CMIR in mature Brahman cattle.

Brahman Cows (early pregnant)	0.00	5.35	> 0.05	Cook et al., 2017 unpublished data
Brahman Cows (middle pregnant)	-0.11	5.8	> 0.05	Cook et al., 2017 unpublished data
Brahman Cows (late pregnant)	-0.07	6.5	> 0.05	Cook et al., 2017 unpublished data
Holstein Cows (non-pregnant)	-0.13	0.37	> 0.05	Thompson-Crispi et al., 2012

The benefits of selecting for high immune responding cattle have been evaluated in various studies. One of the advantages of selecting for AMIR and CMIR is the low to moderate heritability associated with these traits (Thompson-Crispi et al., 2012; Abdel-Azim et al., 2005; Wagter et al., 2000; Hernández et al., 2006). Another benefit of high immune responding animals that has been seen in dairy cattle is an association with decreased occurrence of disease and improved response to vaccination. Thompson-Crispi et al. (2012) reported that cows that had high AMIR and CMIR had deceased instances of mastitis, metritis, and other illness. The improvement seen in production and reproductive aspects of dairy cattle by selecting for enhanced immunity is something that needs further elucidation in beef cattle.

CHAPTER V

CONCLUSIONS AND IMPLICATIONS

Selecting for high immune responding cattle has been shown to have benefits for the individual as well as the herd. It is important to understand which factors play a role in determining the immune status of cattle. Our studies demonstrate that weaned Brahman calves can be separated by AMIR and CMIR class and that AMIR and CMIR should be investigated further as selection tools in beef cattle production. Heifer calves had higher weaning temperament scores than the bull calves, while the bull calves had greater body weights than the heifers. There was no difference between sexes regarding AMIR but there was a sexual dimorphism in CMIR with the bull calves having a greater response than the heifers.

In mature Brahman cattle, the mean CMIR was the lowest in non-pregnant cows relative to pregnant cows (early, middle, and late) and bulls. Stage of pregnancy did not affect CMIR nor did fertile bulls and pregnant cows differ in CMIR. Regarding AMIR, fertile bulls and non-pregnant cows did not differ. Although AMIR did not differ between non-pregnant and early pregnant cows, stage of pregnancy was a factor as AMIR was least in the middle and late pregnant cows. Based on these results, it is also important to note that physiological status, stage of pregnancy, and sex should be considered when evaluating either cellular or antibody mediated immune response in mature Brahman cattle.

Our data elucidate the influence of sex, temperament, and pregnancy status on immune traits in Brahman cattle. However, future studies would be required to fully

understand the relationship between sex, physiological status, and the immune system in beef cattle. The evidence discovered regarding the factors influencing antibody and cellular mediated immune response can ultimately be used to modify beef cattle management practices to:

1) improve breeding stock by culling low immune responding individuals and breeding moderate to high responders,
2) minimize negative influences of illness on production that are increased in low immune responding cattle, and
3) enhance immune function and overall health of cattle.

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APPENDIX A

BOVINE IgG ELISA PROTOCOL TO MEASURE IgG IN RESPONSE TO VACCINATION WITH SALMONELLA NEWPORT EXTRACT VACCINE

A. Buffers

1. Carbonate coating buffer
    i. Add 3.03 g Na2CO3 and 6.0 g NaHCO3 to 950 mL of DI water and stir until dissolved
    ii. Adjust pH to 9.6 using HCl
    iii. Add DI water to a final volume of 1000 mL
    iv. Store at 2-8°C
2. PBS
    i. Add 1.16 g Na2HPO4, 0.1 g KCl, 0.1 g K3PO4, and 4.0 g NaCl in 300 mL of DI water
    ii. Adjust pH to 7.4 using HCl
    iii. Add DI water to a final volume of 500 mL
    iv. Store at room temperature
3. PBST
    i. Dissolve 8g of NaCl, 0.2g of KCl, 1.44g of Na2HPO4, 0.24g of KH2PO4, and 2 mL of tween-20 in 800 mL of DI water
    ii. Adjust pH to 7.2 with HCl
    iii. Adjust volume to 1 L with additional DI water

iv. Store at room temperature

4. Milk Block
    i. Dissolve 5g of boxed milk into 20 mL of DI water
    ii. Adjust volume to 50 mL
    iii. Store at room temperature for use the same day
5. *Salmonella* Newport extract vaccine coat
    i. Add 10 mL vaccine to 30 mL of Carbonate coating buffer
    ii. Mix well and store at 4°C for use the same day

B. Bovine IgG ELISA

1. Coat *Salmonella* Newport vaccine on 96 well plates at 100 uL/well in Carbonate Coating Buffer
    i. Cover and incubate overnight at 4°C
2. Dump plate, pat dry then wash 6 times with PBST
3. Dump plate, pat dry, block using 200 uL/well of milk block
4. Cover and incubate 1 hour at 37°C
5. Dilutions of the serum samples were prepared in milk block at 1:700 (Samples tested in triplicate)
6. Cover and incubate plates at 37°C for one hour
7. Wash plates 6 times with 0.05% PBS-Tween 20
8. Following this wash step add 100 uL of sheep anti-bovine IgG diluted at 1:8,000 in milk block to each well

9. Cover and incubate plates at 37°C for one hour
10. Wash plate 6 times with PBST and 6 times with PBS
11. Add 100 uL of sure blue to each well and protect from light
12. After 15 minutes read the absorbance at 450 nm using an ELISA reader after adding 100 uL of HCl to each well