THE EFFECTS OF MATERNAL DIET ON PASSIVE TRANSFER OF IMMUNITY TO THE NEONATAL BEEF CALF, AND CALF IMMUNE RESPONSE TO VACCINATION AND IMMUNOSTIMULANTS

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ABSTRACT

To investigate the effects of maternal diet on passive transfer of immunity to the neonatal beef calf, and calf immune response to vaccination and immunostimulants, three experiments (Exp.) were completed. Results from Exp. 1 indicated that there was no effect of supplemental starch to cows on colostrum production, offspring birth weight, incidence of dystocia, respiratory acidosis, and the passive transfer of immunity. Results from Exp. 2 indicated that both the needle – free injection devices (NFs) and needle and syringe (NS) initiate a haptoglobin (Hp) inflammatory response ($P < 0.001$). After injection bovine viral diarrhea virus (BVDV) type 2 antibody titers continually declined from d 0, 7, 28, and 115 across both NS and NF treatment groups ($P < 0.001$). After secondary booster vaccination on d 115, BVDV type 2 antibody titers significantly increased by d 143 ($P < 0.001$). The use of NFs will maintain a level of immunity that has been seen by the use of conventional NS vaccine administration methods. In Exp. 3, treatments containing a modified live vaccine (MLV) antigen initiated an Hp inflammatory response over time ($P < 0.001$). Interferon gamma was not significantly different across treatment over time ($P = 0.39$); however, there was a threefold increase in treatment three over time. Treatments containing a MLV BVDV antigen maintained BVDV type 2 antibodies over time ($P <0.001$). Feed intake and feeding behavior were unaffected by the use of the vaccine and/or immunostimulant.

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CHAPTER 1. LITERATURE REVIEW

Introduction

The immune system protects animals against microbial invasion and is essential for life. Fetal calves are able to respond early in gestation to foreign invaders; however, the dams' own immune system and the placenta provide additional protection to the fetal calf. The ruminant placenta is morphologically classified as cotyledonary and histologically classified as syndesmochorial (Lemley et al., 2015). The syndesmochorial placenta of the cow forms a syncytium between the maternal endometrium and the fetal trophectoderm; thus, separating the maternal and fetal blood supplies (Arthur, 1996). This separation of maternal and fetal blood supplies prevents the transmission of immunoglobulins in utero (Weaver et al. 2000). Prevention of the transfer of immunoglobulins in utero means calves are born agammaglobulinemic; thus, the calves have very little to no immunoglobulins in circulation (Weaver et al. 2000), and are characterized as being immuno-naïve. (Barrington and Parish, 2001). Although immuno-naïve in utero, as the fetus develops so do the various innate and adaptive immune defenses (Barrington and Parish, 2001). By the time parturition takes place and the calf is born, it can respond to a variety of antigens, but still not completely as when the calf is fully mature (Barrington and Parish, 2001). This is due to a lack of immunological memory that is achieved by the immune system responding to a threat and activating the immune system.

Intake and absorption of colostral components plays a critical role in passive immune transfer and ultimately survival rate of the neonatal calf (Stelwagen et al., 2009). Colostrogenesis occurs prepartum and results in the formation of colostrum in the mammary gland (Stark et al., 2015). Colostrum contains a complex of cells and proteins that actively

protect the neonate from pathogens and other extra-uterine challenges (Bendixen et al., 2011). The bovine mammary gland plays an active role in regulating the concentration of various immunoglobulins in colostrum.

The passive transfer of colostral immune-proteins (PIT), specifically immunoglobulins, to the calf plays a critical role in short-term calf health (Smith and Little, 1922). Success of colostral transfer, reported by calf serum immunoglobulin concentrations, has been shown to be an important indicator of preweaning morbidity and mortality (Perino, 1997). Calves with inadequate serum IgG concentrations (<8 mg/ml) at 24 h after birth are from 3.2 to 9.5 times more likely to become sick and 5.4 times more likely to die before weaning compared to calves with adequate concentrations of serum IgG (Perino et al., 1993).

The passive immune transfer is critical to immediate survival of the calf, while the continued health of the animal is paramount to long term productivity. In livestock, the major causes of death preceding slaughter are due to infectious diseases (Babiuk, 2002). Bovine respiratory disease complex (BRDC) persists as the single most costly disease syndrome associated with commercial beef production in the United States. The appropriate use of vaccines can reduce the risk of BRDC (Urban-Chmiel and Grooms, 2012). Disease control requires the stimulation of the immune system in a sufficient proportion of the population or herd (Siegrist, 2013). Immunization is achieved by inducing protection, through stimulation of the adaptive immune system (Siegrist, 2013). This immunity is achieved by the maintenance of antigen-specific immune effectors and/or by the induction of immune memory cells that can reactivate if re-exposure to the antigen occurs (Siegrist, 2013).

This literature review will discuss: 1) the in utero development of the calf and its immune system; 2) the formation of colostrum in the mammary gland; 3) the digestive

physiology of the neonatal calf gut and the absorption of the immune components found in colostrum; 4) factors that affect the efficiency of absorbing the immune components of colostrum; 5) the immune response in relation to vaccination and its effect on prolonged calf health; and 6) the immune response in relation to immunostimulants and its effects on prolonged calf health. When possible, examples from research performed on beef cattle will be used. However due to limited information and similarities observed in the mammalian immune systems, knowledge from across species will be included in this literature review. Following this literature review, the main experimental objectives of this thesis will be discussed in their own chapters, followed by a general discussion and considerations for future research.

In Utero Development

The cow is one of the "eutherian" species, i.e. those that have placentas (Schlafer et al. 2000). "Placenta" is taken from the Latin term meaning "flat cake" due to apparent similarity of the human discoid placenta to the round flat loaves of unleavened bread commonly made in ancient times (Schlafer et al. 2000). The ruminant placenta is morphologically classified as cotyledonary and histologically classified as syndesmochorial (Lemley et al., 2015). The syndesmochorial placenta of the cow forms a syncytium between the maternal endometrium and the fetal trophectoderm; thus, separating the maternal and fetal blood supplies (Arthur, 1996). This separation of maternal and fetal blood supplies prevents the transmission immunoglobulins in utero (Weaver et al. 2000). The bovine placenta contains a population of fetal macrophages (Schlafer et al. 2000), in human medicine these phagocytes are termed Hofbauer cells (Benirschke and Kaufmann, 1995). These cells play an important role in utero fetal defenses (Schlafer et al., 2000). Fetal placenta macrophages originate from the chorionic mesenchyme early in gestation or from fetal bone marrow-derived macrophages (Schlafer et al.,

2000). Prevention of immunoglobulin transfer in utero means calves are born agammaglobulinemic; thus, the calf has very little to no immunoglobulins in circulation (Weaver et al. 2000) and are characterized as being immuno-naïve (Barrington and Parish, 2001).

Although immuno-naïve in utero, as the fetus develops so do the various innate and adaptive immune defenses (Barrington and Parish, 2001). These mechanisms can be characterized into those dependent on antigen recognition by antibody and or lymphocytes, adaptive immunity, and those that occur independent of recognition events, innate immunity (Barrington and Parish, 2001). The innate immune defense mechanisms include effects such as secreted enzymes, acids in the stomach, epithelium, and normal flora that colonize the mucosal surfaces once the neonate is born (Barrington and Parish, 2001). The innate immune system also includes the complement system and phagocytic cells, both neutrophils and macrophages derived from pluripotent stem cells (Tizard, 2013). Neutrophils and macrophages contribute minimal protection in early fetal life since they remain in their derivation sites until being released into the fetal blood at approximately d 130 of gestation (Banks and McGuire, 1989). Complement activity has been reported at approximately d 90 of gestation (Osborn et al., 1974). Innate immunity defense mechanisms increase in their effectiveness throughout gestation and though they are functional by birth, they can be suppressed by stress, malnutrition, low level infections, or exposure to toxins (Barrington and Parish, 2001). The adaptive immune defense system consists of antibodies, memory lymphocytes, and effector cells (Tizard, 2000). Lymphocytes, also developed from pluripotent stem cells, are initially released into the circulation where they later migrate to specific locations to undergo further differentiation (Barrington and Parish, 2001). T-lymphocytes mature in the thymus and B-lymphocytes mature

in the bone marrow and Peyer's patches (Barrington and Parish, 2001). During the first trimester of gestation, T and B-lymphocytes move from primary lymphoid organs to populate the lymph nodes, spleen, and mucosal lymphoid tissues; this activity occurs independent of antigen exposure and stimulation (Barrington and Parish, 2001). By the time parturition takes place and the calf is born it can respond to a variety of antigens, but still not as completely as when the calf is fully mature due to a lack of immunological memory (Barrington and Parish, 2001).

In addition to growth and development of the fetal immune system, fetal growth overall is crucial and can be affected by nutritional status of the dam. In rats, Godfrey and Barker (2000) demonstrated that the maternal diet can have significant long- and short-term impacts on offspring health. Maternal nutrition is crucial to fetal and placental development, which ultimately influences lifetime performance of that animal (Funston et al., 2010). Despite improved management techniques and extensive research on mammalian nutrition, suboptimal nutrition during gestation continues to be problematic for many livestock species (Wu et al., 2004). Nutrition demand during the early phases of fetal development are critical to accommodate maximal placental growth, differentiation, and vascularization (Funston et al., 2010). Normal fetal development follows an exponential pattern, such that 75% of growth in the bovine fetus takes place in the last 2 mo (NRC 2000; Robinson et al., 1977). Nutrient demands of the fetus parallel the exponential growth of the fetal tissues (NRC, 2000). By d 250, uterine uptake from maternal nutrient supply is 46%, 72%, and 12% for glucose, amino acids, and acetate, respectively (Bell, 1995). In order to meet these requirements the dam may need to dramatically shift basal metabolism (Bell, 1995).

The protein requirement of the dam will drastically increase with the increase in amino acid uptake in utero. Bull et al. (1979) reported that cows maintained on diets deficient in crude protein produced calves that exhibited symptoms classically seen of Weak Calf Syndrome. Calves born to heifers that received a protein-restricted diet had decreased heat production of 11.4% compared to calves born to dams receiving adequate protein levels in the diet (Carstens et al. 1987). More recent research has shown positive results for cow weight and body condition in calves born from cows supplemented with a protein supplement (Larson et al., 2009), as well as improved performance and health of offspring (Martin et al., 2007; Stalker et al., 2006; Larson et al., 2009). Weaning weights, both actual and adjusted, were greater in calves from supplemented dams (Larson et al., 2009; Kennedy et al., 2016). Heifers from protein-supplemented dams were heavier at weaning, pre-breeding, first pregnancy, and had greater pregnancy rates than those from non-supplemented dams (Martin et al., 2007). Steers from protein-supplemented dams had increased weaning and carcass weights (Stalker et al., 2006; Larson et al., 2009). The carcasses from protein-supplemented dams also had greater intramuscular fat and increased percent choice quality grades (Larson et al., 2009). Earlier work by Martin et al. (2007) did not show a difference between protein- and non-supplemented dams, however, more recent research by Larson et al. (2009) indicates that protein-supplementation during late gestation did tend to affect calf birth weight. Later work by Kennedy et al. (2016) showed increased calf birth weight of dams supplemented protein during late gestation.

In addition to protein requirements of the dam and fetus; uterine uptake of glucose and acetate by the fetus also increases by 46% and 12% respectively (Bell, 1995). Unlike amino acid placental uptake, which is through active transport (Bell, 1993), glucose placental uptake occurs through facilitated diffusion (Stacey et al., 1978). Thus, glucose transport is dependent

upon the maternal – fetal plasma glucose concentration gradients and responds to changes in maternal plasma glucose (Bell, 1995). Ewes deprived of energy are susceptible to hypoglycemia during late pregnancy (Bergman, 1973), which results in a reduction in uterine and fetal glucose uptake (Hay et al., 1984; Leury et al., 1990). If nutrient available glucose is limited in ewes, most glucose available to oxidation is made up of increased catabolism of amino acids, at the expenditure of protein synthesis and deposition of the fetus (Lemons and Schreiner, 1983). This results in reduced fetal growth associated with placental synthesis and excretion of urea (Lemons and Schreiner, 1983). Supplying adequate levels of starch to the dam during late gestation can directly supply the placental and fetal tissues with glucose and indirectly supply the placental and fetal tissues with amino acids. Underwood et al. (2010) saw improved final body weights, average daily gain (ADG), adjusted 12^{th} rib fat thickness, and hot carcass weights (HCW) of calves whose dams were provided improved forage quality pastures during late gestation compared to dams grazing native low quality forage quality. They did not see any differences in birth weight and adjusted 205 d weaning weights. However; Radunz et al. (2012) reported that calves from corn supplemented dams had increased birth weight, 100 d weights, and tended to have improved weaning weights. Radunz et al. (2012) did not observe the improved carcass characteristics that was noted by Underwood et al. (2010). These differences in outcomes may be due to the differences in starch availability from pasture forages and corn.

Birth weight is commonly used as an initial reference point when regarding the development of an individual animal, and it represents the culmination of the most dynamic growth and development process in mammalian biology (Holland and Odde, 1992). At fertilization, the weight of the zygote is approximately 1.0 ng, by parturition, the fetal weight

averages 38.5 kg, which equates to a 38 trillion fold increase in weight over an average 283 d period (Holland and Odde, 1992). Since gestation length is relatively constant, variations in birth weight (BW) are the result of differences in fetal growth rate (Holland and Odde, 1992). Offspring born at above-average BW have an increased chance of survival compared to those born at below-average BW (Funston, et al., 2010). However, above-average BW may increase the incidence of dystocia (Laster et al., 1973), leading to increased birth asphyxia, metabolic and respiratory acidosis (Szenci, 1985), depressed immunoglobulin absorption (Odde, 1988), and an increased predisposition to disease (Roy, 1990). Offspring with below-average BW may lack vigor, tolerance to cold-stress (Carstens et al., 1987), resistance to infectious agents (Roy, 1990), or the ability to overcome stresses of parturition during adaptation to extra uterine life (Woodward and Clark, 1959; Herschler et al., 1962). Increased BW in livestock species can be advantageous or detrimental depending on production environment; thus, it is critical to recognize the demand environment places on the animal genetics (Jenkins and Ferrell, 2006).

Colostrogenesis

As previously mentioned, the syndesmochorial placenta of the cow separates the maternal and fetal blood supplies (Arthur, 1996); thus, preventing the transmission of immunoglobulins in utero and categorizing the neonatal calf as agammaglobulinemic (Weaver et al. 2000). Consequently, intake and absorption of colostral components plays a critical role in passive immune transfer and ultimately survival rate of the neonatal calf (Stelwagen et al., 2009). Colostrogenesis occurs prepartum and results in the formation of secretory colostrum in the mammary gland (Stark et al., 2015). The mammary gland begins concentrating colostral components during the final 4-5 wk of pregnancy (Stark et al., 2015). Colostrum is a combination of diverse components, such as fat, lactose, vitamins, and minerals that have a high

quality nutritional composition (Ontsouka et al., 2003). In addition to and more important than the nutritional value, colostrum contains a complex of cells and proteins that actively protect the neonate from pathogens and other extra-uterine challenges (Bendixen et al., 2011). In colostrum the concentration of immunoglobulins is particularly high; in ruminants the major immunoglobulin class is IgG (Stelwagen et al., 2009). In addition to immunoglobulins, colostrum contains viable cells such as neutrophils and macrophages, which secrete numerous immune-related components, as well as oligosaccharides, gangliosides, acute phase proteins, immunomodulatory factors, ribonucleases, and a range of peptide and proteins with antimicrobial activity (Stelwagen et al., 2009). These immune-related components secreted by neutrophils and macrophages include cytokines, antimicrobial proteins and peptides, and reactive oxygen species (Stelwagen et al., 2009). Mammary epithelial cells also contribute to the host defense by secreting innate immune effector molecules such as lactoferrin, β-defensin, and lipopolysaccharide-binding protein (LBP) (Stelwagen et al., 2009).

Research on the hormonal regulation of immunoglobulin transport into colostrum has been investigated but remains incomplete (Wheeler et al. 2007). Smith et al. (1971) suggested that changing serum concentrations of estrogen and progesterone in late pregnancy exerted a controlling influence on the transport of IgG. Other research suggests the IgG transfer is a consequence of mammary gland development, which is controlled by estrogen and progesterone. (Lascelles and McDowell, 1974).

The accumulation of immunological cells and proteins enter the bovine mammary gland in various ways. The bovine mammary gland plays an active role in regulating the concentration of various immunoglobulins in colostrum, predominantly IgG and to a lesser extent IgA, however the mammary epithelium itself does not synthesize immunoglobulins

(Stelwagen et al., 2009). A small amount of the immunoglobulins enter the colostrum from the blood serum through the paracellular route as a result of "leaky" intercellular tight junctions (Lacy-Hulbert et al., 1999). The vast majority of immunoglobulins enter through a selective receptor-mediated intracellular route (Stelwagen et al., 2009). These immunoglobulins may be blood-derived or produced in situ by intramammary plasma cells (Stelwagen et al., 2009). Mayer et al. (2005) demonstrated the presence of a specific IgG receptor, neonatal Fc receptor (FcRN), in the mammary epithelial cell (MEC) that plays an active role in transporting IgG into the lactating bovine mammary gland. IgA found in bovine colostrum is produced by intramammary plasma cells (Stelwagen et al., 2009). These plasma cells move to the mammary gland through the blood, where their transport is mediated by chemokines (Wilson and Butcher, 2004). The movement of IgA across the MEC is facilitated by the polymeric immunoglobulin receptor (pIgR) expressed in the mucosal epithelium (Apodaca et al., 1994). On the apical side of the MEC, pIgR is cleaved and IgA is released into the alveolar lumen along with a secretory component of pIgR (Apodaca et al., 1994).

The movement of neutrophils and macrophages into the alveolar lumen is mediated in a separate fashion than that seen with IgG and IgA. Serum amyloid A3 (SAA-3), a variant of the serum amyloid A (SAA) family, is expressed by MEC in response to pathogens and is present in milk and colostrum (McDonald et al., 2001). The concentration of SAA-3 is increased 200 fold in colostrum than in later lactation milk (McDonald et al., 2001). The SAA protein has numerous proinflammatory actions including chemoattractant to neutrophils, monocytes, and T lymphocytes, causing adhesion to the MEC (Badolato et al., 1994; Xu et al., 1995; Su et al., 1999). These findings suggest a crucial function for SAA to establish and maintain inflammation (He et al., 2009). The inflammatory status produced by SAA allows a continued

flow of neutrophils and macrophages into the alveolar lumen, which directly kill bacteria via phagocytosis, but also produce numerous cytokines, reactive oxygen species and antimicrobial peptides (Stelwagen et al., 2009). Cytokines are immunological regulatory proteins that affect a wide variety of cells and tissues (Tizard, 2000). Cytokines are redundant in their biological activities and many of them have similar effects. This has given rise to the concept of the cytokine network (Hirako et al., 2005). Further explanation is beyond the scope of this review. Reactive oxygen species are highly reactive metabolites of oxygen, including superoxide anion, hydroxyl radicals, and hydrogen peroxide, that are produced by active phagocytes (Abbas et al., 2014). Reactive oxygen species are used by phagocytes, such as neutrophils and macrophages, to form oxyhalides that damage phagocytized bacteria and may also be released from the cells to promote an inflammatory response (Abbas et al., 2014).

The bovine MEC plays an active role in mammary gland defense and synthesis of lactoferrin, β-defensins, and LBP (Stelwagen et al., 2009). Lactoferrin is an iron binding molecule that transports iron ions; additionally, lactoferrin has a wide variety of biological functions (Brock, 2002). Lactoferrin provides antimicrobial activity, both bactericidal and fungicidal, to the mammary gland and to the neonate (Brock, 2002). The concentration of lactoferrin increases during most inflammatory reactions and some viral infections, and it is classified as an acute phase protein (APP) (Kanyshkova et al., 2001). B-defensins are a subset of antimicrobial peptides that interact with the adaptive immune system, and are produced in response to microbial products or pro-inflammatory cytokines (Ganz and Lehrer, 1998). Lipopolysaccharide binding protein (LBP) is one of the most produced immune system proteins during infections with gram-negative bacteria (Schumann et al., 1990). The main function of LBP is to bind bacteria lipopolysaccharides (LPS) expressed on the cell wall of bacteria, acting

as a transporter for LPS and to help control LPS-dependent monocyte response (Stelwagen et al., 2009).

Bovine Neonatal Digestive Tract Physiology

Nursing by the calf on the dam will cause the closure of the esophageal groove (Dirr and Dirksen, 1989). Closure of the esophageal grooves allows colostrum and milk to bypass the reticulo-rumen and enter the abomasum (Titchen, 1976). Fluid consumed by the neonatal calf must come into contact with the receptors located in the oropharyngeal area (Dirr and Dirksen, 1989). Nursing by the neonate causes vagal stimulation of the afferent nerve located in the mouth and pharynx. This stimulation causes the lips of the esophageal groove extending from the cardia to the reticulo-omasal orifice to contract (Phillipson, 1970). A twisting movement of the lip draws the epithelium to the reticulum adjacent to it forming a tube for colostrum and milk to pass into the abomasum (Phillipson, 1970). Failure of the esophageal groove causes the colostrum or milk to flow into the reticulo-rumen (Dirr and Dirksen, 1989). If the colostrum or milk remains in the reticulo-rumen for a sufficient period of time, bacterial fermentation will take place, which can cause ruminal acidosis that can be fatal (Dirr and Dirksen, 1989).

The abomasum is similar to the monogastric stomach, and it is the only region in the gastrointestinal tract that contains secretory tissues, with fundic and pyloric regions that are histologically similar to the monogastric (Church, 1988). The fundic mucosa contains parietal cells, which secrete hydrogen chloride (HCL), peptic cells that secrete the proteolytic enzyme precursor pepsinogen, and mucous-secreting cells (Church, 1988). The secreted amounts of HCL are low at birth, although the plasma concentration of gastrin, which upregulates the secretion of HCL, is high (Guilloteau et al., 2009). The number of parietal cells, which release HCL are also very low at birth, but increases ten times during the first 72 h of life (Hill, 1956).

The pH of the abomasum is approximately 5.8 at birth before the first nursing and decreases to approximately 3.0 after 42 h (Guilloteau et al., 1985). Chief cells in the abomasum, the only protease producing cells found in the abomasum, secrete an inactive proteolytic enzyme pepsinogen (Church, 1988; Johnson, 2013). Pepsinogen is converted to the active protease pepsin at a pH of 2.1 (Church, 1988). The elevated pH seen in the abomasum at birth and for the first 24 h of life decreases proteolytic activation of pepsin (Guilloteau et al., 1983). This minimizes proteolytic and denaturing activity, which allows immunologic proteins and cells found in colostrum to move through the ruminant abomasum viably (Guilloteau et al., 1983).

Colostrum exits the abomasum via the pyloric valve and enters into the duodenal region of the small intestine. It is at the major duodenal papilla, very close to the pyloric valve in the small intestine, that the bile and pancreatic ducts enter (Church, 1988). At birth the pancreatic gland of the calf is well developed and ready to exert its exocrine function (Guilloteau et al., 2009). In the neonatal calf, secretions of the pancreatic juice are very low at birth (Guilloteau et al., 2009). The secretions respond to feeding from the first day of life. The secretions take place during the cephalic phase, during nursing, with the elevation of plasma concentrations of pancreatic polypeptide (Zabielski and Naruse, 1999). However, pancreatic secretions in the same study were not observed during the gastric and intestinal phases (Zabielski and Naruse, 1999). In the non-nursing neonatal calf, the exocrine pancreas secretes small amounts (about 1 µl/kg BW x min^{-1}) of pancreatic juice (Guilloteau et al., 2009). Pancreatic secretions steadily increase with age and reach 4.0 and 5.5 µL/kg BW/min^{-1} in 1 and 4 week old calves respectively (Zabielski and Naruse, 1999). Pancreatic proteases and trypsin secretions increase following the pancreatic juice volume patterns (Zabielski and Naruse, 1999). Bovine IgG, the most abundant immunoglobulin in colostrum, is most sensitive to degradation by trypsin

(Pineiro, 1978). In order to combat trypsin degradation activity in the small intestine, colostrum contains trypsin inhibitors; colostrum has almost 100 times the level of trypsin inhibitors compared to conventional milk (Sandholm and Hankanen-Buzalski, 1979). The concentration of trypsin inhibitors in colostrum is positively related to the concentration of IgG in the colostrum, which for poor quality colostrum can further reduce absorption due to a reduced defense against the proteolytic activity of trypsin (Quigley et al., 1995).

The amount of immunoglobulins transported across the epithelium of the small intestine increases from duodenum to ileum (Jochims et al., 1994). Absorption of colostral immunoglobulins occurs via transport through the enterocytes by pinocytosis (Jochims et al., 1994). The enterocytes of newborn calves seem to be non-discriminately permeable to all classes of immunoglobulins, as well as other proteins and macromolecules (Jochims et al., 1994; Tizard, 2000; Mayer et al., 2002). Immunoglobulins pass through the glycocalyx on the apical membrane of the enterocyte towards the basolateral membrane where they are exocytosed into the lacteals and intestinal capillaries and enter systemic circulation through the thoracic duct. (Tizard, 2000; Mayer et al., 2002; Weaver et al., 2000). The non-selectivity of this process has been substantiated by the fact that other protein macromolecule concentrations and enzyme activities such as γ-glutamyltransferase (GGT) are increased after ingestion of colostrum (Thompson and Pauli, 1981). However, Goldstein et al. (1951) identified clathrin molecules on the enterocytic microvilli in the jejunum and ileum of the calf near the IgG molecules, which may be evidence of a receptor mediated system of transport. The presence of clathrin molecules implies the existence of the coated pits of vesicles (Stanley et al., 1972). Also, Mayer et al. (2002) determined the FcRN transporter protein is in neonatal ruminant on the apical side of enterocytes. In a similar fashion the specific IgG receptor FcRN in the MEC

transports IgG into the lactating bovine mammary gland (Mayer et al., 2005). Reber et al. (2006) demonstrated the movement of whole leukocytes takes place from the intestinal lumen through the enterocyte and into circulation. They demonstrated that cytokines Cd43 and Cd11, necessary for leukocyte transendothelial migration, are found in colostrum and assist in the migration through the enterocyte and into neonatal circulation.

The cessation of macromolecule absorption in the enterocyte is termed closure (Smith and Little, 1922). In the bovine neonate, closure of the enterocyte to colostral immune-proteins occurs with age at a progressively increased rate after 12 h postpartum (Stott et al., 1979a). Complete closure occurs at approximately 24 h postpartum when calves are not fed colostrum and even earlier on calves that receive colostrum shortly after birth (Stott et al., 1979b). Closure is caused by rapid neonatal enterocyte turn-over (Smeaton and Simpson-Morgan, 1985; Jochims et al., 1994). Lysosomes appear in new enterocytes at 24 h postpartum (Jochims et al., 1994). Lysosomes are membrane-enclosed organelles that contain enzymes capable of breaking down all types of biological polymers (Cooper, 2000). Immune-proteins that are absorbed by the enterocyte will come into contact with this organelle and be degraded (Jochims et al., 1994). Lysosomes first appear in the duodenum, followed by the cranial and middle regions of the jejunum (Jochims et al., 1994). The appearance of lysosomes first in the duodenum then in the upper and middle regions of the jejunum could be the reason that the lower section of the jejunum and the ileum were seen to absorb the largest amount of immunoglobulins (Jochims et al., 1994). Enterocytes will continue to absorb immune-proteins. However, the protein is destroyed proteolytically by the fusion of the lysosome with the vesicles containing the immune-proteins (Jochims et al., 1994).

Passive Immune Transfer and Factors Affecting its Efficiency

The PIT, specifically immunoglobulins, to the calf plays a critical role in short-term calf health (Smith and Little, 1922). Success of colostral transfer, reported by calf serum immunoglobulin concentrations, has been shown to be an important indicator of preweaning morbidity and mortality (Perino, 1997). The primary immunoglobulin found in colostrum is IgG and it accounts for approximately 85% of the total immunoglobulins, with IgG_1 accounting for 80-90% of the total IgG (Larson et al., 1980). Calves with inadequate serum IgG concentrations (<8 mg/ml) at 24 h are from 3.2 to 9.5 times more likely to become sick and 5.4 times more likely to die before weaning compared to calves with adequate concentrations of serum IgG (>16 mg/ml) (Perino et al., 1993). When calves have inadequate serum IgG concentrations (<8 mg/ml) a failure of passive transfer of immunity (FPT) has occurred (Perino et al., 1993). In general, this is due to the inability of the dam to accumulate and secrete immunoglobulins and/or the neonatal calf's inability to nurse and absorb immunoglobulins in the bloodstream. Failure of the passive transfer of immunity itself is not a disease, it is a condition that predisposes the neonate to the development of infectious disease (Weaver et al., 2000). Numerous factors can cause FPT, the two most important are calf age at colostrum consumption and the mass of IgG consumed (Perino, 1997). Associated with these two factors are dystocia, twins, sex of calf, metabolic state of calf, dam disease and vaccination history, dam and calf genetics, dam age, dam body condition score, dam nutrition, and dam udder conformation and health (Perino, 1997; Weaver et al., 2000).

Immunoglobulin transfer across the enterocyte is optimized in the first 4 h postpartum and begins to decline rapidly after 12 h postpartum (Stott et al., 1979; Bush and Staley, 1980). Calves fed earlier will have a significantly higher serum IgG concentration than those fed later

when similar concentrations are fed (Stott et al., 1979). In addition to the calf's ability to nurse and absorb is the dam's ability to accumulate and secrete immune-proteins. IgG comprises roughly 85% of the total immunoglobulins found in colostrum; thus, the concentration of IgG in colostrum has been considered the trademark for evaluating colostrum (Larson et al., 1980; Godden, 2008). High quality colostrum has an IgG concentration greater than 50 mg/mL (McGuirk and Collins, 2004). The average concentration of IgG in dairy breeds (predominantly Holsteins) is 32 mg/mL in dams ranging in age from 2-5 plus years (Foley and Otterby, 1978) and in beef breeds (predominantly Hereford and Angus) is 57.65 mg/mL in dams ranging in age from 2-9 plus years (Odde, 1988). This difference is due to the inverse relationship of milk yield and colostral IgG production (Pritchett el al., 1991).

Incidence of dystocia has been reported to decrease PIT (Odde, 1988; Muggli et al., 1984). However, Stott and Reinhard (1978) did not find a dystocia effect on calf serum IgG concentrations when all calves were fed 1 L of colostrum. Prolonged parturition can cause the development of respiratory or metabolic acidosis, and calves with prolonged calving times are more likely to become acidotic than calves born from normal parturition (Szenci, 1983). Fetal asphyxia is characterized by mixed respiratory-metabolic acidosis (Bleul and Götz, 2013). The respiratory component of acidosis is caused by an accumulation of carbon dioxide in the fetus due to diminished removal by the placenta (Szenci, 1985). Metabolic acidosis in neonatal calves is caused by L-lactate (Bleul and Götz, 2013). L-lactate in blood plasma is the result of anaerobic glycolysis and is formed when pyruvate is converted by lactate dehydrogenase (Lagutchik et al., 1996). Carbon dioxide, a weak acid, and L-lactate, a strong acid, account for neonatal acidosis (Bleul and Götz, 2013). Studies have shown higher concentrations of lactate from calves delivered after calving assistance compared to unassisted calves (Diesch et al,

2004; Sorge et al., 2009; Bleul and Götz, 2013). Bleul and Götz (2013) determined duration of parturition had effects on pH and L-lactate but not on pCO_2. Serum IgG may be reduced in calves that have lower blood pH and elevated pCO_2 when fed the same volume of colostrum per kg body weight (Besser et al., 1990). Duration of metabolic acidosis exceeds that of respiratory acidosis, 48 and 4 h, respectively, in neonatal asphyxiated calves (Bleul and Götz, 2013). Conversely, Boyd (1989) showed duration of metabolic acidosis did not exceed respiratory acidosis, 2 and >24 h, respectively. Most research on the effects of dystocia and PIT have focused on respiratory acidosis, measuring pH and pCO_2, and results have been confounding (Besser et al., 1990; Boyd, 1989; Drewry and Quigley, 1997).

A thorough nutrition strategy can affect cow-calf productivity in many ways; however, there is little evidence of a direct link between gestational cow nutrition and PIT in calves (Perino, 1997). Concentrations of CP in gestational cow diets generally do not have significant effects on colostral IgG concentrations (Olson et al., 1981; Blecha et al., 1981; Hough et al., 1990). However, Odde (1988) did see an increase in both colostral IgG and serum IgG in protein restricted beef heifers. Odde (1988) noted that the effect seen in calf serum IgG was probably the result of all calves being fed 1 L of colostrum. Olson et al. (1981) did not see a significant effect of the concentrations of CP in gestational cow nutrition on serum IgG levels; however, similar effects were reported on colostral IgG levels and Burton et al. (1984) did see reduced serum IgG levels in calves from protein restricted dams. The control diet used by Burton et al. (1984) had dietary CP levels at 110% of NRC requirement. Research on the effect of starch supplementation of dams during late gestation on colostrum quantity and quality has been limited with varying results. Corah et al. (1975) noted an increase in the number of calves dying due to scours ($P = 0.04$) from cows that received decreased energy diets; however,

colostral and calf serum IgG concentrations were not quantified, so it is indefinite that calf mortality is due to a failure of PIT. Ewes carrying singleton lambs and fed 1.5 times their metabolizable energy requirement during the last week of pregnancy produced 185% more colostrum than unsupplemented ewes (Banchero et al., 2007). Most research investigating the effects nutrition has on colostral IgG and serum IgG has focused on dietary restrictions. Further investigation is warranted on supplementation of CP, specific amino acids and/ or starches. General cow nutritional status can be visually measured with the use of BCS (Perino, 1997). Odde (1988) reported that first calf heifers with increased BCS was associated with increased serum IgG concentrations in their offspring ($p = 0.03$); however, BCS in cows of all ages did not significantly affect serum IgG concentrations ($p = 0.19$). Perino et al. (1995) did not see a relationship between BCS and serum IgG levels in the calf at 24 h after birth.

Age of dam is an important factor to successful PIT in beef breeds (Odde, 1988; Perino, 1995; Muggli et al., 1984) and dairy breeds (Morin et al., 2000; Pritchett et al., 1991). In all studies mentioned, first-calf heifers have calves with lower serum IgG concentrations. The data for both dairy and beef breeds shows an increase in calf serum IgG as cow's age increases, plateauing at 3 - 4 yr and beginning to drop off after 6 - 7 yr. The reduction in calf serum IgG was only noted in beef breeds (Odde, 1988; Muggli et al., 1984). Odde (1988) noted that colostral IgG concentration did not differ across age. First-calf heifers have a decreased total colostral volume compared to multiparous dams (Odde, 1988).

Udder and teat confirmation and health in beef cows are not often given consideration (Perino, 1997). Most research on udder and teat conformation have been conducted on dairy cows. In dairy cows, udders suspended closer to the floor led to increased time for calves seeking the teat and duration of first nursing event (Ventorp and Michanek, 1992). Similar to

Ventorp and Michanek (1992), Edwards (1982) noted more pendulous udders did affect the success of calves finding the teat and suckling. Wesselink et al. (1999) did not see significance of udder conformation on calves finding the teat and nursing. Ventorp and Michanek (1992) did not report colostral or 24 h serum IgG, thus PIT or FPT was not reported. Perino (1995) reported that calves born to dams with clinical mastitis any time during lactation had reduced serum IgG concentrations at 24 h of age; however it was not apparent if mastitis was a causative risk factor due to compromised colostrum quality and/or quantity or if this was associated with poor udder confirmation.

The gender of the neonatal calf has been shown to affect colostrum production and later lactation milk production (Joaquin et al., 2015; Hinde et al., 2014). Additionally, Joaquin et al. (2015) found that both total immunoglobulin concentration and colostral volume were affected by the sex of the neonate. Total immunoglobulin concentration was higher in dams carrying males than for those carrying females; however, total colostral volume was higher in dams with female offspring than dams with male offspring (Joaquin et al., 2015). The results of the previous study agree with research conducted by Hinde et al. (2014). They found that dams with female offspring produced 5% more milk during lactation than dams with male offspring. They suggested that hormones from the fetus and placenta may enter maternal circulation to bind to the dam's MEC and influence functional development and subsequent milk synthesis. The mechanism that influences milk production may also affect colostrogenesis (Joaquin et al., 2015). The results from the studies mentioned above were performed in dairy cattle. Kennedy et al. (2016) found in beef cattle that total colostral volume was greater in dams carrying female offspring compared to dams carrying male offspring, however; they did not see a significant difference of colostral IgG concentration between male and female offspring. Odde (1988)

reported a higher incidence of FPT in male beef calves. This could have been due to the higher incidence of dystocia noted in male beef calves (Odde, 1988). Perino et al. (1995) and Filteau et al. (2003) included dystocia in a multivariate analysis, and did not find any variation between male and female serum IgG levels.

Vaccines

Immunization of humans and animals against infectious diseases has been practiced for over 200 years (Babiuk, 2002). The experiences seen through the past two centuries have shown the benefits of immunization and that vaccinations are one of the most cost-effective methods to preventing economic losses and reducing risk of disease in livestock (Babiuk, 2002). In livestock, the major causes of death preceding slaughter are due to infectious diseases (Babiuk, 2002). Bovine respiratory disease complex (BRDC) persists as the single most costly disease syndrome associated with commercial beef production in the United States, accounting for losses in 2010 of 1,055,000 animals valued at $643 million (NASS, 2011). Increased morbidity and mortality, decreased weight gains, decreased feed utilization, and decreased carcass quality account for the economic losses associated with BRDC (Edwards, 2010). Bovine respiratory disease complex was originally termed “shipping fever” since signs often occur shortly after arrival into the feedlot (Urban-Chmiel and Grooms, 2012). The morbidity risk of BRDC in feedlot cattle occurs in the first 45 d after arrival into the feedlot with the highest risk occurring in wk 1 to 3, after that morbidity declines (Buhman et al., 2000; Edwards, 1996). Vaccination for viruses and bacteria associated with BRDC are widespread (Taylor et al., 2010). The viral vaccine components of BRDC consist of bovine herpesvirus type 1, also known as infectious bovine rhinotracheitis (IBRV), bovine viral diarrhea (BVDV), parainfluenza virus type 3 (PIV-3), and bovine respiratory syncytial virus (BRSV) (Urban-

Chmiel and Grooms, 2012). The bacterial vaccine components of BRDC consists of *Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni* (Urban-Chmiel and Grooms, 2012). Killed and modified live vaccines (MLV) are available in different combinations of viral pathogens (Urban-Chmiel and Grooms, 2012). The appropriate use of these vaccines can reduce the risk of BRDC (Urban-Chmiel and Grooms, 2012).

The three most common types of vaccines used for cattle are MLV, killed, and genetically engineered (Tizard, 2013; Cortese, 2002). Modified live vaccines contain living bacterial or viral organisms that are typically collected from field disease cases and then grown and passed through host cells or media for virus and bacteria, respectively, to attenuate the pathogen (Cortese, 2002). Each growth cycle represents a passage, and the modified pathogen is then administered back into the animal to determine its virulence (Cortese, 2002). After several passages, the pathogen loses virulence and can no longer cause disease in the specific species, and is then tested to confer immunity (Cortese, 2002). The final vaccines are passed a number of times beyond virulence in order to reduce the risk of reverting back to a virulent state (Cortese, 2002). Killed vaccines use a pathogen isolated from a disease outbreak, grown in a media, and then chemically or physically killed (Cortese, 2002). The inactivation of the pathogen is achieved using chemicals or ultraviolet rays (Cortese, 2002). A concern using this type of vaccine is that heat inactivation may degrade important epitopes needed for a proper immune response (Cortese, 2002). To improve efficacy and achieve an effective immune response in killed vaccines, adjuvant is added to heighten the immune response (Cortese, 2002). Genetically engineered vaccines have been altered through a mutation that results in a bacterium or virus with altered virulence or growth characteristics (Cortese, 2002). Genetically

engineered vaccines have been engineered to delete a gene and cause an immune response deficient in specific antibody responses (Cortese, 2002).

The term adjuvant is defined as a substance that, when used in combination with an antigen, enhances the immune response beyond those elicited by the antigen alone (Iain et al., 1993). Iain et al. (1993) defined four general ways in which adjuvants promote an immune response. First, adjuvants maintain a depot of antigen at the site of injection. Second, adjuvants promote accumulation of immunoreactive cells at the site of injection and also into the draining lymph nodes. Third, adjuvants modify the activities of cells that generate, promote, and maintain the immune response. Fourth, adjuvants can modify the presentation of antigen to the immune system. The most common adjuvants used in veterinary medicine are aluminum hydroxide and oil and water micelles (Ian et al., 1993). The effects of an aluminum adjuvant depends on the adsorption of antigen on the surface of the precipitate (Hansen et al., 2007). The greater the adsorption the more effective the adjuvant, allowing more antigen in the same volume of solution (Hansen et al., 2007). This adsorption depends on the concentrations of the adjuvant and the pH of the solution (Seeber et al., 1991). At high concentrations of adjuvant and lower pH more adsorption will take place; however, at pH below 6.0 and higher concentration of adjuvant, pain and tissue irritation are produced at the injection site (Makimura and Suzuki, 1982).

Vaccines used specifically against IBR are classified into five types: parental administered MLV vaccines, chemically altered live virus temperature sensitive vaccines, inactivated viral vaccines, combination and inactivated viral vaccines, and MLV intranasal vaccines (Fulton, 2002). MLV parental vaccines are attenuated by multiple passages in a cellular culture that retain the ability to replicate in a vaccinated animal, possibly causing

viremia (Fulton, 2002). MLV vaccines stimulate a rapid immune response and generally one dose is needed to stimulate protective immunity (Sutton, 1980; Fulton, 2002). One dose will stimulate an adequate immune response, which varies in length depending on the form of disease challenge (Fulton, 2002). Long term immunity, shown by antibody and cell-mediated response is detectible after an MLV vaccine is administered (Fulton, 2002). MLV intranasal vaccines can be divided into two attenuation processes, those that are modified by cell culture and those modified by treatment so that they are temperature sensitive (Todd et al., 1972; Fulton 2002). MLV intranasal vaccines stimulate protection with one dose and induce an initial rapid onset of protection, possibly through interferon-γ found in nasal secretions (Todd et al., 1972).

The majority of vaccines administered to cattle are parenteral, either intramuscular or subcutaneous, using a needle and syringe (Babiuk et al., 2018). There are potential drawbacks to this vaccine delivery method. Improper handling of needles can result in accidental injury to the individual operator as well as the animal receiving vaccine (Weese and Jack, 2008). There is also risk of needle fragments may break off during injection and remain in the muscle of the animal to slaughter (van Drunen Little-van den Hurk, 2006). Additionally, blood – born infectious agents such as bovine leucosis and anaplasmosis as well as organisms on the skin can be transmitted between animals if one needle is used to inject multiple animals (Hollis et al., 2005; Reinbold et al., 2010). These concerns have led to the design of alternative vaccination techniques, including the use of needle – free injection devices (NFs) (Rey et al., 2013). As previously mentioned, the use of intranasal vaccines would also be an alternative to parenteral vaccination; however, those vaccines are specifically formulated to be administered via intranasal route. The use of parenteral vaccines administered via intranasal route would be off

label use and will not be discussed. Needle – free injection devices use compressed gas, typically CO_2 or N_2, at pressures ranging from 310.3 – 448.2 kPa (Rey et al., 2015). Upon triggering the device, the desired volume of vaccine is forced through a small orifice which forms a high pressure stream that can penetrate the skin and deposit the vaccine into the desired tissue (Mousel et al., 2008). This vaccination technique removes the issues observed with needles, helps reduce disease transfer, and reduces vaccination time (Chase et al., 2008; Reinbold et al., 2010; Mousel et al., 2008). This vaccination technique has been shown to elicit serological immune responses in cattle to Mannheimia haemolytica, IBRV, and BVDV (Hollis et al., 2005; Rey et al., 2013). Rey et al. (2013) did note that there were significantly higher numbers of injection site reactions over time in calves that received vaccination with the NFs. Rey et al. (2013) observation of this phenomena was measured qualitatively and quantitative measure of injection site reactions may be more appropriate. A novel tool to quantitatively measure injection sight reactivity may be with the use of infrared thermography (IRT) (Hoffman et al., 2018). Hoffman et al. (2018) used IRT technology to effectively measure injection site reactions in patients that received the influenza vaccine. In dairy cattle, IRT has been used to examine inflammation, infections, and lameness associated with digital dermatitis (Alaaod and Buscher, 2012; Harris – Bridge et al., 2018).

Disease control or elimination requires the stimulation of the immune system in a sufficient proportion of the population or herd (Siegrist, 2013). Immunization is achieved by inducing protection, as a result of stimulating the adaptive immune system (Siegrist, 2013). This immunity is achieved by the maintenance of antigen-specific immune effectors and/or by the induction of immune memory cells that can reactivate if re-exposure to the antigen occurs (Siegrist, 2013). Antigen-specific immune effectors are antibodies produced by B lymphocytes

that are able to bind to a specific toxin or pathogen (Cooper and Newmerow, 1984). Other potential effectors are cytotoxic $CD8^+$ T lymphocytes (CTL) that may limit the spread of infectious antigens by recognizing and killing infected cells as well as secreting antiviral cytokines (Siegrist, 2013). The proliferation and maintenance of B lymphocyte and CTL response is supported by growth factors and signals from $CD4^+$ T helper lymphocytes (Th) (Siegrist, 2013). All of these immune effector cells are controlled by regulatory T cells (Treg), involved in immune tolerance (Bacchetta et al., 2005). Most antigens and vaccines trigger a B and T cell response; however, killed vaccines mostly mediate protection through the stimulation of B lymphocytes secreting highly specific IgG serum antibodies (Siegrist, 2013). These antigen specific antibodies have been shown to confirm vaccine-induced protection against numerous diseases (Casadevall, 2004).

Innate and Adaptive Immune Response

The body is equipped with multiple levels of defenses; thus, an organism that breaks through the first line of defense is confronted with a second, higher barrier and so forth (Tizard, 2013). The first line of defense are physical barriers to invasion (Tizard, 2013). Intact skin provides an effective barrier to invasion; however, insults to the skin such as scratches, incisions, microscopic abrasions, burns, and even insect bites may allow microbes to invade (Tizard, 2013). Other body surfaces, such as the mucosal linings of the respiratory and gastrointestinal tracts can act as physical barriers as well (Tizard, 2013). Physical defenses include coughing, sneezing, and mucous flow of the respiratory tract and vomiting and diarrhea (Tizard, 2013). In addition to physical defenses, there is a large population of commensal bacteria on the skin and in the gastrointestinal tract that can outcompete poorly adapted pathogenic organisms (Tizard, 2013).

To elicit a vaccine response, a vaccine must provide enough danger signals from the antigen, typically paired with an adjuvant, to trigger the inflammatory reaction that is mediated by cells of the innate immune system (Hoebe et al., 2004). Upon injection of vaccine antigens, an acute phase inflammation develops within minutes (Tizard, 2013). Upon injection of an antigen, broken cells release molecules known as damage-associated molecular patterns (DAMPs) that trigger the release of cytokines, chemokines, and enzymes from sentinel cells (Tizard, 2013). The antigens also provide pathogen-associated molecular patterns (PAMPs) that trigger sentinel cell response (Tizard, 2013). The swelling at the sight of injection causes sensory nerves to release bioactive peptides (Tizard, 2013). The complex mixture of molecules attracts defensive white blood cells and increases blood flow to the sight of injection. Sentinel cells, such as macrophages, neutrophils, and dendritic cells located near the site of injection are active when DAMPs and PAMPs bind to their pattern-recognition receptors (PRRs) located on the cell surface of the sentinel cells (Tizard, 2013). This causes the sentinel cells to synthesize and secrete a mixture of molecules that trigger inflammation and initiates the first steps of the adaptive immune system (Tizard, 2013). The three major cytokines secreted by sentinel cells include tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) (Tizard, 2013).

The release of TNF-α, IL-1, and IL-6 increases protein synthesis, specifically APPs (Tizard, 2013). APP are a group of blood proteins that change in concentration when an animal is subjected to external or internal challenges, such as infection, inflammation, and stress (Murata et al., 2004). One of the most prominent APP found in beef cattle is haptoglobin (Hp) (Alsemgeest et al., 1994). Haptoglobin concentrations in healthy cattle are often undetected, but during an acute phase response bovine Hp can increase 50-100 times (Conner et al., 1988).

However, Hp concentrations observed post vaccination with killed clostridial 7 – way bacterin toxoids or BRDC 5 – way MLV viral vaccines with a *Mannheimia haemolytica toxoid* have been closer to 4 – 7 fold increases post injection (Stokka et al., 1994; Gaspers et al., 2018). Hp binds to free hemoglobin (Hb) in the plasma and reduces the oxidative damage associated with hemolysis (Yang et al., 2003). The Hp-Hb complex is recognized by receptors on macrophages and phagocytized (Schear et al., 2002). This action prevents bacteria from utilizing iron and thus prevents bacteria from proliferating (Idoate et al., 2015). Haptoglobin also plays a key role in the recruitment of neutrophils in the early phase of inflammation (Riollet et al., 2000). Hp concentrations will increase with both bacterial and viral infections (Schroedl et al., 2001; Ganheim et al., 2003; Heegaard et al., 2000; Idoate et al., 2015), and Hp can be used as a tool to measure respiratory disease in feedlot conditions (Idoate et al., 2015).

The triggering of inflammation by cytokines, and the mobilization of phagocytic cells such as neutrophils and macrophages contributes to the rapid destruction of foreign microbes (Tizard, 2013). Inflammation and the actions of the innate immune system may be sufficient to protect the body, but it cannot be guaranteed to provide complete resistance to infection, or assist the body to learn from the experience (Tizard, 2013). This limitation of the innate immune response is the advantage of the adaptive immune response (Tizard, 2013). Dendritic cells are the link between the innate and adaptive immune system. Dendritic cells act as sentinel cells and activate the innate defenses when they first encounter foreign antigen, they can process antigen and initiate the adaptive immune system. They regulate adaptive immunity by determining whether an antigen will trigger an antibody-mediated and or cell-mediated response (Tizard, 2013). Dendritic cells process and then present foreign protein antigen to T cells (Tizard, 2013). Immature dendritic cells patrol throughout the body as sentinel cells

(Siegrist, 2013). When exposed to an antigen, dendritic cells undergo a maturation process, modulating specific surface receptors and migrate towards the lymphatic system, in secondary lymph nodes (Siegrist, 2013). The central role of mature dendritic cells, in response to a vaccine, is to provide antigen-specific and costimulatory signals to activate naïve T cells (Palucka et al., 2005).

Dendritic cells and T cells that have been upregulated by antigen exposure migrate towards secondary lymphoid tissues, including lymph nodes, spleen, Peyer's patches and mucosal associated lymphoid tissue (Siegrist, 2013). These cells up-regulate specific surface molecules that provide B cell activating signals (Siegrist, 2013). T cells help drive B cell differentiation into immunoglobulin secreting plasma cells that can produce low affinity germline antibodies (Maclennan et al., 2003). When exposed to an antigen, dendritic cells undergo a maturation process, modulating specific surface receptors and migrate towards the lymphatic system, in secondary lymph nodes (Siegrist, 2013). Phagocytosed antigen is loaded onto the MHC class II molecules and presented to T cells (Tizard, 2013). The central role of dendritic cells, in response to a vaccine, is to provide antigen-specific and costimulatory signals to activate naïve T cells (Palucka et al., 2005).

Dendritic cells and CD4+ helper T cells that have been upregulated by antigen exposure migrate towards secondary lymphoid tissues, including lymph nodes, spleen, Peyer's patches and mucosal associated lymphoid tissue (Siegrist, 2013). These cells up-regulate specific surface molecules that provide B cell activating signals (Siegrist, 2013). CD4+ T cell help drives B cell differentiation into immunoglobulin secreting plasma cells that can produce low affinity germline antibodies, termed the extrafollicular reaction. (Maclennan et al., 2003). During B cell differentiation, immunoglobulin class switching from IgM towards IgG, IgA, or

IgE occurs with the upregulation of activation-induced deaminase enzyme (Siegrist, 2014). CD4+ T cells exert helper functions during the extrafollicular pathway, and engagement on B cells skew class-switch recombination into particular immunoglobulin classes and subclasses (Siegrist, 2013). Antigen specific B cells that receive sufficient help from antigen specific T cells proliferate in structures known as germinal centers, where differentiation into plasma cells takes place (Siegrist, 2013). This stimulation of the germinal centers as a few B cells upregulate and migrate towards B cell follicles, due to the attraction follicular dendritic cells (Siegrist, 2013). Follicular dendritic cells play a key role in the B cell response, they attract antigen specific B and T cells as well as retain antigen for extended periods of time (Siegrist, 2013). This time allows for a massive proliferation of the antigen specific B cells, which results in the large production of antibodies with increased antigen binding affinity (Siegrist, 2013).

B cells process vaccine antigen into small peptides that are displayed on the MHC class II, in a similar fashion to dendritic cells (Siegrist, 2013). The MHC-peptide complex is displayed on their surface and binds to a subset of CD4+ T cells, follicular helper T cells (Siegrist, 2013). These follicular helper T cells have different sets of chemokine receptors, transcription factors, surface markers, and interleukins that are uniquely equipped to provide the most efficient B cell help through a series of coststimulatory molecules (Vinuesa et al., 2005). The interactions between antigen-specific germinal center B cells, antigen bearing follicular dendritic cells, and follicular helper T cells results in the production of B cells with the highest level of antigen-specific affinity (Siegrist, 2013). This combination provides signals necessary for the differentiation of germinal center B cells either towards plasma secreting specific antibodies or towards memory B cells (Siegrist, 2013). Antigen specific antibodies have been

formally demonstrated as conferring vaccine-induced protection against many disease, and the production of memory B cells allows for the long term disease protection (Casadevall, 2004).

Immunostimulants

As previously mentioned, BRDC persists as the single most costly disease syndrome associated with commercial beef production in the United States (NASS, 2011). Bovine respiratory disease complex is a leading cause of morbidity and mortality to the cattle industry worldwide (Ilg, 2017). In addition to vaccination for the bacterial and viral components to reduce the incidence of BRDC, the prophylactic use of antibiotics has been commonly used in United States beef production to control BRDC (Panciero and Confer, 2010; Murray et al., 2016). With increasing public concerns about widespread use of antimicrobials and governmental policy regarding the use of antimicrobials, alternative preventative treatments have been investigated (Ilg, 2017). Activation of specific areas of the immune system in cattle has been considered as a potential mechanism and area of research (Ackermann et al., 2010). Administration of an exogenous substance that has the ability to augment and/or stimulate certain immune responses may have the ability to increase disease resistance (Blecha, 1988). Substances that exert these functions are known as immunostimulants (Blecha, 2001). The ability to enhance or initiate an immune response to benefit the animal and thus the production efficiency is the goal of immunostimulation in food producing animals (Hudson, 2017). This concept has raised interest in the beef industry as a potential novel preventative measure that may reduce antimicrobial use (Hudson, 2017). There are a limited number of immunostimulatory products that have been licensed by the United States Department of Agriculture for use in food animals and few have achieved Food and Drug Administration approval (Huenefeld, 1988; Blecha, 2001).

The first, and only, immunostimulant on the market approved for aiding in the treatment of BRDC in beef cattle is Zelnate (Ilg, 2017). Research completed by Bayer (2014) showed a reduction in lung lesions and mortality associated with BRDC with the use of Zelnate. Zelnate is a plasmid DNA rich in non – methylated cysteine and guanine (CpG) motifs that is encased in a cationic liposome shell (Ilg, 2017). Zelnate is indicated for use to aid in treatment of BRDC caused by *Mannheimia haemolytica* in cattle 4 mo of age or older, when administered at the time of or within 24 h of a perceived stressful event (Nickell et al., 2016). Ilg (2017) researched Zelnate's potential mechanism of action by investigating cellular DNA recognition pathways in cell culture using knockout human and mouse cell lines and reporter gene assays. Ilg (2017) reported that Zelnate, despite being rich in CpG islands, did not activate toll like receptor 9 (TLR9) and the downstream proinflammatory nuclear factor kappa – light – chain – enhancers of activated B cells (NF_KB) pathway. Ilg (2017) did report that Zelnate did initiate the interferon regulatory transcript factor 3 (IRF3) pathway, known to lead to a strong type I interferon response. Rogers et al. (2017) evaluated health outcomes of at risk heifers for the first 60 d upon arrival to the feedlot. Rogers et al., (2017) did not report differences among treatment groups on the incidence of heifers treated for respiratory disease; however, the inclusion of Zelnate did reduce the percentage of BRDC incidence as well as overall mortality by d 60. Roger et al. (2017) reduced morality results correspond with the results reported by Bayer (2014), which may suggest that the DNA immunostimulant has the potential to positively affect survivability and health outcomes of high risk feedlot calves.

Objectives

To: 1) determine the effects of starch supplementation to mid – and late – gestating beef cows on colostrum production, offspring birth weight, incidence of dystocia, respiratory

acidosis, and the passive transfer of immunity; 2) determine the effects of maternal starch supplementation and vaccination strategies on the serologic immune response in calves at turnout; and 3) determine the serologic immune response of vaccination protocols and immunostimulants for bovine respiratory disease complex on feeding behavior and feedlot performance in previously vaccinated, weaned, backgrounding steers.

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CHAPTER 2. EVALUATION OF CORN SUPPLEMENTATION FOR OVERWINTERED BEEF COWS DURING MID – TO LATE - GESTATION ON INCIDENCE OF DYSTOCIA, METABOLIC AND RESPIRATORY ACIDOSIS, AND THE PASSIVE TRANSFER OF IMMUNITY

Abstract

Objectives were to investigate effects of starch supplementation on colostrum production, offspring birth weight, incidence of dystocia, respiratory acidosis, and passive transfer of immunity. Forty – seven Angus – based multiparous beef cows carrying bull calves were divided randomly into two dietary treatments. Treatments were control (CON; n = 23) which received ad libitum access to a low – quality, forage based basal TMR only (57.54% TDN, 6.4% CP) and a treatment group (SUP; n = 24) receiving additional corn at 0.2% of BW (94.5% TDN, 7.64% CP) in addition to ad libitum access to the basal TMR. Calving ease, calf vigor, and mother scores, were assigned. The back right quarter of each dam's mammary gland was completely emptied of colostrum and a sample was collected. Udder and teat score were assigned. Blood samples were collected from calves at 0 and 24 h post calving. Calves were weighed at 0, 6, 12, and 24 h post calving. Dams were weighed at 0 and 24 h and were assigned a BCS at 24 h post calving. Colostrum quantity (695.89 vs. 670.64 ± 119.5g; $P = 0.88$) was and quality (111.1 vs. 126.7 ± 7.56 mg/ml; $P = 0.16$) was not significantly different in CON and SUP fed dams respectively. Calving ease score was not significantly different in offspring from SUP and CON fed dams ($P = 0.15$). There were no differences in any blood gas parameters associated with metabolic and respiratory acidosis in calves born to SUP and CON fed dams. At 24 h calves from SUP and CON fed dams achieved an appropriate level of maternal IgG to be considered a successful passive transfer of immunity (50.63vs. 51.50 ± 3.45 mg/ml; $P = 0.86$).

Introduction

The syndesmochorial placenta of the cow forms a syncytium between the maternal endometrium and the fetal trophectoderm, thus separating the maternal and fetal blood supplies (Arthur, 1996). This separation of maternal and fetal blood supplies prevents the transmission immunoglobulins in utero (Weaver et al. 2000). Prevention of immunoglobulin transfer in utero means calves are born agammaglobulinemic, thus the calf has very little to no immunoglobulins in circulation (Weaver et al. 2000), and is characterized as being immuno-naïve (Barrington and Parish, 2001). Although immuno-naïve in utero, as the fetus develops so do the various innate and adaptive immune defenses (Barrington and Parish, 2001). These mechanisms can be characterized into those dependent on antigen recognition by antibody and or lymphocytes, adaptive immunity, and those that occur independent of recognition events, innate immunity (Barrington and Parish, 2001).

In addition to growth and development of the fetal immune system, fetal growth overall is crucial and can be affected by nutritional status of the dam. In rats, Godfrey and Barker (2000) demonstrated that the maternal diet can have significant long- and short-term impacts on offspring health. Maternal nutrition is crucial to fetal and placental development, which ultimately influences lifetime performance of that animal (Funston et al., 2010). Despite improved management techniques and extensive research on mammalian nutrition, suboptimal nutrition during gestation continues to be problematic for many livestock species (Wu et al., 2004). By d 250, uterine uptake from maternal nutrient supply is 46%, 72%, and 12% for glucose, amino acids, and acetate, respectively (Bell, 1995). In order to meet these requirements the dam may need to dramatically shift basal metabolism (Bell, 1995).

Uterine uptake of glucose and acetate by the fetus also increases by 46% and 12% respectively (Bell, 1995). Unlike amino acid placental uptake, which is through active transport (Bell, 1993), glucose placental uptake occurs through facilitated diffusion (Stacey et al., 1978). Thus, glucose transport is dependent upon the maternal – fetal plasma glucose concentration gradients and responds to changes in maternal plasma glucose (Bell, 1995). Ewes deprived of energy are susceptible to hypoglycemia during late pregnancy (Bergman, 1973), which results in a reduction in uterine and fetal glucose uptake (Hay et al., 1984; Leury et al., 1990). If nutrient available glucose is limited in ewes, most glucose available to oxidation is made up of increased catabolism of amino acids, at the expenditure of protein synthesis and deposition of the fetus (Lemons and Schreiner, 1983). This results in reduced fetal growth associated with placental synthesis and excretion of urea (Lemons and Schreiner, 1983). Supplying adequate levels of starch to the dam during late gestation can directly supply the placental and fetal tissues with glucose and indirectly directly supply the placental and fetal tissues amino acids. Underwood et al. (2010) saw improved final body weights, average daily gain (ADG), adjusted 12^{th} rib fat thickness, and hot carcass weights (HCW) in calves whose dams were provided improved forage quality pastures during late gestation compared to dams grazing native marginal forage quality. They did not see any differences in birth weight and adjusted 205 d weaning weights. However; Radunz et al. (2012) saw calves from corn supplemented dams had increased birth weight, 100 d weights, and tended to have improved weaning weights. Radunz et al. (2012) did not see the improved carcass characteristics that was noted by Underwood et al. (2010). These differences in outcomes may be due to the differences in starch availability from pasture forages and corn.

Research on the effect of starch supplementation of dams during late gestation on colostrum quantity and quality has been limited with varying results. Corah et al. (1975) noted an increase in the number of calves dying due to scours ($P = 0.04$) from cows that received decreased energy diets; however, colostral and calf serum IgG concentrations were not quantified, so it is indefinite that calf mortality is due to a failure of PTI. Ewes carrying singleton lambs and fed 1.5 times their metabolizable energy requirement during the last week of pregnancy produced 185% more colostrum than unsupplemented ewes (Banchero et al., 2007). Most research investigating the relationships nutrition has on colostral IgG and serum IgG has focused on dietary restrictions. Further investigation is warranted on supplementation of CP, specific amino acids and/ or starches. General cow nutritional status can be visually measured with the use of BCS (Perino, 1997). Odde (1988) reported that first calf heifers with increased BCS was associated with increased serum IgG concentrations in their offspring ($p = 0.03$); however, BCS in cows of all ages was not significantly ($p = 0.19$). Perino et al. (1995) did not see a relationship between BCS and serum IgG levels in the calf at 24 h.

Birth weight is commonly used as an initial reference point regarding the development of an individual animal, and it represents the culmination of the most dynamic growth and development process in mammalian biology (Holland and Odde, 1992). Offspring born at above-average BW have an increased chance of survival compared to those born at below-average BW (Funston, et al., 2010). However, above-average BW at birth may increase varying incidence of dystocia (Laster et al., 1973), leading to increased birth asphyxia, metabolic and respiratory acidosis (Szenci, 1985), depressed immunoglobulin absorption (Odde, 1988), and increased predisposition to disease (Roy, 1990). Offspring with below-average BW at birth may lack vigor, tolerance to cold-stress (Carstens et al., 1987), resistance to infectious agents (Roy,

1990), or the ability to overcome stresses of parturition during adaptation to extra uterine life (Woodward and Clark, 1959; Herschler et al., 1962). Increased BW at birth in livestock species can be advantageous or detrimental depending on production environment, thus it is critical to recognize the demand environment places on the animal genetics (Jenkins and Ferrell, 2006).

We hypothesize that starch supplementation, in the form of ground corn, to cows fed a low quality forage will alter colostrum production, offspring birth weight, incidence of dystocia, respiratory acidosis, and the passive transfer of immunity. The objectives were to investigate the effects of starch supplementation on colostrum production, offspring birth weight, incidence of dystocia, respiratory acidosis, and the passive transfer of immunity.

Materials and Methods

All procedures were approved by the North Dakota State University Animal Care and Use Committee. As previously described by Tanner et al. (2018), 47 Angus – based multiparous beef cows carrying bull calves were divided randomly into two dietary treatments. Treatments were control (CON; n = 23) receiving ad libitum access to a low – quality, forage based basal TMR only (57.54% TDN, 6.4% CP) and a treatment group (SUP; n = 24) receiving additional corn at 0.2% of BW (94.5% TDN, 7.64% CP) in addition to ad libitum access to the basal TMR. Cows were stratified by BW and BCS across treatments. Cows weighed 661 ± 7.8 kg, had a BCS of 5.2 ± 0.1 (9 – point scale), and were 7.5 ± 0.2 yr old at the start of the trial. The cows were housed in four adjacent pens (11 or 12 cows per pen), two for each treatment at the NSDU Beef Cattle Research Complex.

After a 3-wk acclimation period, intake was monitored and controlled by Insentec Roughage Feeders (Hokofarm, B. V., Markanesse Netherlands) beginning on d 110 of gestation for 22 wk. Cows were fitted with radio-frequency identification tags to monitor intake. The

Insentec Roughage Feeders are an automated system that identifies cows radio-frequency identification tags individually to control and monitor intake. The basal diet was provided ad libitum to each cow for the duration of the trial; however, the SUP was limited to 0.2% of BW on a 24 h period.

Feed was provided three times daily at 0800, 1200, and 1600. Bunks were checked and additional basal diet added when empty. All pens had free access to water and trace mineralized salt blocks (95.5 to 98.5% NaCl, 3,500 mg of Zn/kg, 2,000 mg of Fe/kg, 1,800 mg of Mn/kg, 280 to 420 mg of Cu/kg, 100 mg of I/kg, 60 mg of Co/kg). Gestation diet 1 consisted of a basal diet of 45% hay, 45% wheat straw, and 10% concentrated separator byproduct on a DM basis; (56.45% TDN; 6.42% CP) and was fed from d 110 of gestation until d 153. Due to the loss of a cow from an impacted abomasum, diets were adjusted on d 154 to 60% hay, 30% wheat straw, and 10% concentrated separator byproducts (Gestation diet 2; 58.63% TDN, 7.11% CP DM basis) until 2 weeks prior to calving (d 265 of pregnancy). Corn was supplemented to the SUP group at 0.2% (87.6% TDN, 7.64% CP on DM basis) of BW from d 110 until d 265. On d 265 of gestation, approximately 2 wk before expected parturition, all cows were fed the same diets (45% straw, 25% DDGS, 30% corn silage; 62.4% TDN and 11.6% CP on a DM basis) for *ad libitum* intake for a period of 5 wk; corn supplementation ceased.

All feed samples were analyzed for ash, CP, NDF, ADF, EE, Ca, and P. Forage samples were dried in a 55° C oven for 48 h and ground to pass through a 1-mm screen. Forage and DDGS samples were analyzed for DM, ash, N (Kjehldahl method), Ca, P, and EE by standard procedures (AOAC, 1990). Crude protein was calculated by multiplying N concentration x 6.25. NDF (using heat stable amylase and sodium sulfite and expressed inclusive of residual ash) and ADF (expressed inclusive of residual ash) concentration were analyzed sequentially by

methods of Robertson and Van Soest (1981) using a fiber analyzer (Ankom Technology Corp., Fairport, NY).

During calving, cows were allowed to remain in their pens with the group until signs of labor were observed. If it was possible to move the cow inside the barn without causing undo stress, she was brought inside and put in an individual pen for calving, otherwise she was allowed to calve outside with the group and the cow-calf pair was immediately brought inside. Cows had access to hay and water ad libitum in the calving barn. A calving ease score was assigned post labor (1 = no assistance; 5 = caesarian section). Calves were immediately removed from the pen, where a blood sample was taken via jugular venipuncture and a wet weight (kg) was recorded. Excess amniotic fluid was removed and dry weight (kg) was recorded. During this time the cow was removed and placed into the Silencer Hydraulic Squeeze Chute (Moly Manufacturing Inc., Lorraine KS.) For each cow, weight was determined, and the right rear quarter was milked completely to collect a colostrum sample. Total colostral weight was recorded (g). A subsample was placed in cryo-vials and frozen at -20° C until later analysis. An udder score (1 = very pendulous with a broken floor; 9 = very tight) and teat size scores (1 = very large, balloon–shaped; 9 = very small) was assigned to each dam. The cow calf pair was then placed back into the pen. Calves were allowed to nurse from dam. A calf vigor score (1 = normal; 5 = stillborn) was assigned to each calf and mothering score (1 = the dam was up within ten minutes after delivery, is actively licking the calf to stimulate standing and is vocalizing to the calf encouraging it to nurse; 4 = cow shows aggression towards calf, will not let calf nurse, and does not vocalize) was assigned to each dam. Each cow calf pair was monitored for signs of general health. Calves were removed from the pen and weighed at 6, 12, and 24 h post calving. At 24 h post calving calves were once more removed from the pen and a

second blood sample was collected via jugular venipuncture. Cows were weighed and assigned a BCS (1 = emaciated and 9 = obese; Wagner et al., 1988) by three technicians 24 h post calving.

All blood samples were collected via 7 ml red top CORVAC serum separation tubes (VWR, Radnor, P. A.). Immediately following collection, 100 µL of whole blood was pipetted from the CORVAC serum separation tubes, approximately 80 - 100 µL was pipetted into a CG4+ i-STAT cartridge (measuring blood pH, pCO_2, pO_2, Base Excess, HCO_3, TCO_2, sO_2, and Lactate). Cartridges were placed into the Vetscan i-STAT 1 handheld analyzer (Abaxis North America, Union City, California, 94587.). Remaining blood samples were placed in a refrigerator at 1° C for 30 min and centrifuged for 20 min at 1,380 x g to separate serum, which was then pipetted into cryo-vials and frozen at -20° C until later analysis. Serum and colostrum samples in cryo-vials were taken out of freezer and placed in sample tube holders at room temperature (21° C) until completely thawed. Subsamples were pipetted into new cryo-vials and placed in Styrofoam coolers with icepacks and shipped overnight to The Saskatoon Colostrum Co. LTD where radial immunodiffusion (U.S. Veterinary Biological Permit No. 448A) was used to measure total IgG concentration in the colostrum and serum samples. The Saskatoon Colostrum Co. LTD also analyzed a subsample of colostrum to determine a Brix percentage using a Palm Abbe digital refractometer #PA202X (Misco, Solon, Ohio, 44139)

Data were analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary N.C.). The model included fixed effects of maternal diet (SUP vs. CON), time, pen, and there interactions. Sire was included in the model as a random effect. Variables were analyzed at 0 and 24 h. 0 h was used as a covariate for 24 h parameter. Calf body weight included fixed

effects of maternal diet (SUP vs. CON), time and their interactions, pen, calving ease score, and gestation length. Significance was determined at an alpha $P \leq 0.05$.

Results

As previously reported by Tanner et al. (2018), there was a day by treatment interaction on maternal BCS ($P = 0.03$) where SUP dams gained BCS at a faster rate than CON dams which maintained BCS over pregnancy. Tanner et al. (2018) also noted a day by treatment interaction on maternal BW ($P < 0.01$), both SUP and CON dams increased BW over time. At partition, on d 278, dam BW was not different between SUP and CON dams ($P = 0.30$; Table 2.1.). At 24 h post – partum dam BW ($P = 0.18$) and BCS ($P = 0.71$) was not significantly different between SUP and CON dams. Mothering ($P = 0.13$), udder ($P = 0.08$), and teat ($P = 0.83$) scores did not between SUP and CON dams.

Table 2.1. Influence of treatment on dam parturition parameters.

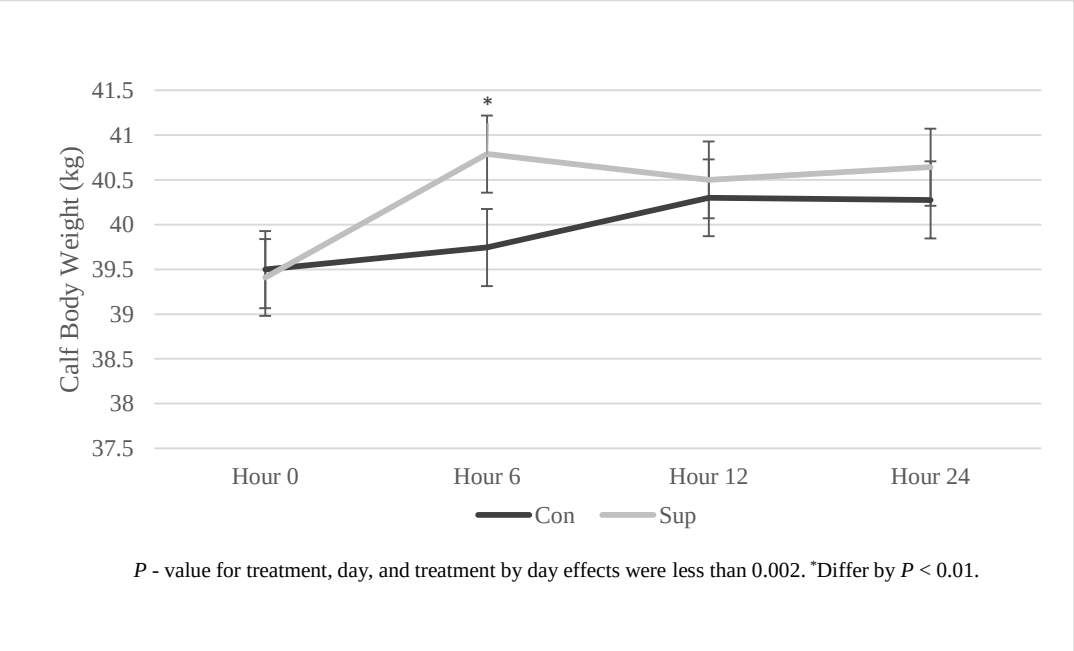
	Treatment			
Item	Control	Supplement	SEM[a]	*P* Value
0 Hour				
Dam weight, kg[b]	697	718	14.0	0.30
Mothering Score	1.12	0.97	0.07	0.13
Udder Score	6.18	5.27	0.36	0.08
Teat Score	6.16	6.27	0.37	0.83
24 Hour				
Dam weight, kg[b]	694	701	3.9	0.18
Dam BCS[b]	5.36	5.41	0.14	0.71

[a]Standard error of the mean (n = supplement 24; control 23).

[b]Tanner et al., 2018

Maternal diet tended to affect calf BW ($P = 0.08$; Figure 2.1.). Six h post – partum offspring form SUP dams had an increased BW compared to offspring from CON dams ($P =$

0.01). However, by 12 and 24 post – partum that there was no significant differences calf BW (P = 0.08). As previously reported by Tanner et al. (2018), at an average age of 3 wk post – partum calves from SUP dams were heavier (P = 0.05) than calves from CON dams. However, by weaning there was no significant difference (P = 0.64) in calves born either SUP or CON dams.



P - value for treatment, day, and treatment by day effects were less than 0.002. *Differ by *P* < 0.01.

Figure 2.1. Calf body weight (kg) by maternal dietary treatment across time

At 0 h, pH, partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), bicarbonate (HCO_3), total carbon dioxide (TCO_2), oxygen saturation (SO_2), lactate, and base excess from whole blood were not different in offspring born to SUP or CON fed dams (Table 2.2.). Also, at 24 h, pH, pCO_2, pO_2, HCO_3, TCO_2, SO_2, lactate, and base excess from whole blood were not different in offspring born to SUP or CON fed dams.

Table 2.2. Influence of treatment on blood parameters in offspring.

	Treatment			
Item	Control	Supplement	SEM[a]	*P* Value
0 Hour				
pH	7.27	7.27	0.02	0.99
pCO_2, mmHg	63.0	65.9	2.52	0.29
pO_2, mmHg	24.0	21.7	1.15	0.17
HCO_3 mmol/L	29.1	30.5	0.70	0.16
TCO_2 mmol/L	31.1	32.4	0.75	0.24
SO_2 %	34.2	28.6	2.62	0.14
Lactate, mmol/L	4.20	4.60	0.51	0.60
Base Excess, mmol/L	2.54	3.66	0.84	0.36
24 Hour				
pH	7.01	7.51	0.20	0.09
pCO_2, mmHg	45.28	44.66	1.54	0.78
pO_2, mmHg	25.75	28.15	2.14	0.33
HCO_3 mmol/L	25.53	27.10	0.92	0.13
TCO_2 mmol/L	26.82	28.62	0.67	0.08
SO_2 %	44.18	5122	5.59	0.27
Lactate, mmol/L	4.12	4.21	0.91	0.94
Base Excess, mmol/L	0.08	2.11	1.03	0.07

[a]Standard error of the mean (n = supplement 24; control 23).

Total colostral weight was not different between SUP and CON fed dams (*P* = 0.88; Table 2.3.). Also, colostral IgG was not different between SUP and CON fed dams (*P* = 0.16; Table 2.3.). However, Brix% tended to be greater in colostrum of SUP dams (126.7 vs. 111.1 ± 7.56%, P = 0.056; Table 2.3.) compared to CON fed dams. At 24 h, there was no differences in serum IgG (*P* = 0.86; Table 2.3.) and serum protein (*P* = 0.55; Table 2.3.) between offspring born from SUP and CON dams.

Table 2.3. Influence of treatment on maternal colostrum and serum protein and IgG in offspring.

	Treatment			
Item	Control	Supplement	SEM[a]	*P* Value
0 Hour				
Colostrum weight, g[b]	696	671	112	0.88
Colostrum Brix%	21.8	25.1	1.17	0.056
Colostral IgG, mg/mL	111	127	7.6	0.16
24 Hour				
Serum IgG, mg/mL	50.6	51.5	3.45	0.86
Serum protein, g/dL	6.95	7.15	0.23	0.55

[a]Standard error of the mean (n = supplement 24; control 23).

[b]Tanner et al., 2018

Calving ease score was not different for offspring from SUP and CON fed dams (*P* = 0.15; Table 2.4.). Calf vigor score was not different for offspring from SUP and CON fed dams (*P* = 0.77; Table 2.4.).

Table 2.4. Influence of treatment on incidence of dystocia and vitality of offspring.

	Treatment			
Item	Control	Supplement	SEM[a]	P - Value
Calving Ease Score	1.00	1.10	0.05	0.15
Calf Vigor Score	1.09	1.06	0.08	0.77

[a]Standard error of the mean (n = supplement 24; control 23).

Discussion

We reject our hypothesis that starch supplementation, in the form of ground corn, offered to cows fed a low quality forage will alter colostrum production, offspring birth weight, incidence of dystocia, respiratory acidosis, and the passive transfer of immunity.

As previously mentioned by Tanner et al. (2018), TDN and NE_m intake was increased in SUP cows compared to CON dams; however, there was a forage intake suppression in SUP cows. The inverse relationship of corn supplementation on forage intake has been demonstrated in beef cattle fed forage – based diets (Lusby and Wagnar, 1986). This inverse relationship between corn supplementation on forage intake is known as substitution (Caton and Dhuyvetter, 1997). Though there was some evidence of improved weight gain and BCS earlier in the trial, maternal weight and BCS were not different at parturition or 24 h post – partum. Odde (1988) reported that first calf heifers with increased BCS was associated with increased serum IgG concentrations in their offspring; however, BCS in cows of all ages was not. Perino et al. (1995) did not see a relationship between BCS and serum IgG levels in the calf at 24 h.

There is little evidence of a direct link between gestational cow nutrition and passive immune transfer (PIT) in calves (Perino, 1997). There were no differences in colostrum quantity (695.89 vs. 670.64 ± 119.5g; $P = 0.88$) between cows on CON or SUP diets. Colostral IgG (111.1 vs. 126.7 ± 7.56mg/ml; $P = 0.16$) was not different in CON and SUP fed dams respectively. High quality colostrum has an IgG concentration greater than 50 mg/mL (McGuirk and Collins, 2004). The average concentration of IgG in beef breeds (predominantly Hereford and Angus) is 57.65 mg/mL in dams ranging in age from 2-9 plus years (Odde, 1988). Both SUP and CON dams in this trial had twice the concentration of IgG in their colostrum noted by McGuirk and Collins (2004). Interestingly, colostrum Brix % tended to be greater in SUP dams compared to CON dams (25.10 vs. 21.77 ±1.17%; $P = 0.056$). Brix refractometers measures total dissolved solids in colostrum, which is different than RID which only measures bioactive IgG (Bartier et al., 2015). Total solids in milk and colostrum is made up of fat, proteins, lactose, and minerals (IDFA, 2018). In addition to and more important than the

nutritional value of total solids, colostrum contains a complex of white blood cells and proteins that actively protect the neonate from pathogens and other extra-uterine challenges (Bendixen et al., 2011). In addition to IgG, colostrum contains viable cells such as neutrophils and macrophages, which secrete numerous immune-related components, as well as oligosaccharides, gangliosides, acute phase proteins, immunomodulatory factors, ribonucleases, and a range of peptide and proteins with antimicrobial activity (Stelwagen et al., 2009). All of these solids are quantified in the Brix % (Bartier et al., 2015). Though bioactive IgG was not significant between treatment groups, total solids tended to be increased in SUP dams. It should be noted that no other immune cells or proteins were quantified in colostrum during this trial, so no determinations can be made if increases in total solids of colostrum are nutritional or immune components.

Birth weight is commonly used as an initial reference point when regarding the development of an individual animal, and it represents the culmination of the most dynamic growth and development process in mammalian biology (Holland and Odde, 1992). Offspring born at above-average BW have an increased chance of survival compared to those born at below-average BW (Funston, et al., 2010). However, above-average BW at birth may increase varying incidence of dystocia (Laster et al., 1973), leading to increased birth asphyxia, metabolic and respiratory acidosis (Szenci, 1985), depressed immunoglobulin absorption (Odde, 1988), and increased predisposition to disease (Roy, 1990). In the current trial, calf BW at birth tended to be different ($P = 0.08$). Six h post – partum offspring form SUP dams had an increased BW compared to offspring from CON dams ($P = 0.01$; Figure 2.1.). However, by 12 and 24 post – partum that there was no significant differences calf BW ($P = 0.08$).

Incidence of dystocia, as measured by calving ease score, was not different across maternal dietary treatment groups. Only three dams needed birthing assistance, which was minimally invasive and was measured as a two for calving ease score. Calf activity post – partum, as measured by calf vigor score, was not significantly different across treatment groups. Again, similar to calving ease score, there was very little variation in this variable, which could strengthen the fact that dystocia was a factor in this trial. All blood gas parameters used to quantify metabolic and respiratory acidosis were not significant different across maternal dietary treatment groups. Ranges for venous blood pH, pCO_2, pO_2, HCO_3, and base excess were all within normal ranges at 0 h for new born calves (Bluel et al., 2007). Ranges for venous blood pCO_2, pO_2, HCO_3, and base excess were all within normal ranges at 24 h for neonatal calves (Bluel et al., 2007). Worth noting is that pH in venous blood at 24 h for calves born to CON dams was 7.01, which is considered acidotic (Bluel et al., 2007). This could be related to the 6 h BW of calves, were calves born to SUP dams had increased BW. Calves born to CON dams did not gain 6 h post calving at the rate of calves from SUP dams. This could be due to the CON calves acidic blood pH. Calves with inadequate serum IgG concentrations (<8 mg/ml) at 24 h are from 3.2 to 9.5 times more likely to become sick and 5.4 times more likely to die before weaning compared to calves with adequate concentrations of serum IgG (>16 mg/ml) (Perino et al., 1993). When calves have inadequate serum IgG concentrations (<8 mg/ml) a failure of passive transfer (FPT) of immunity has occurred (Perino et al., 1993). Calves born from CON and SUP dams had 24 h serum IgG levels three times the 16 mg/ml threshold for an adequate immune transfer (Perino et al., 1993). Calves born from CON and SUP dams had serum protein levels at 24 h that exceed 5.2 g/dl, which is also attributed to a FPT at 24 h (Tyler et al., 1996).

Conclusion

Though starch supplemented to cows fed a low quality forage did not alter colostrum production, offspring birth weight, incidence of dystocia, respiratory acidosis, and the passive transfer of immunity, it adds to a lacking body of literature. The majority of more recent colostrum research has focused around the dairy calf. There have been very few research trials examining how nutrient supplementation of the dam and the effects on neonatal calf health. The vast majority of research has examined maternal nutrient restriction and effects on calf health. Much of the data is from decades earlier and it is important to continue updating the body of literature using cattle genetics in current production beef herds. With more universities and research institutes acquiring systems such as GrowSafe and Insentec Roughage Feeders, very accurate and precise rations can be fed to individual animals. This allows researchers to develop precision research trials looking at factors that affect colostrum production and the passive transfer of immunity in neonatal beef calves.

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CHAPTER 3. IMPACT OF NEEDLE – FREE INJECTION DEVICE ON SEROLOGICAL IMMUNE RESPONSE AND INJECTION SITE REACTION TO A MODIFIED LIVE BVDV TYPE ONE AND TWO VACCINE WITH A *MANNHEIMIA HAEMOLYTICA* TOXOID

Abstract

The objective of this study was to evaluate the impact of a needle – free injection device (NF) on serological innate and adaptive immune responses to a MLV BVDV type one and two vaccine with a *Mannheimia haemolytica* toxoid. At 60 d of age, steers (129.1±6.98kg, n = 43) born from dams that were supplemented with corn during mid – to late – gestation and steers born from cows on the control diet were randomly assigned to either vaccination using needle and syringe (NS) (Non – supplemented = 10 and supplemented = 11; n = 21) or using a NF (Non – supplemented = 11 and supplemented = 11; n = 22). On d 0 of the trial, both treatment groups received a 2 cc dose of MLV BVDV type one and two vaccine with a *Mannheimia haemolytica* toxoid. Haptoglobin (Hp) and BVDV type 2 antibody titers were used as a proxy to measure serological innate and adaptive vaccine immune responses. Maternal diet did not significantly affect Hp ($P = 0.78$) or BVDV type 2 antibody titers ($P = 0.23$). Inflammatory response was observed for both NS and NF treatment groups ($P < 0.001$). After injection, BVDV type 2 antibody titers continually declined from d 0, 7, 28, and 115 across both NS and NF treatment groups ($P < 0.001$). After secondary booster vaccination on d 115, BVDV type 2 antibody titers significantly increased by d 143 ($P < 0.001$).

Introduction

Observations through the past two centuries have shown the benefits of immunization and that vaccinations are one of the most cost-effective methods to preventing economic losses

and reduce the risk of disease in livestock (Babiuk, 2002). In livestock, the major causes of death preceding slaughter are due to infectious diseases (Babiuk, 2002). Bovine respiratory disease complex (BRDC) persists as the single most costly disease syndrome associated with commercial beef production in the United States, accounting for losses in 2010 of 1,055,000 animals valued at $643 million (NASS, 2011). Increased morbidity and mortality, decreased weight gains, decreased feed utilization, and decreased carcass quality account for the economic losses associated with BRDC (Edwards, 2010). The morbidity risk of BRDC in feedlot cattle occurs in the first 45 d after arrival into the feedlot with the highest risk occuring in wk 1 to 3, after that morbidity declines (Buhman et al., 2000; Edwards, 1996).

To help combat this costly inefficiency, cattle producers have implemented vaccination protocols for their beef herds. The viral vaccine components of BRDC consist of bovine herpesvirus type 1, also known as infectious bovine rhinotracheitis (IBR), bovine viral diarrhea (BVD), parainfluenza virus type 3 (PI-3), and bovine respiratory syncytial virus (BRSV) (Urban-Chmiel and Grooms, 2012). The bacterial vaccine components of BRDC consists of *Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni* (Urban-Chmiel and Grooms, 2012). Killed and modified live vaccines (MLV) are available in different combinations of viral pathogens (Urban-Chmiel and Grooms, 2012). The appropriate use of these vaccines can reduce the risk of BRDC (Urban-Chmiel and Grooms, 2012). Disease control or elimination requires the stimulation of the immune system in a sufficient proportion of the population or herd (Siegrist, 2013). Immunization is achieved by inducing protection, as a result of stimulating the adaptive immune system (Siegrist, 2013). This immunity is achieved by the maintenance of antigen-specific immune effectors and/or by the induction of immune memory cells that can reactivate if re-exposure to the antigen occurs (Siegrist, 2013).

In order to elicit a vaccine response, a vaccine must provide enough danger signals from the antigen, typically paired with an adjuvant, to trigger the inflammatory reaction that is mediated by cells of the innate immune system (Hoebe et al., 2004). Upon injection of vaccine antigens, an acute phase inflammation develops within minutes (Tizard, 2013). Upon injection of an antigen, broken cells release molecules known as damage-associated molecular patterns (DAMPs) that trigger the release of cytokines, chemokines, and enzymes from sentinel cells (Tizard, 2013). The antigens also provide pathogen-associated molecular patterns (PAMPs) that trigger sentinel cell response (Tizard, 2013). This causes the sentinel cells to synthesize and secrete a mixture of molecules that trigger inflammation and initiates the first steps of the adaptive immune system (Tizard, 2013). The three major cytokines secreted by sentinel cells include tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) (Tizard, 2013).

The release of TNF-α, IL-1, and IL-6 increases protein synthesis, specifically acute phase proteins (APP) (Tizard, 2013). Acute phase proteins are a group of blood proteins that change in concentration when an animal is subjected to external or internal challenges, such as infection, inflammation, and stress (Murata et al., 2004). One of the most prominent APP found in beef cattle is haptoglobin (Hp) (Alsemgeest et al., 1994). Hp concentrations in healthy cattle are often undetected, but during an acute phase response bovine haptoglobin can increase 50-100 times (Conner et al., 1988). However, Hp concentrations observed post vaccination with killed clostridial 7 – way bacterin toxoids or BRDC 5 – way MLV viral vaccines with a *Mannheimia haemolytica toxoid* have been closer to 4 – 7 fold increases post injection (Stokka et al., 1994; Gaspers et al., 2018). Hp concentrations will increase with both bacterial and viral infections (Schroedl et al., 2001; Ganheim et al., 2003; Heegaard et al., 2000; Idoate et al.,

2015), and Hp can be used as a tool to measure respiratory disease in feedlot conditions (Idoate et al., 2015).

Inflammation and actions of the innate immune system may be sufficient to protect the body, but it cannot be guaranteed to provide complete resistance to infection, or assist the body to learn from the experience (Tizard, 2013). This limitation of the innate immune response is the advantage of the adaptive immune response (Tizard, 2013). Dendritic cells are the link between the innate and adaptive immune system. When exposed to an antigen, dendritic cells undergo a maturation process, modulating specific surface receptors and migrate towards the lymphatic system, in secondary lymph nodes (Siegrist, 2013). Phagocytosed antigen is loaded onto the MHC class II molecules and presented to T cells (Tizard, 2013). The central role of dendritic cells, in response to a vaccine, is to provide antigen-specific and costimulatory signals to activate naïve T cells (Palucka et al., 2005).

The MHC-peptide complex is displayed on their surface and binds to a subset of CD4+ T cells, follicular helper T cells (Siegrist, 2013). These follicular helper T cells have different sets of chemokine receptors, transcription factors, surface markers, and interleukins that are uniquely equipped to provide the most efficient B cell help through a series of coststimulatory molecules (Vinuesa et al., 2005). The interactions between antigen-specific germinal center B cells, antigen bearing follicular dendritic cells, and follicular helper T cells results in the production of B cells with the highest level of antigen-specific affinity (Siegrist, 2013). This combination provides signals necessary for the differentiation of germinal center B cells either towards plasma secreting specific antibodies or towards memory B cells (Siegrist, 2013). Antigen specific antibodies have been formally demonstrated as conferring vaccine-induced

protection against many diseases, and the production of memory B cells allows for the long term disease protection (Casadevall, 2004).

The majority of cattle vaccines administered to cattle are parenteral, either intramuscular or subcutaneous, using a needle and syringe (Babiuk et al., 2017). There are potential drawbacks to this vaccine delivery method. Improper handling of needles can result in accidental injury to the individual operator as well as the animal receiving vaccine (Weese and Jack, 2008). There is also risk that needle fragments may break off during injection and remain in the muscle of the animal to slaughter (van Drunen Little-van den Hurk, 2006). Additionally, blood – born infectious agents such as bovine leucosis and anaplasmosis as well as organisms on the skin can be transmitted between animals if one needle is used to inject multiple animals (Hollis et al., 2005; Reinbold et al., 2010). These concerns have led to the design of alternative vaccination techniques, including the use of needle – free injection devices (NFs) (Rey et al., 2013).

NF's use compressed gas, typically CO_2 or N_2, at pressures ranging from 310.3 – 448.2 kPa (Rey et al., 2015). Upon triggering the device, the desired volume of vaccine is forced through a small orifice which forms a high pressure stream that can penetrate the skin and deposit the vaccine into the desired tissue (Mousel et al., 2008). This vaccination technique removes the issues observed with needles, helps reduce disease transfer, and reduces vaccination time (Chase et al., 2008; Reinbold et al., 2010; Mousel et al., 2008). This vaccination technique has been shown to elicit serological immune responses in cattle to *Mannheimia haemolytica*, IBRV, and BVDV (Hollis et al., 2005; Rey et al., 2013). The objective of this study was to evaluate the influence of NF's on serological innate and adaptive immune responses to a MLV BVDV type one and two vaccine with a *Mannheimia haemolytica*

toxoid. We hypothesized that the both NF as well as needle and syringe (NS) will induce a significant innate and adaptive immune response which will result in long term immunological memory.

Materials and Methods

All procedures were approved by the North Dakota State University (NDSU) and University of Manitoba (UM) Animal Care and Use Committees. Forty – three Angus – based Cow – calf pairs, from the colostrum trial described in chapter 2, were moved as a single group from NDSU's Beef Cattle Research Complex to pasture in mid – May at the Central Grassland Research and Extension Center. At 60 d of age (mid – June) steers (129.1±6.98kg, n = 43) born from dams that were supplemented corn during mid – to late – gestation and steers born from cows on the control diet were randomly assigned to either vaccination using NS (n = 21) (Non – supplemented = 10 and supplemented = 11) or using a NF (n = 22) (Non – supplemented = 11 and supplemented = 11). On d 0 of the trial, both treatment groups received a 2 cc dose of MLV BVDV type one and two vaccine with a *Mannheimia haemolytica* toxoid (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932). The location of vaccination, for both administration methods, was subcutaneous on the right side of the neck 10.2 – 12.7 cm behind the ear in the anterior angle of the triangular zone. For the NS group, vaccinations were administered with a multi – dose pistol – grip syringe fitted with an 18 G 2.54 cm needle. For the NF group, vaccinations were administered using a Pulse 250 NeedleFree Injection System (Pulse Needle Free System, Lenexa, K. S.), using compressed CO_2 set to 310 – 345 kPa. Both treatment groups also received a 7 – way clostridial bacterin–toxoid (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932) administered via subcutaneous route on left side of neck. Steers were weaned on 150 d of age and transported to the Manitoba Beef and Forage Initiative's Johnson

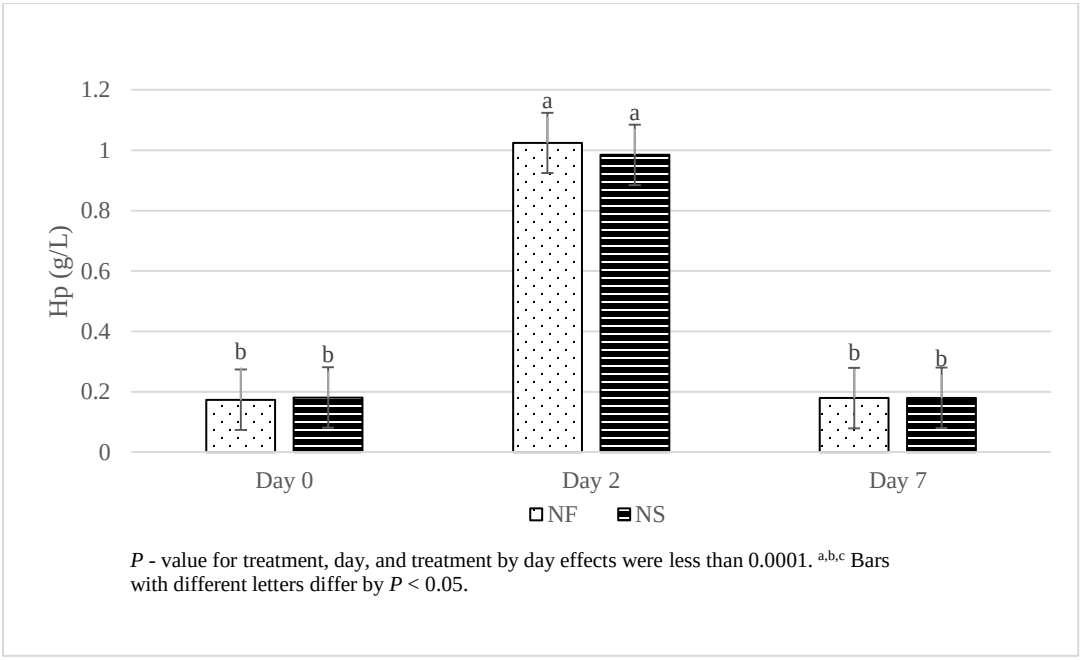
Farm. All steers were backgrounded on pasture and revaccinated with the same MLV BVDV type one and two vaccine with a *Mannheimia haemolytica* toxoid, using the same administration technique and location as the primary dose.

Blood samples were collected via jugular venipuncture in the morning on d 0, 2, 5, 7, 115, and 143 post booster vaccination in 7 ml red top CORVAC serum separation tubes (VWR, Radnor, P. A.). Samples were placed on ice for 30 min and centrifuged for 20 min at 1,380 x g was pipetted into cryo-vials and frozen at -4° F (-20° C) until later analysis. All serum samples in cryo-vials were taken out of freezer and placed in sample tube holders at room temperature 70° F (21° C) until completed thawed. Subsamples were pipetted into new cryo-vials placed in Styrofoam coolers with icepacks and shipped overnight to University of Guelph and Texas A&M Veterinary diagnostic lab for Hb and antibody titers, respectively. Serum samples from d 0, 2, and 7 were sent to the University of Guelph diagnostic lab for Hp concentrations analysis using a Roche Cobas 6000 c501 biochemistry analyzer (Makimura and Suzuki, 1982; Skinner et al., 1991). Serum samples from day 0, 7, 28, 115 and 143 were sent to Texas A&M Veterinary diagnostic lab for BVDV type 2 antibody titers using the serum neutralization test method (Mahy and Kangro, 1996).

Data were analyzed using the mixed procedure of SAS (SAS Ins. Inc., Cary, N. C.) The model included fixed effects of maternal diet, vaccine administration technique, day and their interactions with a repeated measure statement for Hp and BVDV type 2. Maternal diet and the interaction of maternal diet and vaccine administration technique were not significant so maternal diet was removed from the model. Antibody titers were converted using the natural log to normalize data. Significance was determined with an alpha of $P \leq 0.05$.

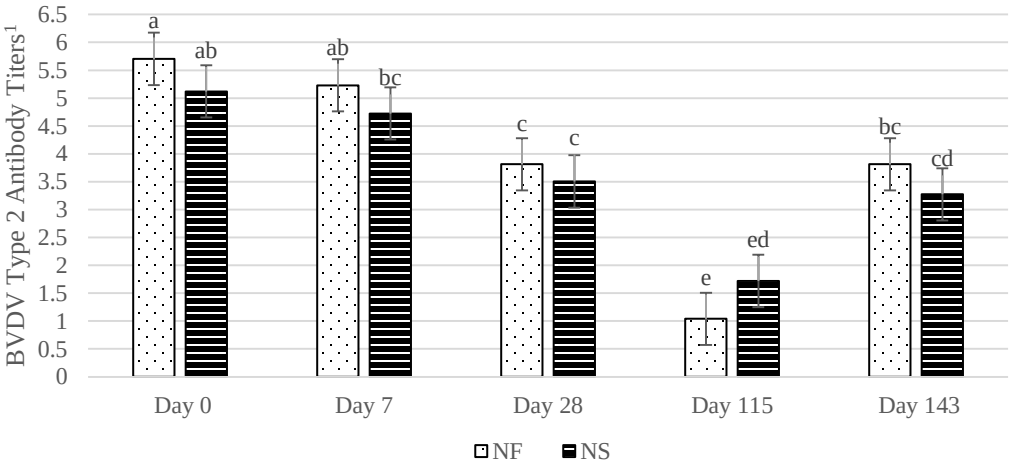
Results

The inflammatory response was observed for both NS and NF treatment groups over time ($P < 0.001$; Figure 3.1). Haptoglobin levels increased d 2 post injection for both NS and NF treatment groups ($P < 0.001$). At d 7 the inflammatory response of the innate immune system returned to homeostatic levels observed on d 0. After injection BVDV type 2 antibody titers continually declined from d 0, 7, 28, and 115 across both NS and NF treatment groups ($P < 0.001$; Figure 3.2). After secondary booster vaccination on d 115, BVDV type 2 antibody titers increased by d 143 ($P < 0.001$). Bovine viral diarrhea virus type 2 antibody titers after primary and secondary vaccination never achieved titer levels seen on d 0 of trial ($P < 0.001$).



P - value for treatment, day, and treatment by day effects were less than 0.0001. [a,b,c] Bars with different letters differ by $P < 0.05$.

Figure 3.1. Haptoglobin levels (g/L) by vaccination treatment across time



[1]SN titer value were normalized using the Natural Log. *P* - value for treatment, day, and treatment by day effects were less than 0.0001. [a,b,c] Bars with different letters differ $P < 0.05$.

Figure 3.2. BVDV type 2 antibody titers by vaccination treatment across time

Discussion

We accept our hypothesis that both NF as well as NS administration of a MLV BVDV type one and two vaccine with a *Mannheimia haemolytica* toxoid will induce a significant innate and adaptive immune response which will result in long term immunological memory. Long term immunity, shown by antibody and cell-mediated response is detectible after a MLV vaccine is administered (Fulton, 2002). The main body of literature that has shown these results of long – term humoral and cell – mediated immunity using MLV vaccines has been completed using parenteral administration techniques. There are very few research trials that have been conducted on MLV BRDC vaccines using NF's, and to the authors knowledge none of them have examined the inflammatory response or the cell mediated response.

The inflammatory response was observed for both NS and NF administration techniques. One of the most prominent APP found in beef cattle is Hp (Alsemgeest et al., 1994). Haptoglobin concentrations increase with both bacterial and viral infections (Schroedl et

al., 2001; Ganheim et al., 2003; Heegaard et al., 2000; Idoate et al., 2015). Haptoglobin concentrations in healthy cattle are often undetected, but during an acute phase response bovine Hp can increase 50-100 times (Conner et al., 1988). However, Hp concentrations observed post vaccination with killed clostridial 7 – way bacterin toxoids or BRDC 5 – way MLV viral vaccines with a *Mannheimia haemolytica toxoid* have been closer to 4 – 7 fold increases post injection (Stokka et al., 1993; Gaspers et al., 2018). The Hp response for both NS and NF administration was similar to Stokka et al. (1994) and Gaspers et al. (2018). There was roughly a 5 fold increase on d 2 post injection. This peak Hp response to a MLV BRDC viral vaccine on d 2 has been shown previously (Gaspers et al., 2018).

Antigen specific antibodies have been formally demonstrated as conferring vaccine-induced protection against many diseases, and the production of memory B cells allows for long term disease protection (Casadevall, 2004). MLV vaccines stimulate a rapid immune response and generally one dose is needed to stimulate protective immunity (Sutton, 1980; Fulton, 2002). One dose will stimulate an adequate immune response, which varies in length depending on the form of disease challenge (Fulton, 2002). In the current trial, the immune response post primary vaccination was not sufficient enough to induce a humoral response. The secondary booster vaccination did induce an improved humoral immune response; however, the antibody response on d 143 never reached the antibody titer levels observed on d 0. The elevated BVDV type 2 antibodies on d 0 is likely the result of maternal antibodies derived from colostrum that were absorbed by the calf in the first 12 – 24 h of life (Morein et al., 2002).

These maternally derived antibodies in circulation of the calf can impede the calf's own immune system to mount an effective response (Morein et al., 2002). This impedance to an

effective vaccine response is known as maternal interference (Chase et al., 2008). Maternal interference has been demonstrated on several vaccine antigens, including BVDV type 2 (Ellis et al., 2001). Thus, the timing of parenteral administered vaccines involves estimating when maternal antibody levels have diminished enough for an individual immune response to progress sufficiently to provide immunological memory (Chase et al., 2008). In a review, Chase et al., (2008) recommended that BVDV control programs that use a MLV should begin around 2 to 3 mo of age and followed by booster vaccination at around 4 – 5 mo of age. The current trial followed that recommendation closely. Based on the antibody levels and lack of response to the primary vaccination, initial vaccination of a MLV BVDV virus may have an improved vaccine response in calves closer to 3 – 4 mo of age.

The majority of cattle vaccines administered to cattle are parenteral, either intramuscular or subcutaneous, using a needle and syringe (Babiuk et al., 2018). There are potential drawbacks to this vaccine delivery method. Improper handling of needles can result in accidental injury to the individual operator as well as the animal receiving vaccine (Weese and Jack, 2008). There is also risk that needle fragments may break off during injection and remain in the muscle of the animal until slaughter (van Drunen Little-van den Hurk, 2006). Additionally, blood – born infectious agents such as bovine leucosis and anaplasmosis as well as organism on the skin can be transmitted between animals if one needle is used to inject multiple animals (Hollis et al., 2005; Reinbold et al., 2010). These concerns have led to the design of alternative vaccination techniques, including the use of NFs (Rey et al., 2013). This vaccination technique removes the issues observed with needles, helps reduce disease transfer, and reduces vaccination time (Chase et al., 2008; Reinbold et al., 2010; Mousel et al., 2008). NF's do have limitations and drawbacks. At the time of the trial, NF's were drastically more

expensive than a conventional multi – dose syringe and needle. Also, changing the dosage of NF's requires the user to change out the pistons of the NF's device, which is additional time and costs. This vaccination technique has been shown to elicit serological immune responses in cattle to Mannheimia haemolytica, IBRV, and BVDV (Hollis et al., 2005; Rey et al., 2013). The current study did confirm that the use of NF's did elicit an inflammatory immune response after primary vaccination and a humoral immune response post – secondary vaccination.

Conclusion

The use of NF's is a viable alternative vaccine administration technique that elicits an immune response comparable to traditional parenteral vaccine techniques. As NF's technology advances, costs of NF's decrease, and the body of research increases, NF devises may become more commonplace in the industry. The benefits of this technology are compelling and further investigation is warranted. Primary vaccination with a MLV BVDV using either technique on calves 60 d of age or less may not result in an adequate humoral immune response due to maternal antibodies already in circulation. A secondary vaccination was needed to stimulate the immune system enough to increase antibody titers. It is common to vaccinate calves when animals are already being handled, such as turnout and weaning. This limits stress to the animals and also limits labor required to handle each individual animal. However, it is also important to understand how vaccines will interact with the animal's immune system at different time periods in life.

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CHAPTER 4. EVALUATION OF SEROLOGICAL IMMUNE RESPONSE TO A BACTERIAL – PRODUCED PLASMID DNA, ZELNATE, AND A MODIFIED LIVE IBRV, BVDV, BRSV, PI3V, AND *MANNHEIMIA HAEMOLYTICA* VACCINATION ON THE FEEDING BEHAVIOR AND FEEDLOT PERFORMANCE OF WEANED CALVES

Abstract

The objective was to evaluate the serological immune response to a bacterial – produced plasmid DNA, Zelnate, and a modified live IBRV, BVDV, BRSV, PI3V, and *Mannheimia haemolytica* vaccination on the feeding behavior and feedlot performance of weaned calves. Weaned commercial Angus and Simmental beef steers (361.5±48.5kg, n=65) were blocked by weight and randomly assigned to one of four treatments. Treatment one (T1) was a 2 cc sterile saline negative control subcutaneously injected in the neck. Treatment two (T2) was adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with *M. haemolytica* bacterin–toxoid administered via subcutaneous route. Treatment three (T3) was bacterial – produced plasmid DNA administered the intramuscular route. Treatment four (T4) was adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with *M. haemolytica* bacterin–toxoid in combination with bacterial – produced plasmid DNA administered via subcutaneous and intramuscular routes, respectively. Blood samples were collected via jugular venipuncture in the morning on days 0, 1, 3, 6, and 28 post vaccination. Individual feed intake and feeding behavior was monitored using the Insentec roughage intake control system. Haptoglobin (Hp), Interferon gamma (IFNγ), and antibody titers for BVDV type 2 were used as a proxy to measure vaccine response. T2 and T4 initiated an Hp inflammatory response over time ($P < 0.001$). Interferon gamma was not significantly

different across treatment over time ($P = 0.39$); however, showed a threefold increase in treatment three over time. T2 and T4 maintained BVDV type 2 antibodies over time ($P < 0.001$). Feed intake and feeding behavior were unaffected by the use of the vaccine and/or immunostimulant.

Introduction

The experiences observed through the past two centuries have shown the benefits of immunization and that vaccinations are one of the most cost-effective methods to preventing economic losses and reduce the risk of disease in livestock (Babiuk, 2002). In livestock, the major causes of death preceding slaughter are due to infectious diseases (Babiuk, 2002). Bovine respiratory disease complex (BRDC) persists as the single most costly disease syndrome associated with commercial beef production in the United States, accounting for losses in 2010 of 1,055,000 animals valued at $643 million (NASS, 2011). Increased morbidity and mortality, decreased weight gains, decreased feed utilization, and decreased carcass quality account for the economic losses associated with BRDC (Edwards, 2010). The morbidity risk of BRDC in feedlot cattle occurs in the first 45 d after arrival into the feedlot with the highest risk occuring in wk 1 to 3, after that morbidity declines (Buhman et al., 2000; Edwards, 1996).

To help combat this costly inefficiency, cattle producers have implemented vaccination protocols for their beef herds. The viral vaccine components of BRDC consist of bovine herpesvirus type 1, also known as infectious bovine rhinotracheitis (IBRV), bovine viral diarrhea virus (BVDV), parainfluenza virus type 3 (PI-3V), and bovine respiratory syncytial virus (BRSV) (Urban-Chmiel and Grooms, 2012). The bacterial vaccine components of BRDC consists of *Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni* (Urban-Chmiel and Grooms, 2012). Killed and modified live vaccines (MLV) are available in different

combinations of viral pathogens (Urban-Chmiel and Grooms, 2012). The appropriate use of these vaccines can reduce the risk of BRDC (Urban-Chmiel and Grooms, 2012). Disease control or elimination requires the stimulation of the immune system in a sufficient proportion of the population or herd (Siegrist, 2013). Immunization is achieved by inducing protection, as a result of stimulating the adaptive immune system (Siegrist, 2013). However, exposing an animal to an antigen can negatively affect their performance in the feedlot (Stokka et al., 1994). This immunity is achieved by the maintenance of antigen-specific immune effectors and/or by the induction of immune memory cells that can reactivate if re-exposure to the antigen occurs (Siegrist, 2013).

In order to elicit a vaccine response, a vaccine must provide enough danger signals from the antigen, typically paired with an adjuvant, to trigger the inflammatory reaction that is mediated by cells of the innate immune system (Hoebe et al., 2004). Upon injection of vaccine antigens, an acute phase inflammation develops within minutes (Tizard, 2013). Upon injection of an antigen, broken cells release molecules known as damage-associated molecular patterns (DAMPs) that trigger the release of cytokines, chemokines, and enzymes from sentinel cells (Tizard, 2013). The antigens also provide pathogen-associated molecular patterns (PAMPs) that trigger sentinel cell response (Tizard, 2013). This causes the sentinel cells to synthesize and secrete a mixture of molecules that trigger inflammation and initiates the first steps of the adaptive immune system (Tizard, 2013). The three major cytokines secreted by sentinel cells include tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6) (Tizard, 2013).

The release of TNF-α, IL-1, and IL-6 increases protein synthesis, specifically acute phase proteins (APP) (Tizard, 2013). APP are a group of blood proteins that change in

concentration when an animal is subjected to external or internal challenges, such as infection, inflammation, and stress (Murata et al., 2004). One of the most prominent APP found in beef cattle is haptoglobin (Hp) (Alsemgeest et al., 1994). Hp concentrations in healthy cattle are often undetected, but during an acute phase response bovine haptoglobin can increase 50-100 times (Conner et al., 1988). However, Hp concentrations observed post vaccination with killed clostridial 7 – way bacterin toxoids or BRDC 5 – way MLV viral vaccines with a *Mannheimia haemolytica toxoid* have been closer to 4 – 7 fold increases post injection (Stokka et al., 1993; Gaspers et al., 2018). Hp concentrations will increase with both bacterial and viral infections (Schroedl et al., 2001; Ganheim et al., 2003; Heegaard et al., 2000; Idoate et al., 2015), and Hp can be used as a tool to measure respiratory disease in feedlot conditions (Idoate et al., 2015).

Inflammation and the actions of the innate immune system may be sufficient to protect the body, but it cannot be guaranteed to provide complete resistance to infection, or assist the body to learn from the experience (Tizard, 2013). This limitation of the innate immune response is the advantage of the adaptive immune response (Tizard, 2013). Dendritic cells are the link between the innate and adaptive immune system. When exposed to an antigen, dendritic cells undergo a maturation process, modulating specific surface receptors and migrate towards the lymphatic system, in secondary lymph nodes (Siegrist, 2013). Phagocytosed antigen is loaded onto the MHC class II molecules and presented to T cells (Tizard, 2013). The central role of dendritic cells, in response to a vaccine, is to provide antigen-specific and costimulatory signals to activate naïve T cells (Palucka et al., 2005).

The MHC-peptide complex is displayed on the surface dendritic cells and binds to a subset of CD4+ T cells, follicular helper T cells (Siegrist, 2013). These follicular helper T cells have different sets of chemokine receptors, transcription factors, surface markers, and

interleukins that are uniquely equipped to provide the most efficient B cell help through a series of costimulatory molecules (Vinuesa et al., 2005). The interactions between antigen-specific germinal center B cells, antigen bearing follicular dendritic cells, and follicular helper T cells results in the production of B cells with the highest level of antigen-specific affinity (Siegrist, 2013). This combination provides signals necessary for the differentiation of germinal center B cells either towards plasma secreting specific antibodies or towards memory B cells (Siegrist, 2013). Antigen specific antibodies have been formally demonstrated as conferring vaccine-induced protection against many diseases, and the production of memory B cells allows for the long term disease protection (Casadevall, 2004).

In addition to vaccination for the bacterial and viral components to reduce the incidence of BRDC, the prophylactic use of antibiotics has been commonly used in United States beef production to control BRDC (Panciero and Confer, 2010; Murray et al., 2016). With increasing public concerns about widespread use of antimicrobials and governmental policy regarding the use of antimicrobials, alternative preventative treatments have been investigated (Ilg, 2017). Activation of specific areas of the immune system in cattle has been considered as a potential mechanism and area of research (Ackermann et al., 2010). Administration of an exogenous substances that has the ability to augment and/or stimulate certain immune responses may have the ability to increase disease resistance (Blecha, 1988). Substances that exert these functions are known as immunostimulants (Blecha, 2001). The ability to enhance or initiate an immune response to benefit the animal and thus the production efficiency is the goal of immunostimulation in food producing animals (Hudson, 2017). This concept has raised interest in the beef industry as a potential novel preventative measure that may be reduce antimicrobial use (Hudson, 2017). There are a limited number of immunostimulatory products that have been

licensed by the United States Department of Agriculture for use in food animals and few have achieved Food and Drug Administration approval (Huenefeld, 1988; Blecha, 2001).

The first, and only, immunostimulant on the market approved for the aid in treatment of BRDC in beef cattle is Zelnate (Ilg, 2017). Research completed by Bayer (2014) showed a reduction in lung lesions and mortality associate with BRDC. Zelnate is a plasmid DNA rich in non – methylated CpG motifs that is encased in a cationic liposome shell (Ilg, 2017). Zelnate is indicated for use to reduce the incidence of BRDC caused by *Mannheimia haemolytica* in cattle 4 mo of age or older, when administered at the time of or within 24 h of a perceived stressful event (Nickell et al., 2016). Ilg (2017) researched Zelnate's potential mechanism of action by investigating cellular DNA recognition pathways in cell culture using knockout human and mouse cell lines and reporter gene assays. Ilg (2017) reported that Zelnate, despite being rich in CpG islands, did not activate TLR9 and the downstream proinflammatory NF_KB pathway. Ilg (2017) did report that Zelnate did initiate the IRF3 pathway, known to lead to a strong type I interferon response. Rogers et al. (2017) evaluated health outcomes of at risk heifers for the first 60 d upon arrival to the feedlot. Rogers et al., (2017) did not report differences among treatment groups on the incidence of heifers treated for respiratory disease; however, the inclusion of Zelnate did reduce the percentage of BRDC associated morbidity as well as overall mortality by d 60. Roger et al. (2017) reduced morality results correspond with the results reported by Bayer (2014), which may suggest that the DNA immunostimulant has the potential to positively affect survivability and health outcomes of high risk feedlot calves.

The objective of this study is to evaluate the serological immune response to a bacterial – produced plasmid DNA, Zelnate, and a modified live IBRV, BVDV, BRSV, PI3V, and *Mannheimia haemolytica* vaccination on the feeding behavior and feedlot performance of

weaned calves. We hypothesized that the bacterial – produced plasmid DNA, Zelnate, and a modified live IBRV, BVDV, BRSV, PI3V, and *Mannheimia haemolytica* vaccination will initiate an immune response to previously vaccinated, newly weaned, backgrounding steers and will not have an effect on feeding behavior and feedlot performance.

Materials and Methods

All procedures were approved by the North Dakota State University Animal Care and Use Committee. This study utilized 65 weaned commercial Angus and Simmental beef steers (361.5±48.5kg) born (January. 1, 2015, to March 31, 2015). At birth, calves were vaccinated with MLV IBRV, PI3V, and BRSV (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932) administered via intranasal route and a killed clostridial type C and D bacterin –toxoid (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932) administered via subcutaneous route. On 1 April 2015, calves were vaccinated with an adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with *M. haemolytica* bacterin-toxoid (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932), a 7 – way clostridial bacterin–toxoid (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932) administered via subcutaneous route and received the macrocyclic lactone, doramectin (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932) administered topically. On 1 September 2015, calves received a MLV IBRV, BVDV (type 1 and 2) PI3V, and BRSV vaccine combined with *Campylobacter* - *Leptospira* bacterin (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932) and a killed *Cl. chauvoei, Cl. septicum, Cl. novyi, Cl. sordelli, Cl. perfringens* (types C and D) and *M. haemolytica* type A1 (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932) subcutaneously and received the macrocyclic lactone, doramectin (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932) administered topically. Calves were weaned for 30 d

and then shipped to the NDSU's Beef Cattle Research Complex. Upon arrival on 15 October 2015, calves (n = 65, body weight [BW] = 336.1 ± 31.6 kg) were trained for 21 d to the Insentec Roughage Feeders (Insentec; Insentec B. V. Repelweg 10, 8316 PV Marknesse, Netherlands).

Insentec Roughage Feeders measure dry matter intake (DMI) kg, time spent at feeder measured in minutes and number of visits, and the number of meals can be calculated. Body weight kg was determined on d -21, 0, 1, 3, 6, 28, and 29 and average daily gain (ADG), feed conversion ratio (FCR), and gain-to-feed (G:F) were calculated. Time spent at feeder, number of visits, and meals were calculated on a 24-h cycle. A meal is defined as a distinct, separate eating period and visit not separated by intervals longer than seven minutes (Forbes, 1995; Montanholi et al., 2010).

On d 0, calves were blocked by weight, randomly assigned to one of four possible treatments. Treatment one (T1) was a 2 cc sterile saline negative control subcutaneously injected in the neck. Treatment two (T2) was a 2 cc adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with *M. haemolytica* bacterin–toxoid (Zoetis; 100 Campus Drive, Florham Park, New Jersey 07932) administered via the subcutaneous route. Treatment three (T3) was bacterial – produced plasmid DNA (Bayer; 100 Bayer Boulevard, Whippany, New Jersey 07981) administered via the intramuscular route. Treatment four (T4) was adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with *M. haemolytica* bacterin–toxoid in combination with bacterial – produced plasmid DNA administered via the subcutaneous and intramuscular routes, respectively. Blood samples were collected via jugular venipuncture in the morning on days 0, 1, 3, 6, and 28 post vaccination in 7 ml red top CORVAC serum separation tubes

(VWR, 100 Matsonford Road, Radnor, Pennsylvania, 19087). Samples were placed on ice for 30 min and centrifuged for 20 min at 1,380 x g to separate serum, which was then pipetted into cryo-vials and frozen at -20° C until later analysis. All serum samples in cryo-vials were taken out of freezer and placed in sample tube holders at room temperature (21° C) until completed thawed. Subsamples were pipetted into new cryo-vials placed in Styrofoam coolers with icepacks and shipped overnight to University of Guelph and Texas A&M Veterinary diagnostic lab for Hp and antibody titers, respectively. Serum samples from d 0, 1, 3, and 6 were sent to the University of Guelph diagnostic lab for Hp concentrations analysis using a Roche Cobas 6000 c501 biochemistry analyzer (Makimura and Suzuki, 1982; Skinner et al., 1991). Serum samples from d 0, 6, and 28 were sent to Texas A&M Veterinary diagnostic lab for BVDV type 2 antibody titer using the serum neutralization test method (Mahy and Kangro, 1996).

Serum samples from d 0, 1, 3, and 6 were analyzed for interferon gamma (IFNγ) using commercially available enzyme – linked immunosorbent assay (ELISA) kit (ABclonal; 86 Cummings Par Drive, Woburn, Massachusetts, 01801) according to the protocol supplied by the manufacturer. Serum was diluted four fold with phosphate buffered saline. Standards, pooled controls, and diluted sample unknowns were added in triplicate to pre – blocked 96 well plate. Optical density (OD) readings at a wavelength of 450 nm were obtained with a Synergy H1 Microplate Reader (Biotek; 100 Tigan Street, Winooski, Vermont, 05404). A standard curve ($R^2 \leq .992$) was produced for each plate which transformed the mean OD values of each sample to the quantity of IFNγ in pg/ml (CV $\geq$ 7.5%).

Data were analyzed using the mixed procedure of SAS (SAS Ins. Inc., Cary, N. C.) The model included fixed effects of treatment, day, breed and their interactions with a repeated measure statement for Hp, IFNγ, and BVDV type 2 antibody titers. Antibody titers were

converted using the natural log to normalize data. Significance was determined with an alpha of $P \leq 0.05$.

Results

The inflammatory response upon injection was observed for both T2 and T4. Both treatments contained the adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with M. haemolytica bacterin–toxoid ($P < 0.001$; Figure 4.1.). Hp levels increased beginning at 1 d post injection ($P < 0.001$) and peak response occurred 3 d post injection ($P < 0.001$). By d 6, Hp levels declined and returned to levels observed on d 0. IFNγ was not different across treatment over time ($P = 0.39$; Figure 4.2.). Serum BVDV type 2 antibody titers levels were greatest for T1, T2, T3, and T4 on d 0 ($P < 0.001$; Figure 4.3.). All BVDV type 2 antibody titer levels decreased by d 6 ($P < 0.001$). By d 28 only T2 and T4 BVDV type 2 antibody titers increased to reach values observed on d 0 ($P < 0.001$). Breed was included in the statistics model for all variables. There were no interactions with breed and treatment across time. Breed did not significantly affect Hp or IFNγ response. Breed was significant on BVDV type 2 antibody titers ($P = 0.008$) where commercial Angus steers had increased titers compared to Simmental steers (4.1 vs. 3.2 ± 0.2). All variables used to quantify feeding behavior and growth performance were not significantly different between all four treatment groups (Tables 4.1. and 4.2.).

Table 4.1. Influence of bacterial - produced plasmid DNA and MLV IBRV, BVDV, BRSV, PI3, and *Mannheimia haemolytica* on feeding behavior in backgrounding steers.

	Treatment[a]					
Item	1	2	3	4	SEM[b]	*P* Value
DMI, kg	8.21	8.30	8.62	8.48	0.27	0.67
Eating events, no./d						
Visits	23.9	28.9	29.7	26.1	2.5	0.35
Meals/d	10.1	10.5	10.9	9.9	0.83	0.54
Eating time, min						
Per visit	6.82	7.17	5.74	7.24	0.55	0.21
Per meal	16.3	17.5	15.2	18.2	1.2	0.25
Per day	157	171	161	173	5.8	0.15
Feed DMI, kg						
Per visit	0.36	0.34	0.31	0.36	0.03	0.47
Per meal	0.86	0.84	0.81	0.91	0.06	0.77
Per min	0.05	0.05	0.05	0.05	0.001	0.06

[a]1: Sterile saline, 2: Adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with *M. haemolytica* bacterin–toxoid, 3: bacterial – produced plasmid DNA, 4: Adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with *M. haemolytica* bacterin–toxoid + bacterial – produced plasmid DNA

[b]Standard error of the mean (n = 65).

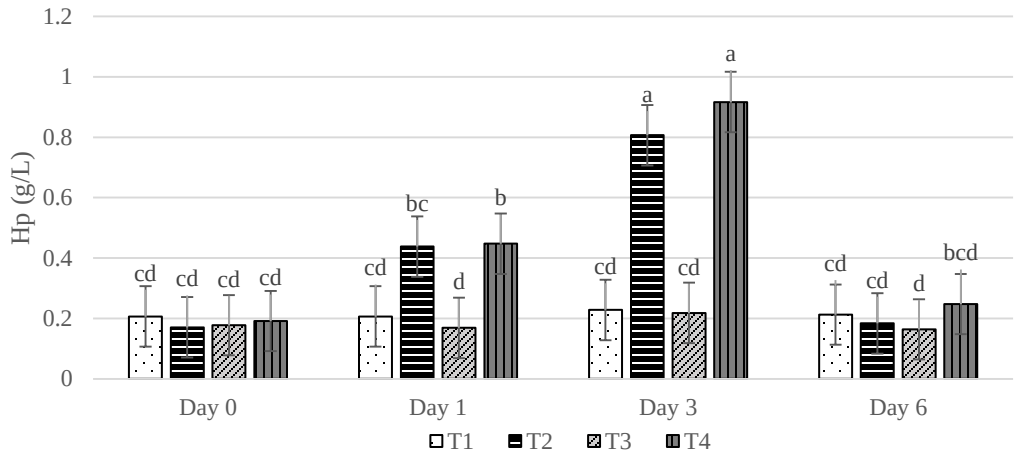
Table 4.2. Influence of bacterial - produced plasmid DNA and MLV IBRV, BVDV, BRSV, PI3, and *Mannheimia haemolytica* on growth performance in backgrounding steers.

	Treatment[a]					
Item	1	2	3	4	SEM[b]	*P* Value
Initial BW, kg	364	355	360	362	9.02	0.89
Final BW, kg	389	384	387	392	9.93	0.95
Gain, kg	24.7	29.1	27.4	29.4	2.82	0.62
Weight change						
3 day	0.56	5.49	2.27	2.32	2.01	0.38
6 day	1.64	5.91	1.84	2.59	1.75	0.29
ADG[c]	0.88	1.04	0.98	1.05	0.1	0.62
G:F	0.05	0.05	0.05	0.05	0.005	0.47

[a]1: Sterile saline, 2: Adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with *M. haemolytica* bacterin–toxoid, 3: bacterial – produced plasmid DNA, 4: Adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with *M. haemolytica* bacterin–toxoid + bacterial – produced plasmid DNA

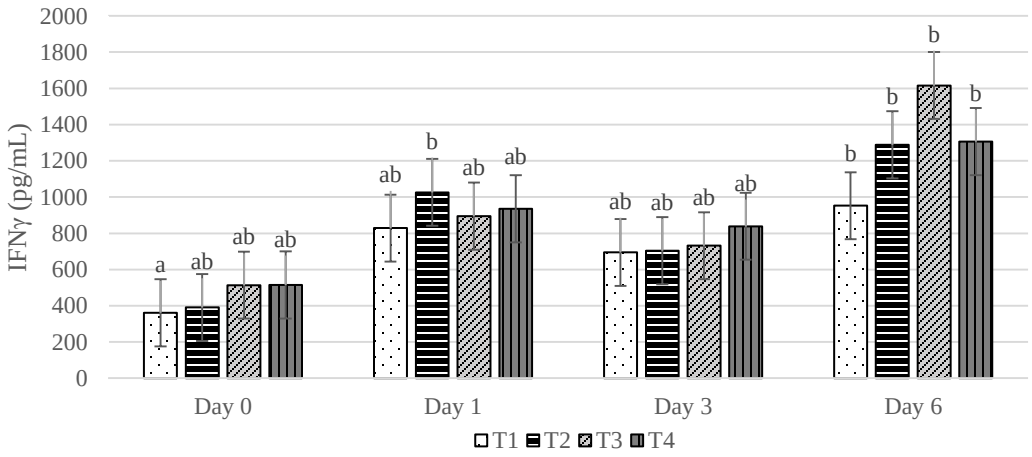
[b]Standard error of the mean (n = 65).

[c]Calculated by dividing the total gain calculated from the average initial and final weights by 28 days



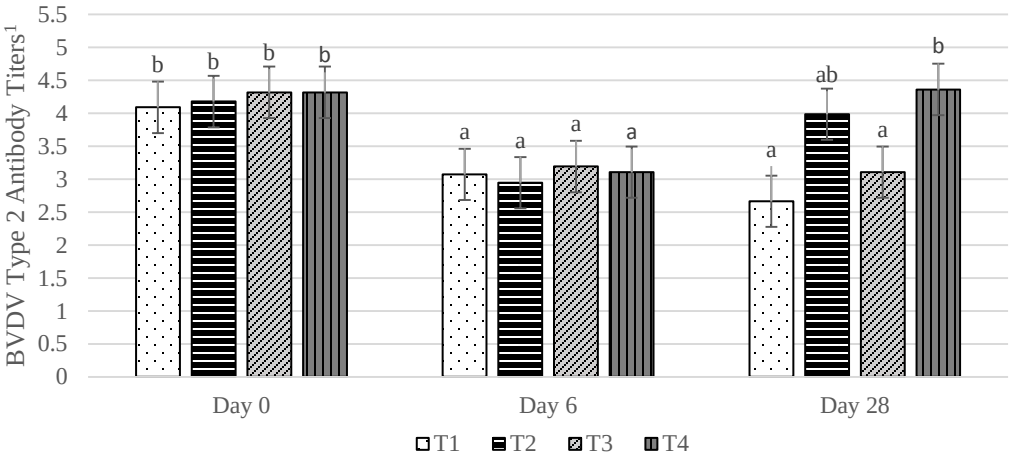
P - value for treatment, day, and treatment by day effects were less than 0.0001. [a,b,c] Bars with different letters differ by $P < 0.05$.

Figure 4.1. Haptoglobin levels (g/L) by vaccination treatment across time



[1]SN titer value were normalized using the Natural Log. *P* - value for treatment, day, and treatment by day effects were less than 0.0001. [a,b,c] Bars with different letters differ by *P* <

Figure 4.2. Interferon Gamma levels (pg/mL) by vaccination treatment across time



[1]SN titer value were normalized using the Natural Log. *P* - value for treatment, day, and treatment by day effects were less than 0.0001. [a,b,c] Bars with different letters differ by $P < 0.05$.

Figure 4.3. BVDV type 2 antibody titers by vaccination treatment across time

Discussion

We accept our hypothesis that the bacterial – produced plasmid DNA, Zelnate, and a modified live IBRV, BVDV, BRSV, PI3V, and *Mannheimia haemolytica* vaccination will initiate an immune response to previously vaccinated, newly weaned, backgrounding steers and will not have an effect on feeding behavior and feedlot performance. Throughout the trial there were no negative effects on feeding behavior or feedlot performance. Also, after completion of the trial and backgrounding phase, we did not observe any signs of respiratory disease leading up to finishing weights and slaughter.

The inflammatory response was observed in both treatment groups that contained an adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with M. haemolytica bacterin–toxoid. One of the most prominent APP found in beef cattle is Hp (Alsemgeest et al., 1994). Hp concentrations will increase with both bacterial and viral infections (Schroedl et al., 2001; Ganheim et al., 2003; Heegaard et al., 2000; Idoate et al.,

2015). T1 (sterile saline) and T3 (bacterial – produced plasmid DNA) did not initiate an Hp inflammatory response. Hp concentrations observed post vaccination with killed clostridial 7 – way bacterin toxoids or multivalent MLV viral vaccines with a *Mannheimia haemolytica toxoid* have been closer to 4 – 7 fold increases post injection (Stokka et al., 1993; Gaspers et al., 2018). The Hp response for both T2 and T4 administration was similar to Stokka et al. (1993) and Gaspers et al. (2018). There was roughly a 5 fold increase on d 3 post injection. This peak Hp response to a MLV BRDC viral vaccine on d 3 has been shown previously (Gaspers et al., 2018).

BRDC is typically initiated with infection of viral pathogens that can predispose the animal to a secondary bacterial infection (Rice et al., 2007). The ability of the bacterial – produced plasmid DNA immunostimulant to activate the innate immune system towards a viral pathogen could prove beneficial in preventing BRDC. Ilg (2017) did report that Zelnate did initiate the IRF3 pathway, known to lead to a strong type I interferon response in human and mouse cell culture. Type I interferons are a major effector cytokine of the host immune response against viral pathogens (Gonzalez – Navajas et al. 2012). Type II interferon, IFNγ, also exhibits antiviral activities ((Gonzalez – Navajas et al. 2012). In addition to antiviral activity, IFNγ plays a major role in the cell mediate immune (CMI) response (Siegrist, 2013). The CMI response initiates CD8+ cytotoxic T cells, bind to virally infected cells and initiate apoptosis directly by release of perforin and granzyme and indirectly thorough antimicrobial cytokine release (Siegrist, 2013). Though IFNγ was not significantly different across treatment over time, further investigation is warranted.

Antigen specific antibodies have been formally demonstrated as conferring vaccine-induced protection against many disease, and the production of memory B cells allows for the

long term disease protection (Casadevall, 2004). MLV vaccines stimulate a rapid immune response and generally one dose is needed to stimulate protective immunity (Sutton, 1980; Fulton, 2002). One dose will stimulate an adequate immune response, which varies in length depending on the form of disease challenge (Fulton, 2002). In the current trial, the humoral immune response post vaccination to an adjuvanted MLV IBRV, BVDV (type 1 and 2), BRSV, and PI3V respiratory vaccine combined with M. haemolytica bacterin–toxoid did not result in an increase to BVDV type 2 antibody titers. Compared to T1 and T3 on d 28, T2 and T4 did maintain an elevated level of BVDV type antibody titers seen on d 0 of the trial. This elevated BVDV type 2 antibody titers seen at the beginning of the trial may have been due to previous vaccinations of steers before the trial. These steers had been exposed to a MLV BVDV antigens twice before this trial, with the last exposure 30 d before the trial start date. This could be the reason for the elevated BVDV type 2 antibody titers seen on d 0. The breed effect on BVDV type 2 antibody titers could be due to heterozygote advantage of MHC polymorphisms (Tizard, 2013). This means that MHC heterozygotes are at an advantage because they can respond to a much larger range of antigens and are better suited to survive infectious diseases (Tizard, 2013). The cattle used on this trial came from a closed NDSU herd. The herd consists of registered Angus, registered Simmentals and commercial SimAngus cattle. The two groups used in this trial were registered Simmental and commercial SimAngus cows with 75% or greater Angus genetics. This could shed some light as to the increased BVDV type 2 antibody titers seen in the commercial SimAngus steer calves. Genetic differences on immune response to vaccine antigens was not the focus of this research and further investigation is warranted to examine the heterozygote advantage of cross – bred calves on immune response to vaccine antigens as well as morbidity and mortality of BRDC.

In addition to vaccination for the bacterial and viral components to reduce the incidence of BRDC, the prophylactic use of antibiotics has been commonly used in United States beef production to control BRDC (Panciero and Confer, 2010; Murray et al., 2016). With increasing public concerns about widespread use of antimicrobials and governmental policy regarding the use of antimicrobials, alternative preventative treatments have been investigated (Ilg, 2017). Activation of specific areas of the immune system in cattle has been considered as a potential mechanism and area of research (Ackermann et al., 2010). Administration of an exogenous substances that has the ability to augment and/or stimulate certain immune responses may have the ability to increase disease resistance (Blecha, 1988). The ability to enhance or initiate an immune response to benefit the animal and thus the production efficiency is the goal of immunostimulation in food producing animals (Hudson, 2017). This concept has raised interest in the beef industry as a potential novel preventative measure that may reduce antimicrobial use (Hudson, 2017). There are a limited number of immunostimulatory products that have been licensed by the United States Department of Agriculture for use in food animals and few have achieved Food and Drug Administration approval (Huenefeld, 1988; Blecha, 2001).

Conclusion

Public concerns about animal agriculture and the widespread use of antimicrobials are not going to go away. Even with improved vaccines and increased number of cattle that are vaccinated prior to weaning and transport, BRDC continues to plague the beef industry. The ability to utilize an exogenous immunostimulant that boost the calf's own immune system in addition to low stress handling techniques, proper vaccination techniques, and management strategies may be beneficial to help reduce the incidence of BRDC and the use of antimicrobials.

Calves used in this study were exposed to BVDV vaccine antigens two times prior to the study, from the same heard, and were allowed to acclimate to their new environment for 21 d before the start of this study. Results on high stress animals that are weaned, vaccinated and comingled with calves from different herds may be different. Further research is needed to evaluate unvaccinated, immune-naïve, calves that are weaned and brought together in a backgrounding feedlot environment.

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CHAPTER 5. GENERAL DISCUSSION AND FUTURE DIRECTIONS

The passive immune transfer is critical to immediate survival of the calf. Colostrum absorption is one of the most important factors in shaping calf health. Calves that fail to absorb enough Ig in colostrum have high pre-weaning mortality rates, up to 89 % in the first week of life (McGuire et al., 1976), as well as other short-term and long-term losses related to animal health, welfare, and productivity (Godden 2008). The continued health of the animal is paramount to long term productivity. In livestock, the major causes of death preceding slaughter are due to infectious diseases (Babiuk, 2002). Bovine respiratory disease complex (BRDC) persists as the single costliest disease syndrome associated with commercial beef production in the United States, accounting for losses in 2010 of 1,055,000 animals valued at $643 million dollars (NASS, 2011). Increased morbidity and mortality, decreased weight gains, decreased feed utilization, and decreased carcass quality account for the economic losses associated with BRD (Edwards, 2010). It is apparent that health in beef calves from birth to weaning is critical to a more efficiently industry. The beef industry is under constant pressure to produce a product that is cost effective, nutritious, and humane. It is our job as researchers and stewards of these livestock species to promote health and productivity.

Chapter two discussed the effects of starch supplementation to beef cows on colostrum production, offspring birth weight, incidence of dystocia, respiratory acidosis, and the passive transfer of immunity. Briefly, colostrum Brix % tended to be greater in SUP dams compared to CON dams (25.10 vs. 21.77 ±1.17%; $P = 0.056$). Incidence of dystocia, as measured by calving ease score, was not different across maternal dietary treatment groups. Calf activity post – partum, as measured by calf vigor score, was not significantly different across treatment groups. Again, similar to calving ease score, there was very little variation in this variable,

which could strengthen the fact that incidence of dystocia was not observed in this trial. All blood gas parameters used to quantify metabolic and respiratory acidosis were not different between maternal dietary treatment groups. Ranges for venous blood pH, pCO_2, pO_2, HCO_3, and base excess were all within normal ranges at 0 h. Ranges for venous blood pCO_2, pO_2, HCO_3, and base excess were all within normal ranges at 24 h. Worth noting is that pH in venous blood at 24 h for calves born to CON dams 7.01, which is considered acidotic. Calves with inadequate serum IgG concentrations (<8 mg/ml) at 24 h are from 3.2 to 9.5 times more likely to become sick and 5.4 times more likely to die before weaning compared to calves with adequate concentrations of serum IgG (>16 mg/ml). When calves have inadequate serum IgG concentrations (<8 mg/ml) a failure of passive transfer (FPT) of immunity has occurred. Calves born from CON and SUP dams had 24 h serum IgG levels three times the 16 mg/ml the threshold for an adequate immune transfer. Calves born from CON and SUP dams had 24 h serum protein levels that exceed the <5.2 g/dl, which is also attributed to a FPT at 24 h.

Chapter three discussed the evaluation of the needle – free injection device (NF) on serological innate and adaptive immune responses to a MLV BVDV type 1 and 2 vaccine with a *Mannheimia haemolytica* toxoid. Maternal diet did not significantly effect on Hp ($P = 0.78$) or BVDV type 2 antibody titers ($P = 0.23$). The inflammatory response was observed for both NS and NF treatment groups ($P < 0.001$). After injection BVDV type 2 antibody titers continually declined from d 0, 7, 28, and 115 across both NS and NF treatment groups ($P < 0.001$). After secondary booster vaccination on d 115, BVDV type 2 antibody titers significantly increased by d 143 ($P < 0.001$).

Finally, chapter four discussed the serological immune response to a bacterial – produced plasmid DNA, Zelnate, and a modified live IBRV, BVDV, BRSV, PI3V, and

Mannheimia haemolytica vaccination on feeding behavior and feedlot performance of weaned calves. Treatments two and four initiated an Hp inflammatory response over time ($P<0.001$). Interferon gamma was not significantly different across treatment over time ($P = 0.39$). Treatments two and four maintained BVDV type 2 antibodies over time ($P <0.001$). Feed intake and feeding behavior were unaffected by the use of the vaccine and/or immunostimulant.

No animal study is without limitations and short comings, and there is certainly room for improvement if these trials were to be repeated. A solution to obtaining more accurate results in trials from chapter two would be to increase the number of animals as well as observations – this would certainly aid in better understanding the passive transfer of immunity and factors that influence it. Integrating corn into the TMR instead of putting it into its own Insentec bunk may have limited the forage substitution effects seen in chapter two. Also, increasing the starch supplementation above 0.2% of BW may have also increased weight and BCS differences at the time of parturition. The continued use of physiologic markers in addition to categorical variables is very important. The use of lactate, base excess, pH, pCO_2, pO_2, Base Excess, HCO_3, TCO_2, sO_2, and lactate assisted in monitoring and quantifying fetal asphyxia and incidence of dystocia. The next step for chapter two would be to examine a wider array of immune factors in the colostrum as well calf sera. The research in chapter four had a large enough number of animals to effectively look at the vaccination response. That trial does have its limitations as well. The steers used in that trial were all previously vaccinated multiple times and were all from the same herd. A similar trial on immune-naïve calves from multiple herds may not respond in the same manner. The stresses of weaning and comingling may depress the immune system and being exposed to a vaccine antigen and/or immunostimulant may negatively affect feeding behavior and feedlot performance. A sex affect was also not

determined in this vaccine trial. Heifers in a similar trial may not react in the same manner. The immune response for the bacterial – produced plasmid DNA was not clearly defined. The IFNγ results for the bacterial – produced plasmid DNA leaves a lot to be desired. Looking at more cytokine profiles over increased number of days beyond six may help elucidate the immunostimulants specific mode of action.

These projects have prospective uses both for future research as well as a benefit to the livestock industry, particularly for those in cow-calf and backgrounding operations. The majority of more recent colostrum research has focused around the dairy calf. There have been very few research trials examining nutrient supplementation of the dam and effects on neonatal calf health. The vast majority of research has looked at maternal nutrient restriction and effects on calf health. Much of that data is decades old and it is important to continue updating the body literature using cattle genetics in current production beef herds. The human and animal welfare benefits of NF's technology are compelling and further investigation is warranted. As NF technology advances, costs of NF's decrease, and the body of research increases, NF devises may become more commonplace in the industry. Public concerns about animal agriculture and the widespread use of antimicrobials are not going to go away. Even with improved vaccines and increased number of cattle that are vaccinated prior to weaning and transport, BRDC continues to plague the beef industry. The ability to utilize an exogenous immunostimulant that boost the calf's own immune system in addition to low stress handling techniques, proper vaccination techniques, and management strategies may be beneficial to help reduce the incidence of BRDC and the use of antimicrobials. The ability to design precise feeding programs during late gestation to help the calf transition healthily into extra-uterine life as well as assist in thorough vaccination and immunostimulant regimes that will keep calves

immunologically sound to progress into the next phases of life while decreasing the use of antimicrobials is of importance to the long-term sustainability of the beef industry.